PROGRAM

TUESDAY, OCTOBER 20, 2009

PALAIS DES CONGRÈS DE VERSAILLES

OPENING SESSION

19:00 ***Greetings:***
Mr. François de Mazières. Versailles, France
Mayor of Versailles

Prof. Dominique Maraninchi, Boulogne-Billancourt, France
President, Institut National du Cancer

Dr. Margaret Foti, Philadelphia, USA
Chief Executive Officer – American Association for Cancer Research

Prof. Fabien Calvo, Boulogne-Billancourt, France
Scientific Director – Institut National du Cancer

Opening Lectures:

19:30 **Isaac P. Witz**, Tel Aviv, Israel – Introductory Lecture
The Tumor Microenvironment: The Making of a Paradigm

19:50 **Jeffrey W. Pollard**, New York, USA – Keynote Lecture
Macrophages and Metastasis

20:30 **Welcome Reception – *Sponsored by the City of Versailles***

WEDNESDAY, OCTOBER 21, 2009

PLENARY SESSION 1: Regulation of Gene Expression in Tumor and Non-Tumor Cells in the Microenvironment

AUDITORIUM RICHELIEU

Session Dedicated to the Memory of Mary A. Pikovski

Chairperson: Margaret Foti, Philadelphia, PA, USA

08:30 **Moshe Oren**, Rehovot, Israel
Involvement of the p53 Tumor Suppressor in Tumor-Stroma Interactions

08:55 **Avraham Raz**, Detroit, MI, USA
Cleavage of Galectin-3 by Matrix Metalloproteinases Regulates Breast Cancer Progression and Metastasis

09:20 **Valerie Marie Weaver**, San Francisco, CA, USA
Extracellular Matrix Remodeling Forces Tumor Progression

09:45 **Yoel Kloog**, Tel Aviv, Israel
Intercellular Transfer of Ras and microRNAs: New Mechanisms of Non-Autonomous Protein Functions and Post-Transcriptional Control

10:10 **Mary Hendrix**, Chicago, IL, USA
Reprogramming Metastatic Tumor Cells with an Embryonic Microenvironment: Convergence of Embryonic and Tumorigenic Signaling Pathways

10:35–11:00 Coffee – Sponsored by TEVA **Pharmaceutical Industries Ltd**

PLENARY SESSION 2: Therapeutic Targeting of Tumor-Microenvironment Interactions: Pre Clinical and Clinical Studies

AUDITORIUM RICHELIEU

Chairperson: Fabien Calvo, Boulogne-Billancourt, France

11:00 **Jacques Pouysségur**, Nice, France
Hypoxia and Tumor progression: New Metabolic Anti-Cancer Targets

11:25 **Amato Giaccia**, Stanford, CA, USA
Identifying New Anti-Cancer Therapeutics Using Synthetic Lethality

11:50 **Frances R. Balkwill**, London, UK
Targeting Cancer-Related Inflammation

12:15 **Benjamin Sredni**, Ramat Gan, Israel
Interference with VLA4 and Microenvironmental Interactions by the Tellurium Compound AS101 Results in the Sensitization of AML Cells to Chemotherapy

12:40 **Eitan Yefenof**, Jerusalem, Israel
Sensitizing Hemopoietic Malignant Cells to Glucocorticoid Induced Apoptosis by Protein Kinase Inhibitors

13:05 **Yona Keisari**, Tel Aviv, Israel
Treatment of Solid Malignant Tumors by Intra-Tumoral Diffusing Alpha-Emitting Sources: Role of Tumor Micro- and Macro-Environmental Traits

13:30–14:45 Business Meeting and Lunch – Auditorium Richelieu

PLENARY SESSION 3: Interactions of Tumor Cells with Microenvironmental Cells and Molecules

AUDITORIUM RICHELIEU

Chairperson: Wolf H. Fridman, Paris, France

14:45 **Yves A. DeClerck**, Los Angeles, CA, USA
Interleukin-6 and the Tumor Microenvironment

15:10 **Adit Ben-Baruch**, Tel Aviv, Israel
Inflammatory Chemokines in Malignancy: Regulation by Microenvironmental and Intrinsic Factors

15:35 **Eli Keshet**, Jerusalem, Israel
Angiogenic Accessory Cells: VEGF-induced Recruitment and Re-programming

16:00 **Robert Kerbel**, Toronto, ON, Canada
Therapy-Induced Alteration of the Tumor Microenvironment: Impact of Bone Marrow Derived Cells

16:25 **Margareta M. Mueller**, Heidelberg, Germany
Characterization of Factors Activating Gr-1+ Inflammatory Cells in Squamous Cell Carcinoma Towards a Tumor-supporting, Pro-angiogenic Phenotype

16:50–17:15 Coffee – Sponsored by "Cancer Microenvironment" the official journal of the International Cancer Microenvironment Society

PLENARY SESSION 4: *Inflammation & Protective Immunity in the Tumor Microenvironment I*

AUDITORIUM RICHELIEU

Chairperson: Eiichi Tahara, Hiroshima, Japan

17:15 **Catherine Sautès-Fridman**, Paris, France
Role of Inflammation and Immune Privilege Microenvironment in Tumor Development

17:40 **Salem Chouaib**, Villejuif, France
Interaction of CTLs with Stroma Components: Endothelial Cell Cross-Recognition by Specific CTL and Influence of Hypoxic Stress

18:05 **Ron N. Apte**, Beer-Sheva, Israel
The Role of IL-1R, TLR2 and TLR4 Signaling in the Malignant Process

20:00 Conference Dinner

THURSDAY, OCTOBER 22, 2009

SYMPOSIUM 1: Regulation of Gene Expression in the Tumor Microenvironment I

AUDITORIUM RICHELIEU

Chairperson: Ruth Lupu, Rochester, MN, USA

08:30 **Andrei Thomas-Tikhonenko**, Philadelphia, PA, USA
Attenuation of TGFβ Signaling by c-Myc-regulated microRNAs

08:50 **Ruth Lupu**, Rochester, MN, USA
Knockout of Heregulin (HRG) Expression Reverts Paclitaxel-Resistance and Promotes Mesenchymal Epithelial Transition (MET) of Triple Negative Breast Cancer Cells

09:10 **Stefano Indraccolo**, Padova, Italy
Decoding Tumor-Host Interactions in Dormancy: Notch3-mediated Regulation of MKP-1 Promotes Tumor Cell Survival

09:22 **Tanya Kalin**, Cincinnati, OH, USA
Role of Foxm1 Transcription Factor in Tumor Microenvironment

09:34 **Florence Brellier**, Basel, Switzerland
Tenascin-W is Overexpressed in Glioma-Associated Blood Vessels and Stimulates Angiogenesis *in vitro*

09:46 **Hagit Turm**, Jerusalem, Israel
Protease activated receptor1, PAR1 Acts via a Novel G_{a13}-DVL Axis to Stabilize b-catenin Levels

09:58 **Matthew Farren**, Buffalo, NY, USA
Tumor-Mediated Suppression of Myeloid to Dendritic Cell (DC) Differentiation via down Regulation of Protein Kinase C βII (PKCβII) Expression

10:10 **Chandana Koorella**, Buffalo, NY, USA
Myeloma Cell Survival and Importance of Crosstalk between Notch1-Jagged2 and CD28-B7 Pathways in Dendritic Cells

10:22 **Olatz Crende**, Leioa, Bizkaia, Spain
Interleukin-18-Dependent Genes of Highly Metastatic Human Melanoma

10:34 **Gwendal Lazennec**, Montpellier, France
Interleukin-8 Expression Is Regulated by Histone Deacetylases through NF-kB Pathway in Breast Cancer

10:46 **Zendra Zehner**, Richmond, VA, USA
Differential Expression of MicroRNA-17-3p Reverts Morphology of Prostate Cells in lrECM Gels, Reduces Tumor Growth *in vivo* and Correlates with Prostate Tumor Expression by LCM Analysis

10:58–11:30 Coffee

SYMPOSIUM 2: Interactions of Tumor Cells with Microenvironmental Cells & Molecules I

ROOM LULLI

Chairperson: Raffaella Giavazzi, Milan, Italy

08:30 **Jacques Huot**, Québec, QC, Canada
Regulation of Colon Cancer Metastasis by Death Receptor-3 and E-selectin

08:50 **Izhak Haviv**, Prahran, Vic, Australia
The Origin of Carcinoma-Associated Fibroblasts

09:10 **Lu-Yuan Li**, Tianjin, China
VEGI, an Endogenous Antiangiogenic Cytokine, Inhibits Hematopoietic Stem Cell Differentiation into Endothelial Progenitor Cell

09:22 **Beatriz Arteta,** Leioa, Bizkaia, Spain
Colon Carcinoma Cell Interaction with Liver Sinusoidal Endothelium Inhibits Organ-Specific Anti-Tumor Immunity via Interleukin-1-Induced Mannose Receptor

09:34 **David Allard**, Cambridge, UK
Reversal of the Transformed Phenotype and Normalisation of Oncogene-Regulated Genes through Contact with Normal Cells

09:46 **Claudia Andl**, Nashville, TN, USA
Fibroblast-Dependent Epithelial Cell Invasion in a Reconstruct Model for Esophageal Cancer

09:58 **Catherine Muller**, Toulouse, France
Cancer-Associated Adipocytes: New Key Players in Breast Tumour Invasion

10:10 **Charlotte Anderberg**, Stockholm, Sweden
Paracrine Signaling by PDGF-CC Promotes Tumor Growth by Recruitment of Cancer-associated Fibroblasts Secreting Osteopontin

10:22 **Didier Dréau**, Charlotte, NC, USA
Interplay between Stroma Chemokines and Endothelin-1 in Breast Cancer Cell Migration and Monocyte Recruitment

10:34 **Ellen Van Obberghen-Schilling**, Nice, France
Autocrine Fibronectin is Essential for Matrix Assembly, Integrin Usage and Adherens Junction Formation in Endothelial Cells

10:46 **Pampee Young**, Nashville, TN, USA
Tumor-Derived, Low-Level TNFa Expression Augments the Formation of Tumor-Promoting Myeloid Subtype of Vascular Leukocytes Through the Upregulation of Integrin a_5 and Enhanced Binding to Fibronectin

10:58–11:30 Coffee

SYMPOSIUM 3: Inflammation & Protective Immunity in the Tumor Microenvironment I

ROOM COLBERT

Chairperson: Jan Bubenik, Prague, Czech Republic

08:30 **Sharon Evans**, Buffalo, NY, USA
Overcoming Obstacles to Cancer Immunity at the T Cell – Tumor Microvascular Checkpoint

08:50 **Jan Bubenik**, Prague, Czech Republic
Depletion of Treg Cells Enhances Inhibition of Tumour Growth by Cyclophosphamide Derivatives and IL-12-producing Cellular Vaccines

09:10 **Melody Swartz**, Lausanne, Switzerland
Lymph Node Mimicry by Tumors Induces Immunological Tolerance

09:30 **Paola Larghi**, Rozzano, Italy
Role of P50 NF-kappaB in Dendritic Cell Functions

09:42 **Marie-Laure Arcangeli**, Marseille, France
JAM-B and JAM-C: Ying and Yang of Metastasis and Anti-Tumor Immune Response

09:54 **Nadira Delhem**, Lille, France
Epstein Barr Virus Infection in Hodgkin's Lymphoma: A Mechanism Facilitating Induced Regulatory T Cells Recruitment

10:06 **Rinat Rotem-Yehudar**, Yavne , Israel
CT-011, a Humanized Monoclonal Antibody, Interacts with the PD-1 Receptor and Modulates Survival and Trafficking Signals in Effector/memory T Lymphocytes

10:18 **Jean Edouard Gairin**, Toulouse, France
Tumor Cell Plasticity under Immune Micro-Environment Pressure

10:30 **Guerric Epron**, Rennes, France
Macrophages, IL-15, and Follicular Lymphoma: Towards a Better Understanding of the Interface Between Tumor B Cells and their Microenvironment

10:42 **Riad Abes**, Paris, France
Anti-Tumor Treatment of Tumor-Bearing Immunocompetent Mice with Anti-CD20 mAb Induces an Adaptive Immune Response that can be Strengthened by IL-2 Infusion

10:54–11:30 Coffee

SYMPOSIUM 4: Molecular Pathways in the Tumor Microenvironment I

AUDITORIUM RICHELIEU

Chairperson: Michael Micksche, Vienna, Austria

11:30 **Mircea Ivan**, Indianapolis, IN, USA
Hypoxia-Regulated MicroRNAs, New Players in Tumorigenesis

11:50 **Pierre Sonveaux**, Brussels, Belgium
Role of Lactate as a Fuel in a Unique Microenvironmentally Controlled Metabolic Symbiont

12:02 **Emily Chen**, Stony Brook, NY, USA
Hypoxia Tolerance and Breast Cancer Metastasis

12:14 **Shoukat Dedhar**, Vancouver, BC, Canada
Silencing Hypoxia Mediated Expression of Carbonic Anhydrase IX Induces Regression of Primary Breast Tumor Growth and Metastasis

12:26 **Ludwig Dubois**, Maastricht, The Netherlands
Specific Sulfonamide Inhibitors of CA IX are able to Image Hypoxia Response and Enhance the *in vivo* Therapeutic Effect of Conventional Cancer Treatments

12:38 **Marina Konopleva**, Houston, TX, USA
Targeting Hypoxic Microenvironment in Acute Lymphocytic Leukemia (ALL)

12:50 **Nathalie M. Mazure**, Nice, France
Mitochondrial VDAC3 Splice Variant is Induced in Hypoxia and Protects from Apoptosis

13:02 **Sarah Crawford**, New Haven, CT, USA
Biomechanical Model of Stress-Dependent Formation of Tissue Organizing Structures (TOS) Associated with Solid Tumor Formation, Invasion and Metastasis

13:14 **Paola Mazzarelli**, Rome, Italy
EGFR Signaling Mediates Metabolism-Dependent Epigenetic Control in a Model of Human Breast Cancer. CPT1A is a Novel Partner of Histone Deacetylase 1 in Cell Death Escaping Mechanisms

13:26 **Constantinos Koumenis**, Philadelphia, PA, USA
The GCN2-ATF4 Pathway is a Key Determinant of Tumor Cell Survival and Proliferation in Response to Amino Acid and Glucose Deprivation

Afternoon free

SYMPOSIUM 5: The Role of the Tumor Microenvironment in Tumor Progression I

ROOM LULLI

Chairperson: Avraham Raz, Detroit, MI, USA

11:30 **Dave Hoon**, Santa Monica, CA, USA
Epigenetic Regulation of SPARC in Tumor Microenvironment Stromal Cells is Associated with Vascular Status of Early Stage Colon Cancer

11:50 **Ugo Cavallaro**, Milan, Italy
The New Identity of L1: from a Neural Adhesion Molecule to a Central Modulator of Tumor/Microenvironment Crosstalk?

12:10 **David Barron**, Houston, TX, USA
Further Defining Reactive Stroma in Prostate Cancer

12:22 **Virginie Dangles-Marie**, Paris, France
Newly Characterised *ex vivo* Colospheres as a Three-Dimensional Colon Cancer Cell Model of Tumour Aggressiveness

12:34 **Steven Mittelman**, Los Angeles, CA, USA
Adipocytes Protect Acute Lymphoblastic Leukemia Cells from Chemotherapy

12:46 **Dimitris Kletsas**, Athens, Greece
Human Lung Fibroblasts Prematurely Senescent after Exposure to Ionizing Radiation Enhance the Growth of Malignant Epithelial Cells *in vitro* and *in vivo*.

12:58 **Ann-Charlotte Johansson**, Linköping, Sweden
Cancer-Associated Fibroblasts Protect Head and Neck Squamous Cell Carcinoma Cells from Cetuximab-Induced Cytotoxicity

13:10 **Magdalena Dutsch-Wicherek**, Krakow, Poland
RCAS1 Protein Involvement in Creation of Suppressive Tumor Microenvironment in Salivary Gland Adenocarcinoma

13:22 **Sumanta Goswami**, Bronx, NY, USA
Tumor Microenvironment Induced Drug and Radio Resistance in Invasive Breast Cancer Cells

Afternoon free

SYMPOSIUM 6: Phenotypic & Functional Alterations in the Tumor Microenvironment

ROOM COLBERT

Chairperson: Theresa L. Whiteside, Pittsburgh, PA, USA

11:30 **Viktor Umansky**, Heidelberg, Germany
Immunosuppressive Tumor Microenvironment in *ret* Transgenic Mouse Melanoma Model

11:50 **Theresa L. Whiteside**, Pittsburgh, PA, USA
Mechanisms of Tumor-escape from the Immune System: Adenosine-producing Treg, Exosomes and Tumor-associated TLRs

12:10 **Curzio Rüegg**, Lausanne, Switzerland
Radiation-Induced Modifications of the Tumor Microenvironment Promote Metastasis

12:30 **Dan Mercola**, Irvine, CA, USA
The Microenvironment Adjacent to Prostate Cancer Exhibits Numerous Differential Expression Changes that are Useful for Diagnosis without Tumor Cells

12:50 **Raj Tiwari**, Valhalla, NY, USA
Bone Marrow Endothelial Progenitor Cells are Systemic Sensors of Breast Cancer

13:02 **Nancy Boudreau**, San Francisco, CA, USA
Stabilization of the Breast Tumor Microenvironment Using Hox Genes

13:14 **Jing Yang**, Houston, TX, USA
Macrophages are an Important Component of Myeloma Microenvironment and Protect Myeloma Cells from Chemotherapy Drug-Induced Apoptosis

13:26 **Ralph R. Weichselbaum**, Chicago, IL, USA
Blockade of TNFα Signaling in Tumor-associated Macrophages: a New Radiosensitizing Strategy

Afternoon free

FRIDAY, OCTOBER 23, 2009

SYMPOSIUM 7: Regulation of Gene Expression in the Tumor Microenvironment II

AUDITORIUM RICHELIEU

Chairperson: Eva Klein, Stockholm, Sweden

08:30 **Eva Klein**, Stockholm, Sweden
The Role of Microenvironment on the Regulation of Epstein-Barr Virus Latent Gene Expression

08:50 **Ben-Zion Katz**, Tel-Aviv, Israel
Adhesive Interactions Regulate Transcriptional Diversity in Malignant B-cells

09:10 **Kerstin Junker**, Jena, Germany
Changes in Epigenetic Expression Patterns of Tumour Associated Fibroblasts (TAF)

09:22 **Emilie Buache**, Illkirch, France
Cancer Cell-adipocyte Cross-talk: Role of Matrix Metalloproteinase-11/stromelysin-3

09:34 **Corinne Bousquet,** Toulouse, France
Forced Hemidesmosome Assembly as a Novel Mechanism for Somatostatin Receptor sst2 Tumor Suppressive Activity in Pancreatic Cancer

09:46 **Vincent Frontera**, Marseille, France
Anti-JAM-C Tumor Growth Inhibition Occurs through Modulation of Thrombomodulin Expressing Stromal Cells

09:58 **Véronique Machelon**, Clamart, France
Identification of Glucocorticoid-Induced Leucine Zipper as a Key Regulator of Tumor Cell Proliferation in Epithelial Ovarian Cancer

10:10 **Sarah Pringels**, Ghent, Belgium
Correlated Expression Analysis of VEGF Family Members and Lipid Inflammatory Mediators in Human Colon Polyps and Carcinomas and Liver Metastases

10:22 **Gertraud Orend**, Strasbourg, France
Tenascin-C in the Tumor Microenvironment Triggers Oncogenic Signaling

10:34 **Rami Aqeilan**, Jerusalem, Israel
WWOX Expression Suppresses Tumorigenecity by Inducing Apoptosis and Attenuating Migration of Metastatic Cells

10:46 **Jozefa Wesierska-Gadek**, Vienna, Austria
Oncogenes do not Fully Override the Cellular Programme: Pronounced Impact of Cellular Microenvironment

10:58–11:30 Coffee

SYMPOSIUM 8: Interactions of Tumor Cells with Microenvironmental Cells & Molecules II

ROOM LULLI

Chairperson: Angelo Messina, Catania, Italy

08:30 **Karin Joehrer**, Innsbruck, Austria
The Role of Myeloma-Derived Chemokine CCL27 on Tumor Progression and Immune Escape

08:50 **Medhat Shehata**, Vienna, Austria
Reconstitution of PTEN Activity and Inhibition of the PI3-K/Akt Signaling Prevent the Pro-Survival Effect of Bone Marrow Microenvironment and Induce Apoptosis in CLL Cells

09:10 **Harry L. Ioachim**, New York, NY, USA
Interactions of Microenvironment with Carcinomas Depend on Tumor Type, Grade and Stage

09:22 **Abdelilah Aboussekhra**, Riyadh, Saudi Arabia
Role of the Tumor Suppressor p16 Protein in Tumor-Stromal Interactions in Breast Cancer

09:34 **Michael Elkin**, Jerusalem, Israel
Role of Heparanase in Colitis Associated Cancer

09:46 **Karen Hunter**, New York, NY, USA
The Role of Heparanase in Promoting Multistage Pancreatic Islet Tumorigenesis

09:58 **Margarida Bernardo,** Detroit, MI, USA
Maspin Restores Redifferentiation of Prostate Cancer Cells in Collagen I

10:10 **Lingtao Wu**, Los Angeles, CA, USA
Osteoblastic Maturation-dependent Microenvironment Mediated by Retinoid Signaling Inhibits Proliferation and Induces Terminal Differentiation of Leukemia Cells

10:22 **Laura Gibson**, Morgantown, WV, USA
VE-cadherin Regulates Philadelphia Chromosome Positive (Ph+) Acute Lymphoblastic Leukemia (ALL) Sensitivity to Apoptosis

10:34 **Ayaka M. Silverman**, Los Angeles, CA, USA
Galectin-3 Binding Protein Produced by Neuroblastoma Cells Stimulates the Expression of Interleukin-6 in the Tumor Microenvironment

10:46 **Hao-Wei Wang**, New York, NY, USA
Tumor-Derived IL-4 Upregulates Cathepsin Activity in Tumor-Associated Macrophages to Promote Cancer Development and Progression

10:58–11:30 Coffee

SYMPOSIUM 9: Inflammation & Protective Immunity in the Tumor Microenvironment II

ROOM COLBERT

Chairperson: Jan Żeromski, Poznan, Poland

08:30 **Michal Baniyash**, Jerusalem, Israel
Chronic Inflammation-Induced Immunosuppression: Micro and Macro Environmental Factors and Implications for Cancer Therapy

08:50 **Jan Żeromski**, Poznan, Poland
Functional Studies on Toll-Like Receptor Expression on Cell Lines of Laryngeal Carcinoma

09:10 **Karin de Visser**, Amsterdam, The Netherlands
Functional Assessment of the Inflammatory Tumor Microenvironment during Spontaneous Breast Cancer Progression and Metastasis Formation

09:22 **Moshe Elkabets**, Beer-Sheva, Israel
A Novel Tumor-Derived Inflammatory Myeloid Suppressor Cell Subset Inhibits Anti-Tumor Activity of T and NK Cells

09:34 **Isabelle Cremer**, Paris, France
Triggering of TLR7 and 8 on Human Lung Cancer Induces Cell Survival and Chemoresistance

09:46 **Guillaume Sarrabayrouse**, Nantes, France
Tumor-Specific CD4CD8ab T Cells Infiltrating Human Colorectal Tumors

09:58 **Vladislava O. Melnikova**, Houston, TX, USA
The Signaling Pathway PAR1-PAFR-MUC18 Links Inflammation with Melanoma Metastasis

10:10 **Samuel Lundin**, Gothenburg, Sweden
Extensive Upregulation of Proinflammatory Cytokines in the Gastric Mucosa of Stomach Cancer Patients

10:22 **Pi-Ling Chang**, Birmingham, AL, USA
Host Osteopontin Maintains an Acute Inflammatory Response in the Tumor Microenvironment to Suppress Extrinsic Cancer Cell Progression

10:34 **Esther N. Arwert**, Cambridge, UK
Tumour Formation Initiated by Nondividing Epidermal Cells via an Inflammatory Infiltrate

10:46 **Aline M. Betancourt**, New Orleans, LA, USA
The Human Pro-inflammatory Antimicrobial Peptide LL-37 Supports Ovarian Tumor Progression by the Recruitment of Multipotent Mesenchymal Stromal Cells and other Immunosuppressive Cells

10:58–11:30 Coffee

SYMPOSIUM 10: The Role of the Tumor Microenvironment in Tumor Progression II

AUDITORIUM RICHELIEU

Chairperson: **Ruth J. Muschel**, Oxford, UK

11:30 **Dario Marchetti**, Houston, TX, USA
Heparanase: A Critical Determinant of Breast Cancer Metastasis to Brain

11:50 **Adriana Haimovitz-Friedman**, New York, NY, USA
A Ceramide Rheostat Balances Angiogenesis and Anti-angiogenesis

12:10 **Yoav Leiser**, Haifa, Israel
Heparanase Role in Oral Cancer Prognosis and Cellular Differentiation

12:22 **Mariagrazia Uguccioni**, Bellinzona, Switzerland
Perivascular Expression of CXCL9 and CXCL12 in Primary Central Nervous System Lymphoma: Chemokine Synergism Controls Cell Infiltration and Positioning

12:34 **Sivan Izraely**, Tel-Aviv, Israel
A Molecular Signature of Melanoma Brain Metastasis: Development and Characterization of a Novel Human Melanoma Mouse Model

12:46 **Ashleigh Hill**, Belfast, Northern Ireland, UK
Characterization of Interleukin-8 Promoted Protease Expression and Activity in Relation to Prostate Cancer Metastasis to the Bone

12:58 **Lukas Hawinkels**, Leiden, The Netherlands
MMP-14 (MT1-MMP) Mediated Endoglin Shedding Regulates Tumour Angiogenesis

13:10 **Shelly Maman**, Tel-Aviv, Israel
Neuroblastoma Macro- and Micro-Metastasis: Interactions with the Microenvironment

13:22 **Yong Li**, Sydney, NSW, Australia
Co-expression of Invasive Markers (uPA, CD44) and Multiple Drug Resistance Proteins (MDR1, MRP2) is Correlated with Epithelial Ovarian Cancer Progression

13:34–14:30 Lunch

SYMPOSIUM 11: Therapeutic Targeting of the Tumor Microenvironment – Pre-clinical and Clinical Studies I

ROOM LULLI

Chairperson: Daniel Zagury, Paris, France

11:30 **Daniel Zagury**, Paris, France
Kinoid Vaccine, a New Immunotherapeutic Generation to Target Tumor Released Ectopic Cytokines

11:50 **Albrecht Reichle**, Regensburg, Germany
Comparative Uncovering of Tumors' Systems Biology by Modular Targeting of Tumor-Associated Inflammation

12:10 **Raymond Frade**, Evry, France
Tumor Micoenvironment Is Controled by Procathepsin L Secretion: A New GeneTherapy to Inhibit Progression of Tumors Induced by Human Melanoma Cells

12:22 **Michael Andreeff**, Houston, TX, USA
Disruption of Leukemia/Stroma Cell Interactions by CXCR4 Antagonists Enhances Chemotherapy and Signal Transduction-Induced Apoptosis in Leukemias

12:34 **Shijie Cai**, Oxford, UK
Role of Tetrahydrobiopterin in Regulation of Tumor Angiogenesis Mediated by PI3K/Akt, eNOS and Ras Pathway

12:46 **Katherine Cook**, Winston-Salem, NC, USA
Angiotensin-(1-7) Inhibits Breast Tumor Growth in an Orthotopic Murine Model by Reducing Angiogenesis and Fibrosis

12:58 **Patricia E. Gallagher**, Winston-Salem, NC, USA
Angiotensin-(1-7) Inhibits VEGF and PlGF to Reduce Tumor Angiogenesis in Triple Negative Breast Cancer in an Orthotopic Mouse Model

13:10 **Charlotta Dabrosin**, Linköping, Sweden
Tamoxifen and the Lignan Enterolactone Increase *in vivo* Levels of IL-1Ra and Decrease Tumor Angiogenesis in Estrogen Dependent Breast Cancer Explants

13:22–14:30 Lunch

SYMPOSIUM 12: Proteomics, Imaging & Biomarkers of the Tumor Microenvironment

ROOM COLBERT

Chairperson: Yona Keisari, Tel Aviv, Israel

11:30 **Francesca Botta**, Lausanne, Switzerland
Non Invasive Molecular Monitoring of Tumor Angiogenesis

11:42 **John D. Lewis**, London, ON, Canada
Intravital Imaging of Human Prostate Cancer Using Bombesin-Targeted Viral Nanoparticles

11:54 **Christopher Gerner**, Vienna, Austria
Novel Multiple Myeloma Biomarker Candidates Identified in the Secretome of Bone Marrow Fibroblasts and Endothelial Cells

12:06 **Thomas Mohr**, Vienna, Austria
How do Endothelial Cells Shape the Tissue Microenvironment? A Proteomic Approach.

12:18 **Astrid Enkelmann**, Jena, Germany
Changes in Proteomic Expression Patterns of Tumour Associated Fibroblasts (TAF) by Interaction with Urinary Bladder Carcinoma Cells

12:30 **Arie Admon**, Haifa, Israel
The Serum Soluble HLA Class I Peptidome as a Source for Cancer Biomarkers and a Possible Modulator of the Tumor Microenvironment

SYMPOSIUM 13: Molecular Pathways in the Tumor Microenvironment II

ROOM COLBERT

Chairperson: Ben-Zion Katz, Tel-Aviv, Israel

12:42 **Nitza Lahat**, Haifa, Israel
Hypoxia and PMA-Induced Maturation Inhibit TIMP-2 Secretion from Human Monocytes and Enhance Angiogenesis

12:54 **Kasper Rouschop**, Maastricht, The Netherlands
The Unfolded Protein Response Protects Cells during Hypoxia through Preservation of Autophagic Capacity

13:06 **Nic Savaskan**, Berlin, Germany
Molecular and Cellular Characterization of the Brain Tumor Microenvironment with Focus on Peritumoral Brain Swelling

13:18 **David Waugh**, Belfast, Northern Ireland, UK
Importance of Differential Stress-Induced CXC-chemokine Expression and Signalingin Regulating Cancer and Stromal Cell Function in PTEN-deficient Prostate Tumours

13:30–14:30 Lunch

PLENARY SESSION 5: Inflammation & Protective Immunity in the Tumor Microenvironment II

AUDITORIUM RICHELIEU

Chairperson: Menashe Bar-Eli, Houston, TX, USA

14:30 **Alberto Mantovani**, Milan, Italy
Cancer-Related Inflammation: The Seventh Hallmark of Cancer

14:55 **Laurence Zitvogel**, Villejuif Cedex, France
How Anticancer Therapies Switch on the Immune System?

15:20 **Lisa M. Coussens**, San Francisco, CA, USA
Inflammation and Cancer: Insights into Organ-specific Immune Regulation of Cancer Development

15:45 **Jerome Galon**, Paris, France
Intratumoral Immune Reaction: A Novel Paradigm for Cancer

16:10 **Claire Lewis**, Sheffield, UK
Regulation of Macrophage Function by the Tumor Microenvironment: Role of Hypoxia and Angiopoietin-2

16:35–17:00 Coffee

PLENARY SESSION 6: The Role of the Microenvironment in Tumor Progression

AUDITORIUM RICHELIEU

Chairperson: Suresh Mohla, Bethesda, MD, USA

17:00 **Kornelia Polyak**, Boston, MA, USA
Regulation of In Situ to Invasive Breast Carcinoma Transition

17:25 **Adriana Albini**, Milano, Italy ***EACR sponsored speaker***
Role of the Tumour Microenvironment in Angiogenesis and in Prediction of Breast Cancer Metastasis

17:50 **Yosef Yarden,** Rehovot, Israel
Molecular Basis of Growth Factor-Induced Mammary Cell Migration: Implications to HER2-positive Breast Cancer

18:15 **David Lyden**, New York, NY, USA
The Metastatic Niche: Adapting the Foreign Soil

18:40 **Israel Vlodavsky**, Haifa, Israel
Heparanase: One Molecule with Multiple Functions in Cancer Progression

SATURDAY, OCTOBER 24, 2009

SYMPOSIUM 14: Interactions of Tumor Cells with Microenvironmental Cells III

AUDITORIUM RICHELIEU

Chairperson: Fernando Vidal-Vanaclocha, Leioa, Bizkaia, Spain

08:30 **Maty Tzukerman**, Haifa, Israel
Microenvironment-Dependent Support of Self Renewing Ovarian Cancer Stem Cells

08:50 **Fernando Vidal-Vanaclocha**, Leioa, Bizkaia, Spain
Hepatomimetic Properties of Colon Cancer Cells: Microenvironmental Regulation and Clinical Implications

09:10 **Marcelo Ehrlich**, Tel Aviv, Israel
Disabled-2 a Potential Integrator of TGF-β Signaling and Trafficking in Epithelial to Mesenchymal Transition and Dedifferentiated Tumor Cell Lines

09:30 **Andrei Bakin**, Buffalo, NY, USA
Integrins in EMT and Tumor Microenvironment

09:50 **Ruth J. Muschel**, Oxford, UK
Vascular Co-option in Brain Metastasis

10:10–10:30 Coffee

SYMPOSIUM 14 (cont'd)

10:30 **Judith Leibovici**, Tel- Aviv, Israel
The Aging Host Microenvironment May Reduce Tumor Progression by Reducing Genomic Instability

10:42 **Roy-Akira Saito**, Stockholm, Sweden
FoxF1 Regulates Tumor-promoting Properties of Cancer-associated Fibroblasts in Lung Cancer

10:54 **Li Yang**, Bethesda, MD, USA
Effect and Regulation of Gr-1+CD11b+ Immature Myeloid Cells in Tumor Microenvironment and Beyond

11:06 **Jillian L. Werbeck**, Columbus, OH, USA
Ets2 in Lung Fibroblasts Promotes the Growth of Metastatic Breast Cancer Cells

11:18 **Qing Yi**, Houston, TX, USA
C-reactive Protein Protects Myeloma Cells from Apoptosis via Activating ITAM-containing FcgRII

11:30 **Rosandra Kaplan**, New York, NY, USA
Bone Marrow-Derived Hematopoietic Progenitor Cells as Mediators of Metastasis

11:42 **Ezio Laconi**, Cagliari, Italy
The Microenvironment of Hepatic Nodules is Necessary for Tumor Progression

SYMPOSIUM 15: The Role of Microenvironmental Molecules in Tumor Progression

ROOM LULLI

Symposium sponsored by Roche, France

Chairperson: Dave Hoon, Santa Monica, CA, USA

08:30 **Elena Voronov**, Beer Sheva, Israel
The Differential Role of Microenvironmental IL-1α and IL-1β in Tumor Angiogenesis

08:50 **Sabina Pucci**, Rome, Italy
VEGF-A165A and IL-6 in Human Colon Cancer: A Microenvironment Cooperation Leading to Cell Death Escape through microRNAS Dysregulation

09:02 **Ji Ming Wang**, Frederick, MD, USA
Receptor "Hijacking" by Malignant Glioma Cells: A Tactic for Tumor Progression

09:14 **Masataka Majima**, Sagamihara, Kanagawa, Japan
Recruitment of Mast Cells to the Tumor Microenvironment via a High Affinity Leukotriene B_4 Recepter Signaling Enhances Tumor-Associated Angiogenesis and Tumor Growth

09:26 **Antonia Patsialou**, Bronx, NY, USA
Invasion of Human Breast Cancer Cells In Vivo Requires both Paracrine and Autocrine Loops Involving the Colony Stimulating Factor-1 Receptor

09:38 **Abdel-Majid Khatib**, Paris, France
Regulation of Tumorigenesis, Angiogenesis and Metastasis by the Proprotein Convertases (PCs)

09:50 **Camille Laurent**, Toulouse, France
Characterization of the Immunological Microenvironment in Follicular Lymphoma

10:02–10:30 Coffee

SYMPOSIUM 15 (Cont'd)

10:30 **Steven Mason**, New York, NY, USA
The Proteolytic Cascade in Metastasis

10:42 **Laura Fung**, London, ON, Canada
EGFL7 Protein Expression Effects Tumor Progression by Influencing the Rate of Angiogenesis

10:54 **Laurie McCauley**, Ann Arbor, MI, USA
A Novel Role for Megakaryocytes in the Bone Marrow Microenvironment of Prostate Cancer Metastasis

11:06 **Amitava Chatterjee**, Kolkata, India
Culture of Human Laryngeal Carcinoma Cell Line Hep-2 in Presence of Fibronectin Increases MMP-9 Expression with the Involvement of Multiple Signaling Pathways

11:18 **Miranda Ween**, Adelaide, South Australia, Australia
Transforming Growth Factor Induced Protein TGFβI Promotes Ovarian Cancer Cell Motility and Adhesion to Peritoneal Cells

11:30 **Grégoire Mignot**, Dijon, France
Membrane Hsp72 from Tumor-Derived Exosomes Mediates p-Stat3 Dependent Function of Myeloid Suppressor Cells through the TLR2-MyD88 Pathway

11:42 **Cornelia Trimble**, Baltimore, MD, USA
Immune Cell Homing in Preinvasive HPV Disease

SYMPOSIUM 16: Therapeutic Targeting of Tumor-Microenvironment Interactions – Pre-clinical and Clinical Studies II

ROOM COLBERT

Chairperson: **W. Gillies McKenna**, Oxford, UK

08:30 **W. Gillies McKenna**, Oxford, UK
Tumor Conditioning: Modulation of the Tumour Microenvironment by Signalling Inhibition as a Strategy for Improving Cancer Therapy

08:50 **Yoshiaki Kubota**, Tokyo, Japan
M-CSF Inhibition Selectively Targets Pathological Angiogenesis and Lymphangiogenesis

09:12 **Sheng-Bin Peng**, Indianapolis, IN, USA
Pre-Clinical Evaluation of a Potent and Selective CXCR4 Peptide Antagonist Currently in Phase 1 Trials for Cancer

09:24 **Tanaya Shree**, New York, NY, USA
Inhibition of Cathepsin Proteases Synergizes with Maximum-Dose and Low-Dose Chemotherapy to Block Malignant Progression in a Mouse Model of Metastatic Breast Cancer

09:36 **Carlton Donald**, Atlanta, GA, USA
The Effect of the PAX2 Oncogene on the Tumor Microinvironment, Tumor Progression and its Potential as a Therapeutic Target for Prostate Cancer

09:48 **Jian Wang**, Bergen, Norway
Targeting the Tumor Stroma - a Novel Therapeutic Strategy Based on Separate Analysis of the Malignant and Stromal Cell Compartments in Brain Tumors

10:00–10:30 Coffee

SYMPOSIUM 16 (Cont'd)

10:30 **Jenny Worthington**, Coleraine, UK
Does Hypoxia Play a Role in the Failure of Androgen Ablation Therapy for Prostate Cancer?

10:42 **Mark Pines**, Bet Dagan, Israel
Inhibition of Fibroblast-to-myofibroblast Transition as a Modality for Cancer Treatment: Effect of Halofuginone

10:54 **Michael P. Lisanti**, Philadelphia, PA, USA
Stromal Caveolin-1 Predicts Recurrence and Clinical Outcome in DCIS and Human Breast Cancers

11:06 **F. Javier Oliver**, Armilla, Granada, Spain
Antimetastasic Action of Parp Inhibition in Melanoma trough Counteracting Angiogenesis and emt Transition

11:18 **Silke Haubeiss**, Stuttgart, Germany
Targeting Cancer-Associated Fibroblasts (CAFs) with Small Molecule Inhibitors to Enhance Sensitivity of Tumors to Conventional Chemotherapy

11:30 **Lucy Allen**, Amersham, Buckinghamshire, UK
Monitoring Tumour Response to the Anti-angiogenic Therapy Sunitinib with an F18-labeled Angiogenesis Imaging Agent

CLOSING PLENARY SESSION

AUDITORIUM RICHELIEU

Chairperson: Isaac P. Witz, Tel Aviv, Israel

12:00 Poster Session – presentation of best posters and awarding of prizes

13:00 Jan-Willem van de Loo, Brussels, Belgium
European Commission
Funding for Translational Research on the Tumour Microenvironment through EU Programmes

13:15 Concluding remarks

13:30 Adjourn

ABSTRACTS

ORAL PRESENTATIONS

O1

Macrophages and Metastasis

Jeffrey W. Pollard[1]
[1]*Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, NY, NY, USA*

Non-malignant cells within the tumor microenvironment play important roles in modulating tumor progression to malignancy. Many of these cells are derived from the hematopoietic system. Particularly abundant are macrophages whose density in many different human tumor types is usually positively correlated with poor prognosis suggesting that macrophages are tumor promoting. Studies in mouse models reinforce this idea since genetic or chemical ablation of macrophages results in a reduction in tumor progression and metastasis (1) (2). Functional studies have identified several tumor-promoting functions for macrophages in primary tumors. These include promotion of angiogenesis, tumor cell invasion, migration and intravasation. In some cases the signaling molecules that are produced by macrophages have been identified at least in the context of these mouse models of breast cancer (3, 4). In addition to these effects of macrophages at the primary tumor site we have recently identified a sub-population of macrophages that are required for metastatic seeding and persistent growth at distant sites. These data together with that of others, suggest that targeting macrophages and their unique signaling pathways could offer new therapeutic strategies against metastatic disease.

1. Pollard, J. W. (2004) Nature Reviews Cancer 4, 71 – 78.
2. Joyce, J. A. & Pollard, J. W. (2009) Nat Rev Cancer 9, 239–252.
3. Condeelis, J. & Pollard, J. W. (2006) Cell 124, 263–266.
4. Lin, E. Y., Li, J. F., Gnatovskiy, L., Deng, Y., Zhu, L., Grzesik, D. A., Qian, B., Xue, X. N., & Pollard, J. W. (2006) Cancer research 66, 11238–11246.

O2

Involvement of the p53 Tumor Suppressor in Tumor-Stroma Interactions

Neta Moskovits[1], Jair Bar[3], Yoseph Addadi[2], Michal Neeman[2], Varda Rotter[1], **Moshe Oren**[1]
[1]*Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel,* [2]*Biological Regulation, Weizmann Institute of Science, Rehovot, Israel,* [3]*Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Israel*

The tumor suppressor functions of p53 have been extensively studied within tumor cells and cells that are at risk of becoming tumorous. However, recent studies indicate that p53 also possesses non cell-autonomous tumor suppressor activities. Thus, we report that p53 can exert its tumor suppressor activity also within the stromal compartment of the tumor. Consequently, co-injection of p53-null fibroblasts together with PC3 human prostate cancer cells selectively augments tumor growth, while wild type fibroblasts fail to exert a similar effect. p53-deficient fibroblasts produce elevated levels of secreted proteins such as SDF-1/CXCL12, which may facilitate tumor growth and spread. Conversely, tumor-associated mutant p53 isoforms increase the expression of SDF-1 in fibroblasts. In addition to quenching SDF-1 production by stromal fibroblasts, p53 also represses the expression of the SDF-1 receptor CXCR4. Of note, siRNA-mediated downregulation of SDF-1 production attenuates the ability of p53-null fibroblasts to augment tumor growth. Quenching p53 function in adjacent stromal fibroblasts may therefore provide tumor cells with a selective growth advantage. Indeed, we found that epithelial tumor cells can repress p53 activation in fibroblasts. This ability is acquired when epithelial cells undergo neoplastic transformation. Interestingly, this p53-repressive effect of tumor cells is exerted more readily in cancer-associated fibroblasts (CAFs). All these findings implicate p53 in a non cell-autonomous tumor suppressor mechanism, exerted from stromal cells and affecting adjacent tumor cells. Activation of stromal p53 might therefore attenuate tumor progression even if the cancer cells themselves do not harbor wt p53 anymore

O3

Cleavage of Galectin-3 by Matrix Metalloproteinases Regulates Breast Cancer Progression and Metastasis

Avraham Raz[1]
[1]*Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, USA*

For reasons largely unknown, Caucasian women are at a significantly higher risk of developing breast cancer than Asian women. Over a decade ago, mutations in BRCA1/2 were identified as genetic risk factors; however, the discovery of additional breast cancer genes and genes contributing to racial disparities are lacking. We report a functional germline mutation (polymorphism) in the galectin-3 gene at position 191 (rs4644) substituting proline with histidine (P64H), which results in susceptibility to matrix metalloproteinase (MMP) cleavage and acquisition of resistance to drug-induced apoptosis. This substitution correlates with incidence of breast cancer and racial disparity. Of note, Cleavage of galectin-3 by MMPs is related to progression of breast and prostate cancer. We show that galectin-3 regulated functions like chemotaxis, chemoinvasion, heterotypic aggregation, epithelial-endothelial cell interactions and angiogenesis are dependent in part on cleavage of the N terminus of galectin-3 followed by its release in the tumor microenvironment. Breast carcinoma cells harboring cleavable galectin-3 species showed increased chemotaxis towards collagen IV, invasion through Matrigel and heterotypic interactions with endothelial cells resulting in angiogenesis and 3-D morphogenesis *in vitro* compared to cells harboring non-cleavable galectin-3. Wound healing studies employing a novel cell culture insert showed increased migration and phosphorylation of focal adhesion kinase in endothelial cells migrating towards H64 cells compared to P64 cells. Using 3-

dimensional co-cultures of endothelial cells with breast cells harboring galectin-3 peptides, we show that amino acids 1-62 and 33–250 stimulate migration and interaction of cells with the endothelial cells. Immunohistochemical analysis of blood vessel density and galectin-3 cleavage in a breast cancer progression tissue array support the *in vitro* findings. These results indicate that cleavage of galectin-3 in tumor microenvironment leads to breast cancer angiogenesis and progression.
In conclusion, we provide novel data implicating a galectin-3 germline nonsynonymous functional polymorphism in breast cancer progression and metastasis.

O4

Extracellular Matrix Remodeling Forces Tumor Progression

Valerie Marie Weaver[1]
[1]*Department of Surgery, UCSF, San Francisco, CA, USA*

Tumor progression is accompanied by a desmoplastic response that is characterized by significant extracellular matrix (ECM) remodeling. We have been studying the role of matrix metalloproteinase and lysyl oxidase-mediated collagen cross-linking in ECM remodeling and tissue desmoplasia during breast tumor progression. Thus far we have established a positive association between lysyl oxidase-dependent collagen cross-linking, the accumulation of linear, oriented collagen fibrils, tissue fibrosis and tissue stiffening during breast transformation. We have demonstrated that either pharmacological or antibody-mediated inhibition of lysyl oxidase-induced collagen cross-linking prevents tissue fibrosis, reduces tissue stiffening, enhances tumor latency and decreases tumor incidence in both the MMTV-Neu and PyMT transgenic mouse models of breast cancer. We also observed that inducing lysyl oxidase-dependent collagen cross-linking promotes tissue desmoplasia, stiffens the tissue and promotes the malignant transformation and invasion of an oncogenically-primed premalignant mammary epithelium. Experiments using three dimensional organotypic models showed that collagen cross-linking per se promotes the invasive behavior of an oncogenically-modified mammary epithelial tissue but is insufficient to induce invasion in normal tissues. Because we previously observed that ECM stiffness can enhance growth factor-dependent mammary epithelial cell (MEC) proliferation and survival and will disrupt mammary tissue morphogenesis by promoting integrin clustering, focal adhesion maturation, integrin-dependent signaling through ERK, and cell-generated force (Paszek et al., Cancer Cell 2005) we explored functional associations between ECM cross-linking and stiffness and integrin signaling. We could show that lysyl oxidase-dependent breast transformation in vivo and ECM cross-linking in culture are functionally-linked to increased actomyosin contractility and focal adhesion assembly and signaling, elevated PI3Kinase activity and reduced PTEN expression and activity. These findings underscore the importance of ECM remodeling in tumor progression and identify mechanical force as a novel molecular mediator and tumorigenesis. (Supported by grants from the Department of Energy DE-FG02-07ER64420, DOD BCRP W81XWH-05-1-330, and NIH CA078731A2)

O5

Intercellular Transfer of Ras and microRNAs: New Mechanisms of Non-Autonomous Protein Functions and Post-Transcriptional Control

Oded Rechavi[1], Yaniv Erlich[2], Hila Avram[3], Fedor V. Kaginov[2], Itamar Goldstein[3], Gregory J. Hannon[2], **Yoel Kloog**[1]
[1]*Department of Neurobiology, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel Aviv, Israel,* [2]*Watson School of Biological Sciences, Howard Hughes Medical Institute, Howard Hughes Medical Institute Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA,* [3]*Immunology Program, Cancer Research Center, Chaim Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel*

Lipidated Ras proteins are highly mobile and redistribute rapidly between the plasma membrane and endomembranes. We postulated that this high mobility might allow also functional "proteome mixing" among interacting cells, particularly between immune cells. If so, then this would support the notion that no cell is an island, and that even these "unsplittable" units are actually non-autonomous. We will present results on cell-contact-dependent intercellular transfer of proteins including oncogenic H-Ras and of microRNAs. Acquisition of oncogenic H-RasG12V by natural killer (NK) and T lymphocytes had important biological functions in the adopting lymphocytes including ERK phosphorylation, increased interferon-γ and tumor necrosis factor-α secretion, enhanced lymphocyte proliferation, and augmented NK-mediated target cell killing. We also will show that upon cell contact, T cells acquire from B cells small RNAs, which impact expression of target genes in the recipient T cells. Both synthetic microRNA (miRNA) mimetics and viral miRNAs expressed by infected B cells can be transferred into T cells. Such mechanisms may allow cell non-autonomous post-transcriptional control, a process, which could be exploited by tumors or virus-infected cells.

O6

Reprogramming Metastatic Tumor Cells with an Embryonic Microenvironment: Convergence of Embryonic and Tumorigenic Signaling Pathways

Mary Hendrix[1], Lynne-Marie Postovit[1], Naira Margaryan[1], Elisabeth Seftor[1], Dawn Kirschmann[1], Alina Gilgur[1], Luigi Strizzi[1], Richard Seftor[1]
[1]*Children's Memorial Research Center, Northwestern University, Chicago, IL, USA*

Embryonic stem cells sustain a microenvironment that facilitates a balance of self-renewal and differentiation. Aggressive cancer cells, expressing a multipotent, embryonic cell-like phenotype, engage in a dynamic reciprocity with a microenvironment that promotes plastic-

ity and tumorigenicity. However, the cancer associated milieu lacks the appropriate regulatory mechanisms to maintain a normal cellular phenotype. Previous work from our laboratory reported that aggressive melanoma and breast carcinoma express the embryonic morphogen Nodal, which is essential for human embryonic stem cells (hESC) pluripotency. Based on the aberrant expression of this embryonic plasticity gene by tumor cells, this current study tested whether these cells could respond to regulatory cues controlling the Nodal signaling pathway, which might be sequestered within the microenvironment of hESCs, resulting in the suppression of the tumorigenic phenotype. Specifically, we discovered that metastatic tumor cells do not express the inhibitor to Nodal, Lefty, allowing them to overexpress this embryonic morphogen in an unregulated manner. However, exposure of the tumor cells to a hESC microenvironment (containing Lefty) leads to a dramatic down-regulation in their Nodal expression concomitant with a reduction in clonogenicity and tumorigenesis accompanied by an increase in apoptosis. Furthermore, this ability to suppress the tumorigenic phenotype is directly associated with the secretion of Lefty, exclusive to hESCs, because it is not detected in other stem cell types, normal cell types, or trophoblasts. The tumor-suppressive effects of the hESC microenvironment, by neutralizing the expression of Nodal in aggressive tumor cells, provide previously unexplored therapeutic modalities for cancer treatment.

O7

Hypoxia and Tumor progression: New Metabolic Anti-Cancer Targets

Jacques Pouysségur[1], Johanna Chiche[1], Renaud LeFloch[1], Karine Ilc[1], Christiane Brahimi-Horn[1], Nathalie M. Mazure[1]
[1]*CNRS UMR6543, Centre A. Lacassagne, University of Nice, Institute of Developmental Biology and Cancer Research, Nice, France*

Nutrient sensing is a fundamental process for life. In its absence, fast growing cells of the developing embryo and of expanding tumors would rapidly outstrip essential nutrients and die. In fact cells sense and respond to variations in the concentration of key nutrients. However, early on in evolution, oxygen sensing has emerged, as a central control mechanism of energy metabolism and vasculogenesis. At the heart of this regulatory system is the Hypoxia-Inducible Factor, HIF, which controls, among other gene products, the expression of VEGF-A and Angiopoïetin-2, two key angiogenic factors in vertebrates. This finding has placed the hypoxia-signaling pathway at the forefront of nutritional control. HIF controls glycolysis, intracellular pH (pHi), angiogenesis, cell migration and invasion, and so has become recognized as a strong promoter of tumor growth.
We will highlight some of the HIF-induced gene products that participate in tumor adaptation, resistance and progression in a nutrient-depleted and acidic microenvironment.
First we will demonstrate that the two HIF-induced 'BH3-only'-proteins (BNIP3, BNIP3L/NIX), in contrast to current belief, do not trigger cell death but, by inducing macro-autophagy, stimulate tumor cell survival.
Second, we will show how tumor cells by expressing two HIF-dependent membrane-bound carbonic anhydrases, CAIX and CAXII, acidify the extracellular milieu, and ensure a more alkaline intracellular pH favoring migration, survival and growth in a hostile acidic microenvironment.
Third, HIF-induced glycolysis in most hypoxic tumor cells is essential to ensure maintenance of ATP levels for growth and cell survival. Two MonoCarboxylate Transporters MCT-1 and MCT-4, stabilized in the plasma membrane by the common chaperon basigin/CD147, play a key role in cancer metabolism.
We propose that appropriate exploitation of these HIF-regulated proteins and new validated cancer targets, which control exacerbated tumor metabolism and intracellular pH, will be at the forefront of anti-cancer therapy.

O8

Identifying New Anti-Cancer Therapeutics Using Synthetic Lethality

Amato Giaccia[1], Sandra Turcotte[1], Patrick Sutphin[1], William Denny[2], Michael Hay[2], Denise Chan[1]
[1]*Radiation Oncology, Stanford University, Stanford, CA, USA,* [2]*Experimental Therapeutics, University of Auckland, Auckland, New Zealand*

Synthetic lethality results when two nonallelic mutations that by themselves are not lethal, answer in cell death when combined. To screen for small molecules that acted in a synthetic lethal manner to the loss of VHL, we needed a means of tracking cell growth in microwell plates when exposed to a library of small molecules. Renal carcinoma cell lines with naturally occurring VHL mutations and their genetically matched wild-type VHL counterparts were stably labeled with enhanced yellow fluorescent protein (EYFP). Cells were then seeded onto 384-well plates and allowed to attach overnight. Baseline fluorescence readings were obtained and a compound library was added at a concentration of 5 μM. Fluorescence intensity was read once a day for four days. An increase in fluorescence intensity was used as a surrogate marker for cell growth. The most promising compounds that inhibited growth of VHL-deficient cells but still had an increase in fluorescence of cells with wild-type VHL were validated by a short-term XTT assay, which measures cellular metabolic activity as a surrogate for cell viability. Those compounds that were confirmed by XTT were then subjected to clonogenic survival assays to further verify specificity for killing VHL-deficient cells. From this screen, we identified several small molecules, which demonstrated selective toxicity against cells that had lost VHL compared to isogenic matched cell lines with wild-type VHL both in vitro and in vivo. One of these small molecules kills VHL deficient cells by inducing autophagy and another kills by inhibiting glucose uptake and retention. Both of these small molecules illustrate the power of using synthetic lethality in mammalian cells to develop new therapeutic strategies.

O9

Targeting Cancer-Related Inflammation

Fran Balkwill[1]
[1]*Centre for Cancer and Inflammation, Barts and The London School of Medicine and Dentistry, London, UK*

The cells and mediators of inflammation form a major part of the epithelial tumour microenvironment. In some cancers, inflammatory conditions precede development of malignancy; in others, oncogenic change drives a tumour-promoting inflammatory milieu. Whatever its origin, this 'smouldering' inflammation aids proliferation and survival of malignant cells, stimulates angiogenesis and metastasis; subverts adaptive immunity, and alters response to hormones and chemotherapy. Cytokines are major mediators of communication between cells in the inflammatory tumour microenvironment and may be important therapeutic targets in cancer patients.

The inflammatory cytokine TNF-a and its receptors are involved in tumour promotion and progression in some experimental cancers and both are found in human cancer biopsies. Mice deficient in TNF-a or TNFR1 are resistant to skin carcinogenesis; TNF-a drives an autocrine cytokine network in ovarian cancer, stimulating production of other cytokines by malignant cells, and TNF-a is important in maintaining the tumour-associated macrophage, TAM, phenotype in ovarian cancer. We hypothesised that neutralising its activity would be of therapeutic benefit and tested this in Phase I/II clinical cancer trials of TNF-a antagonists. We obtained a signal of clinical activity, with stable disease and some partial responses achieved in patients with advanced renal and ovarian cancer.

Interleukin 6 is another inflammatory cytokine that is implicated in cancer progression and host tumour communication. A Phase II trial of a therapeutic antibody against IL-6 in ovarian cancer patients is now complete. Again we see a signal of activity in the clinical trial and have identified potential biomarkers of response. Finally, we have evidence that TNF-a and IL-6 signalling pathways are intricately linked with other pathways involved in host tumour communication, including CXCR4, CXCL12, Notch receptors and ligands.

O10

Interference with VLA4 and Microenvironmental Interactions by the Tellurium Compound AS101 Results in the Sensitization of AML Cells to Chemotherapy

Benjamin Sredni[1], Alain Berrebi[2], Itai Skornik[1], Yona Kalechman[1]
[1]*The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel,* [2]*Hematology Institute, Kaplan Medical Center, Rehovot, Israel*

Drug resistance is induced by the attachment of the integrin receptor VLA-4 on leukemic cells to its ligand fibronectin. We show that the tellurium compound ammonium trichloro(dioxo-ethylene-O,O'-)tellurate AS101, sensitizes AML cells to ARA-C or DNR. Sensitization of AML cells to chemotherapy by AS101 was similar to that obtained by neutralizing anti VLA antibodies. Sensitization to chemotherapy by AS101 could be obtained in leukemic cells expressing VLA-4, from AML patients, while not sensitizing those not expressing this integrin. Treatment of AML cells plated on FN with AS101 and chemotherapy, significantly decreased pAkt and Bcl-2 when AML cells were co-treated by AS101, the decrease correlated with the sensitizing effect of AS101. Suggesting that treatment with AS101 may interfere with the sequence of events in AML in which high VLA-4 in leukemic cells reduces their chemosensitivity through interaction with FN, resulting in a poor induction of remission, ultimately leading to recurrence and short survival.

O11

Sensitizing Hemopoietic Malignant Cells to Glucocorticoid Induced Apoptosis by Protein Kinase Inhibitors

Ronit Vogt-Sionov[1], Shlomit Kfir[1], Hali Spokoini[1], Orly Cohen[1], **Eitan Yefenof**[1]
[1]*Lautenberg Center of General & Tumor Immunology, Hebrew University, Jerusalem, Israel*

Glucocorticoids (GCs) are widely used in the therapy of lymphomas and lymphoblastic leukemias owing to their apoptogenic effects on these cancerous cells. A major impediment of GC therapy is the acquisition of apoptotic resistance to GC treatment. Also, certain lymphomas and leukemias are *a priori* resistant to GC. Therefore, a desirable goal is to develop strategies that confer GC-sensitivity on GC-resistant cells. We observed that the broad-acting protein kinase (PK) inhibitor Staurosporine (STS) confers GC-sensitivity on several GC-resistant lymphoma cells. GC-resistant lymphoma cells express elevated levels of anti-apoptotic Bcl-2 or Bcl-X_L. Transfection with Bcl-2 or Bcl-X_L in sensitive cells confers resistance to GC-induced apoptosis. STS overcomes the anti-apoptotic properties of Bcl-2 but not of Bcl-X_L. STS acts at several levels. It induces the expression of the pro-apoptotic Nur77 orphan receptor, which offsets the anti-apoptotic effects of Bcl-2. STS also leads to phosphorylation of Bim by an ERK-dependent mechanism which results in Bim upregulation. In addition, STS inhibits PI3K/Akt, leading to the activation of GSK3. Inhibition of GSK3 by its specific inhibitor SB216763 or by overexpression of a dominant negative GSK3 attenuated the effect of STS. Our study demonstrates a central role for GSK3a in promoting GC-induced apoptosis. We found that GSK3a is sequestered to the glucocorticoid receptor (GR) in the absence of ligand, but dissociates from the GR complex upon exposure to GC to promote apoptosis. GC-resistance in lymphoma cells can be relieved by inhibiting the PI3K-Akt survival pathway, which exerts a negative effect on GSK3. Our data demonstrate that lymphoma and leukemia therapy can be improved if GCs are combined with Protein Kinase inhibitors that shift the cell's kinome in favor of apoptosis-prone phenotype.

O12

Treatment of Solid Malignant Tumors by Intra-Tumoral Diffusing Alpha-Emitting Sources: Role of Tumor Micro- and Macro-Environmental Traits

Yona Keisari[1], Hadas Bittan[2], Elinor Lazarov[2], Tomer Cooks[1], Shira Reitkopf[1], Galit Horev[1], Margalit Efrati[1], Lior Arazi[2,3], Michael Schmidt[2], Sefi Raab[1], Itzhak Kelson[2,3]
[1]*Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel,* [2]*School of Physics and Astronomy, Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv, Israel,* [3]*Research and Development, Althera Medical, Tel Aviv, Israel*

Alpha radiation is a most lethal form of radiation whose short range limits its use for cancer treatment. We developed a practical solution to treat the entire tumor with this short range radiation using intratumoral wires, with radium-224 atoms fixed below their surface. As radium-224 decays, it releases into the tumor, by recoil, short-lived atoms which spread inside the tumor and release their lethal alpha particles. We termed this treatment Diffusing Alpha-emitters Radiation Therapy (DART). In previous studies we demonstrated DART's ability to control tumor development and extend survival of mice bearing mouse or human-derived tumors, from various histological origins. Tumors of different histotypes responded differently to the treatment, with squamous cell carcinoma (SCC) derived tumors being the most sensitive and pancreatic cell derived tumors the most resistant.
The extent of tumor damage may be affected by several characteristics:
1. Factors that affect the spread of radioactive atoms and their clearance from the tumor, i.e., fibrotic tissue, blood vessels, compactness.
2. Tumor cell characteristics, governing sensitivity to radiation, i.e., cell repair mechanisms.
Dosimetric measurements of the intra-tumoral spread of radioactivity in different tumor models revealed biologically significant doses (asymptotically exceeding 10 Gy) of Pb-212 over a region a few mm in size. The average region diameter was largest in SCC tumors, smallest in pancreatic tumors and intermediate for colon and lung tumors.
Measurements of the mean lethal dose (D0) for human and mouse pancreatic, SCC and colon carcinomas irradiated by alpha particles, showed that SCC cells are about twice as radiosensitive to alpha radiation as all other cell lines examined.
Intratumoral tissue necrosis and tumor growth retardation are in correlation with the distribution of released alpha emitting isotopes and with the radiosensitivity of tumor cells. Further attempts are made to correlate radiosensitivity with DNA repair mechanisms.

O13

Interleukin-6 and the Tumor Microenvironment

Yves A. De Clerck[1]
[1]*Pediatrics and Biochemistry & Molecular Biology, The Saban Research Institute of Childrens Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, USA*

The contribution of cytokines to the tumor microenvironment and to inflammation in cancer has been the focus of much recent attention. Among the cytokines that play a pro-tumorigenic role in cancer is IL-6, a pleiotropic cytokine produced by stromal and inflammatory cells. In many cancers, like multiple myeloma and neuroblastoma, the expression of IL-6 is increased and higher levels are indicators of poorer clinical outcome. Tumor cells stimulate the expression of IL-6 by stromal cells through adhesion dependent and adhesion independent mechanisms. The latter seems to predominate in neuroblastoma. We have shown that Cox-2 mediated production of PGE2 and the expression of Galectin-3 binding protein by neuroblastoma cells are potent mechanisms of IL-6 induction in bone marrow-derived mesenchymal cells and monocytes. IL-6 has multiple effects on cancer progression. In the bone marrow it stimulates the maturation and activation of osteoclast precursor cells and promotes osteolytic bone metastasis. IL-6 also has a paracrine effect on neuroblastoma cells which express the 2 subunits of the IL-6 receptor (IL-6R/gp80 and gp130) that are necessary for IL-6-mediated activation of ERK 1/2 and STAT-3. Signaling is potentiated by soluble IL-6R/gp80 that stabilizes IL-6 and acts as a potent agonist. IL-6 stimulates the proliferation of tumor cells and enhances their survival in the presence of cytotoxicity drugs like etoposide (an inducer of the mitochondrial apoptotic pathway) by increasing the expression of the anti-apoptotic proteins Bcl-2, Bcl-$_{XL}$ and survivin. This effect is dependent on STAT-3 activation. In neuroblastoma, IL-6 is rarely expressed by tumor cells and commonly expressed by bone marrow-derived mesenchymal cells in the bone marrow and monocytes/macrophages in primary tumors, which are also a source of sIL-6R. Thus stromal expression of IL-6 contributes to the protective role that the bone marrow microenvironment has against the cytotoxic effect of chemotherapy on tumor cells. IL-6 or IL-6 mediated signaling could therefore represent valuable targets for therapeutic intervention.

O14

Inflammatory Chemokines in Malignancy: Regulation by Microenvironmental and Intrinsic Factors

Gali Soria[1], Maya Ofri[1], Tal Leibovich-Rivkin[1], Marcelo Ehrlich[1], Tsipi Meshel[1], Neora Yaal-Hahoshen[2], Leonor Trejo-Leider[3], **Adit Ben-Baruch[1]**
[1]*Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel,* [2]*Department of Oncology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel,* [3]*Department of Pathology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel*

The inflammatory milieu of tumors affects considerably cancer development and progression. Inflammatory chemokines, including CCL2 and CCL5 are major contributors to breast malignancy. The two chemokines are expressed by the tumor cells in ~60–70% of biopsies of breast cancer patients, but are minimally detected in normal breast epithelial duct cells. In this study, we have analyzed molecular motif/s that regulate the secretion of CCL5 by breast tumor cells. We focused on a specific region located at the 40 s loop of the chemokine. This region was essential for the release of CCL5 by the

tumor cells, and for the trafficking of vesicles containing the chemokine from the endoplasmic reticulum to post-golgi regions and to secretion. Our studies have also identified the mechanisms by which this motif regulates the release of CCL5 by the tumor cells. Also, we determined the regulation of CCL2 and CCL5 secretion by inflammatory cytokines in breast tumors. Our analyses indicate that TNFa and IL-1b are expressed by the tumor cells in 90% of breast cancer patients, and that both cytokines potently promote the release of CCL2 and CCL5 by breast tumor cells and by normal breast epithelial cells. Combined with additional findings that provided evidence to interactions between inflammatory cytokines and chemokines in breast cancer, we suggest that TNFa and IL-1b that are found at the tumor microenvironment are important up-regulators of CCL2 and CCL5 release in early and advanced stages of disease, as well as of progression-related processes. Together, our findings identified microenvironmental and intrinsic properties that regulate the release of the pro-malignancy chemokines CCL2 and CCL5 by breast tumor cells, and consequently affect disease development and progression.

O15

Angiogenic Accessory Cells: VEGF-induced Recruitment and Re-programming

Eli Keshet[1]
[1]*Department of molecular Biology, The Hebrew University of Jerusalem, Jerusalem, Israel*

Adult angiogenesis, in general, and tumor angiogenesis, in particular, heavily rely on myeloid cells recruited from the bone marrow and homing to the respective target organ or tumor. There, they act as paracrine accessory cell without whom angiogenesis is greatly compromised. Using transgenic systems designed for conditional gain- or loss of function of VEGF we thrive to elucidate the pivotal role of VEGF in the recruitment of pro-angiogenic monocytes and their re-programming. Previously, we have shown that VEGF functions in homing monocytes to the target tissue from which it emanates, in their perivascular positioning, and in their retention therein. The current study addresses dynamic changes that recruited monocytes undergo under the influence of local VEGF. Specifically, we provide evidence supporting the notion that angiogenic monocytes do not represent a dedicated subset of cells but rather 'regular' Gr1+ monocytes that are nevertheless 'educated' by VEGF to become more pro-angiogenic and, importantly, also pro-arteriogenic 'professional' cells. These conclusions are mostly based on following the fate, gene expression profiles and functional performance of genetically-tagged monocytes adoptively-transferred into the circulation of mice in which VEGF has been induced in selected organs.

O16

Therapy-Induced Alteration of the Tumor Microenvironment: Impact of Bone Marrow Derived Cells

Robert Kerbel[1]
[1]*Molecular & Cellular Biology Research, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada*

A common problem associated with cancer therapy using various cytotoxic drugs, including chemotherapy, or other treatments, e.g. radiation, is the property of responding tumors to rapidly repopulate and recover from such therapies (Kim & Tannock, *Nat Rev Cancer* 2005). This can significantly compromise the progression free and overall survival benefits induced by such therapies. Historically, tumor repopulation has been viewed primarily, or exclusively, as an intrinsic tumor cell phenomenon. However, we have obtained evidence for various therapy-induced host responses that can alter the tumor microenvironment in such a way so as to accelerate tumor repopulation after administering therapies such as maximum tolerated dose (MTD) chemotherapy or 'vascular disrupting agents' (Y Shaked et al. *Science* 2006; *ibid Cancer Cell* 2008). These host responses consist of the rapid systemic induction of a variety of growth factors, cytokines, and chemokines such as SDF-1 and G-CSF, among others, which then induce mobilization of a variety of bone marrow derived cell (BMDC) types, including circulating endothelial progenitor cells (CEPs). Such cells subsequently home to and invade the drug treated tumors, in potentially large numbers. The molecular mechanisms responsible for CEP tumor homing and retention at the tumor site are under investigation, and several molecular entities have been implicated including CXCR4/SDF-1, a4b1 integrin, G-CSF, and VE-cadherin. As a result, targeting such molecules to prevent the invasion of tumors by BMDCs becomes a therapeutic option, e.g. targeting CXCR4 or a4b1 concurrently with certain cytotoxic therapies. In addition, certain antiangiogenic drugs such as anti-VEGF(R-2) antibodies may function, at least in part, to enhance MTD chemotherapy or VDA therapy by reducing aspects of the host bone marrow 'tumor response', either by preventing mobilization, tumor homing, or retention at the tumor site.

O17

Characterization of Factors Activating Gr-1+ Inflammatory Cells in Squamous Cell Carcinoma Towards a Tumor-supporting, Pro-angiogenic Phenotype

Nina Linde[1], Dennis Dauscher[1], **Margareta M. Mueller**[1]
[1]*Tumor and Microenvironment, German Cancer Research Center, Heidelberg, Germany*

Inflammatory cell infiltration as an essential contributor to tumor development and progression has gained increasing acceptance. Data from our HaCaT model system for human skin squamous cell carcinoma (SCC) clearly emphasize that a tumor-promoting microenvironment including an activated inflammatory infiltrate is indispensable for tumor formation and progression. In this model as well as in a syngeneic mouse skin SCC model we could demonstrate that the recruitment of Gr-1+ cells into the malignant stroma precedes persistent angiogenesis. We were able to show that CD11b+/Gr1+ immature myeloid cells constitute the majority of the tumor associated inflammatory infiltrate in SCCs of both immunocompetent C57Bl/6 and athymic nude mice. In athymic nude mice depletion of Gr-1+ cells strongly inhibited tumor growth, angiogenesis and invasion. Interestingly, the depletion of Gr-1+ cells correlates with the reduction of MMP-9 in the malignant stroma. These findings

imply that CD11b+/Gr-1+ cells have a tumor supporting role other than being suppressors of an anti-tumor T-cell response. Our current work focuses on the characterization of the functional contribution of Gr-1+ cells to tumor progression and identifies the factors that activate Gr-1+ cells within the tumor microenvironment.

O18

Role of Inflammation and Immune Privilege Microenvironment in Tumor Development

Catherine Sautès-Fridman[1], Isabelle Cremer[1], Sylvain Fisson[1], Wolf H. Fridman[1]
[1]*Department of Immunology, Cancer and Inflammation, Cordeliers Research Center, Paris, France*

Lung cancer develops at the mucosal airway interface. The respiratory epithelium is in contact with the outside environment and exposed continuously to a broad range of pathogen agents including viruses. We describe the expression of TLRs in human lung tumor cells (Non Small Cell Lung Carcinoma) and show that the stimulation by TLR7 and TLR8 agonists leads to increased tumor cell survival and chemoresistance. Transcriptional analysis suggests a TLR chronic stimulation of tumor cells in situ. These data indicate that TLR signaling during infection could directly favour tumor development. Primary intraocular lymphoma (PIOL) is a high grade non-Hodgkin lymphoma which develops in an immunoprivileged site. Using a murine model of intraocular B cell lymphoma we detect an impaired Th1-Tc1 profile and Th17 cells in the eye concomitant to a high proportion of CD4+CD25+Foxp3+ T-cells. Systemic depletion of naturally occurring regulatory T cells induces only a slight decrease of the tumor burden suggesting that nTregs is one of the immune suppressive mechanisms occurring in this microenvironment. Other immune privilege mechanisms are under study.

O19

Interaction of CTLs with Stroma Components: Endothelial Cell Cross-Recognition by Specific CTL and Influence of Hypoxic Stress

Salem Chouaib[1], Houssem Benlalam[1], Muhammed Zaeem N.[1]
[1]*Institut Gustave Roussy, Villejuif, France*

Cellular interactions in the tumor stroma play a major role in cancer progression but can also induce tumor rejection. To explore the role of endothelial cells in these interactions, we used an *in vitro* 3D collagen matrix model containing a cytotoxic T lymphocyte CTL clone autologous tumor cells and an endothelial cell line that are all derived from the same tumor. We demonstrated that specific killing of the endothelial cells by the CTL clone required the autologous tumor cells and involved antigen cross-presentation. The formation of gap-junctions between endothelial and tumor cells is required for antigenic peptide transfer to endothelial cells that are then recognized and eliminated by CTL. We provided evidence indicating that gap-junctions facilitate an effective CTL-mediated destruction of endothelial cells from the tumor microenvironment which may contribute to the control of tumor progression.
How a better understanding of the crosstalk between killer cells and stroma components including hypoxic stress may lead to the development of novel therapeutic strategies will be discussed.

O20

The Role of IL-1R, TLR2 and TLR4 Signaling in the Malignant Process

Ron N. Apte[1], Liat Mann[1], Shahar Dotan[1], Yaron Carmi[1], Moshe Elkabets[1], Charles A. Dinarello[3], Elena Voronov[1]
[1]*The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel,* [3]*Division of Infections Diseases, University of Colorado, Denver, CO, USA*

IL-1 is a pleiotropic pro-inflammatory and immunostimulatory cytokine with diverse effects on malignant processes. At tumor sites, IL-1 is produced by microenvironmental cellular elements as well as by the malignant cells, in response to tissue damage products recognized by TLR receptors on innate cells. We have recently shown the involvement of TLR2 and TLR4 in IL-1 production and in the control of malignant processes. The IL-1 family consists of two agonistic proteins, namely IL-1α and IL-1β, and one antagonistic protein, the IL-1 receptor antagonist (IL-1Ra), which is a physiological inhibitor of pre-formed IL-1. Recombinant IL-1α and IL-1β bind to the same receptor and exert the same biological activities. However, in the physiological milieu, IL-1α and IL-1β differ dramatically in the sub-cellular compartments in which they are active; IL-1α is mainly active as a cell-associated cytokine (cytosolic and membrane-associated forms), while IL-1β is active only in its mature secreted form. We have previously shown that IL-1α expression on the membrane of tumor cells increases their immunogenicity and leads to tumor eradication, while tumor cells which actively secrete IL-1β are more malignant than control cells and also induce anergy mediated by MDSC. 3-MCA-indcued chemical carcinogenesis was further used in IL-1 KO mice. It was shown that IL-1β-mediated inflammation is essential in the process of 3-MCA carcinogenesis, while microenvironmental IL-1β synergizes with tumor cell-derived IL-1β in determining the malignant phenotype of transplantable tumors. Expression of cell-associated IL-1α by the malignant cells or the host increases the immunogenicity of the tumor or controls immunoediting of the arising malignant cells, respectively. Altogether, the results show the differential effects of IL-1β and IL-1α in malignant processes and point to the therapeutic feasibility of using the IL-1Ra in tumor therapy to neutralize soluble IL-1 (mainly IL-1β), in addition to its use in treatment of autoimmune diseases, such as Rheumatoid arthritis.

O21

Attenuation of TGFβ Signaling by c-Myc-regulated microRNAs

Michael Dews[1], **Andrei Thomas-Tikhonenko[1,2]**
[1]*Children's Hospital of Philadelphia, Philadelphia, PA, USA,* [2]*Department of Pathology & Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA*

TGFβ produced within the tumor plays an important role in tissue homeostasis and strongly affects both the stromal and the neoplastic compartments. Some tumors (e.g., colon adenocarcinomas with microsatellite instability) sustain and preserve mutations in the TGFβ-R2, making them refractory to this growth inhibitor. In other cases, the molecular mechanisms underlying resistance to TGFβ are less clear. Previously, we had developed a mouse model of colon cancer based on p53-null murine colonocytes sequentially transformed with Ki-Ras- and c-Myc oncogenes. In this genetically complex system, c-Myc does not appear to be a primary determinant of cell proliferation. Instead it strongly promotes the angiogenic phenotype, at least partly through downregulation of thrombospondin-1 and related thrombospondin type I repeat (TSR) proteins such as clusterin (Thomas-Tikhonenko et al, Cancer Res 2004; Dews et al, Nature Genetics 2006). Many of these Myc-downregulated proteins are concertedly upregulated by TGFβ, leading us to hypothesize that c-Myc somehow attenuates TGFβ signaling. Since Myc can repress gene expression by activating the miR-17-92 microRNA cluster, we asked whether the six microRNAs comprising this cluster directly target components of TGFβ signaling. We discovered that at least two key signaling molecules, TGFβ-R2 and Smad4 are indeed down-regulated by miR-17-92. In addition, down-regulation of thrombospondin-1, which is a direct target of miR-17-92, hinders the release of TGFβ from the complex with the latent TGFβ-binding protein 1 (LTBP1.) Consequently, in tumors with Myc- and miR-17-92 overexpression TGFβ signaling is significantly reduced and the robust angiogenic phenotype ensues. Our findings help explain how tumor cells become resistant to TGFβ and identify relevant molecular intermediates that can be targeted therapeutically.

O22

Knockout of Heregulin (HRG) Expression Reverts Paclitaxel-Resistance and Promotes Mesenchymal Epithelial Transition (MET) of Triple Negative Breast Cancer Cells

Jing Li[1], Ingrid Espinoza[1], **Ruth Lupu[1]**
[1]*Department of Laboratory Medicine and Experimental Pathology, Mayo Cancer Center,, Mayo Clinic, Rochester, MN, USA*

The growth factor Heregulin (HRG) is expressed in about 30% of breast cancer tumors, and induces tumorigenicity and metastasis of breast cancer cells. We have demonstrated previously that HRG overexpression renders breast cancer cells resistance to the microtubule-interfering agent Taxol, a drug of choice for the treatment of metastatic breast cancer. The mechanism by which HRG induces Taxol resistance is largely unknown. It is also known that triple negative breast cancer tumors do express high levels of HRG and unfortunately they do not respond to HRG. Our studies were aimed at targeting HRG with the goal of achieving a therapeutic target as well as restoring the response to Taxol, while preventing the formation of metastasis. Thus, we knocked-down HRG expression in three different breast cancer cell lines: MDA-MB-23, HS578T and BT549. Our data demonstrates that HRG expression is an absolute requirement for the anchorage-independent growth for triple negative breast cancer cells, since none of the breast cancer cells MDA-MB-231, HS578T and BT549 stable expressing the silencing (shRNA) for HRG, were capable of forming colony in soft agar. Furthermore, these cells, not only no longer were not anchorage-independent were no longer resistant to Taxol, to the contrary the shRNA/HRG cells were exquisitely sensitive to Taxol, to induce growth inhibition and apoptosis. More importantly, we observed that the disorganized structured observed in 3D matrigel culture observed for triple negative cells, was completely abolished once HRG was knockdown and a very organized structure. These characteristics resembled an EMT (epithelial-mesenchymal epithelial transition (MET). This should be deemed a potential target in developing therapies for triple negative breast carcinomas.

O23

Decoding Tumor-Host Interactions in Dormancy: Notch3-mediated Regulation of MKP-1 Promotes Tumor Cell Survival

Massimo Masiero[1], Sonia Minuzzo[1], Irene Pusceddu[2], Lidia Moserle[1], Luca Persano[1], Alberto Amadori[1,2], **Stefano Indraccolo[2]**
[1]*Department of Oncology and Surgical Sciences, University of Padova, Padova, Italy,* [2]*Istituto Oncologico Veneto - IRCCS, Padova, Italy*

While it has been recently recognized that signals between endothelial and cancer cells may drive escape from tumor dormancy, little is known regarding the molecular mechanisms behind this phenomenon. Recently, we demonstrated that the Notch ligand Dll4, induced by angiogenic factors in endothelial cells, triggers Notch3 activation in neighbouring T-ALL leukaemia cells and promotes tumorigenesis. Here we show that MKP-1 levels - a broadly expressed dual specificity phosphatase - are controlled by Notch3 by a non-transcriptional mechanism involving regulation of MKP-1 protein stability. Notch3 and MKP-1 levels are consistently up-regulated in aggressive compared to dormant tumors, which is accompanied by opposite variations in the levels of active p38 and ERK1-2 - two canonical MKP-1 targets. A good correlation between Notch3 and MKP-1 levels was observed in T-ALL primary samples from patients and in a panel of T-ALL cell lines. Inhibition of Notch3 by RNA interference or GSI treatment, or stimulation by the Dll4 ligand had marked effects on MKP-1 levels in T-ALL cells in vitro. Attenuation of MKP-1 levels by shRNA did not affect proliferation, whereas it significantly increased T-ALL cell death following drug treatment or serum starvation. Importantly, tumorigenesis of MKP-1 deficient T-ALL cells was markedly impaired compared to controls. Our results elucidate a novel mechanism downstream of Notch3 by which the direct interplay between endothelial and tumor cells promotes survival of T-ALL cells.

O24

Role of Foxm1 Transcription Factor in Tumor Microenvironment

Tanya Kalin[1], David Balli[1], Fu-Sheng Chow[1], Vladimir Kalinichenko[1]
[1]*Pulmonary Biology, Cincinnati Children's Hospital, Cincinnati, OH, USA*

The Forkhead Box m1 (Foxm1) protein is induced in a majority of human cancers, including non-small cell lung cancers. Increased Foxm1 expression is associated with poor prognosis. However, specific requirements for the Foxm1 in each cell type of the cancer lesion during lung tumor formation remain unknown. In this study, we examined the role of Foxm1 in tumor microenvironment using conditional knockout mouse models with Foxm1 deficiency in macrophages (LysM-Cre $Foxm1^{fl/fl}$ mice; *macFoxm1*$^{-/-}$) or endothelial cells (Tie2-Cre $Foxm1^{fl/fl}$ mice; *enFoxm1*$^{-/-}$). Lung tumors in mice were induced using two experimental protocols: 3-methylcholanthrene (MCA) / butylated hydroxytoluene (BHT) or urethane. Conditional deletion of *Foxm1* from macrophages caused a significant decrease in lung inflammation during induction of lung tumors, leading to reduction in the number and size of lung adenomas. Decreased lung tumorigenesis in *macFoxm1*$^{-/-}$ mice was associated with diminished proliferation of tumor cells, decreased numbers of tumor-associated macrophages and reduced expression of pro-inflammatory cytokines in the lung and bronchoalveolar lavage fluid. Furthermore, we demonstrated that *Foxm1*$^{-/-}$ mice displayed a dramatic decrease in proliferation and migration of macrophages *in vivo* and *in vitro*. In our studies, we also demonstrated that deletion of Foxm1 from endothelial cells resulted in accelerated lung tumorigenesis. The increased numbers and sizes of lung tumors in *enFoxm1*$^{-/-}$ mice resulted from increased endothelial leakage and infiltration of inflammatory cells into lung tissue. The *enFoxm1*$^{-/-}$ mice displayed increased tumor cell proliferation and increased mRNA levels of cell cycle regulator cMyc and cyclin D1. Deletion of Foxm1 from endothelial cells caused reduced expression of Foxf1 and Foxf2 transcription factors, both of which are critical regulators of endothelial cell functions and VEGF signaling. Altogether, our studies demonstrated that Foxm1 plays a dual role in tumor microenvironment: it controls cellular permeability in endothelial cells and induces inflammation and migration of macrophages into lung tumors.

O25

Tenascin-W is Overexpressed in Glioma-Associated Blood Vessels and Stimulates Angiogenesis *in vitro*

Florence Brellier[1], Enrico Martina[1], Martin Degen[1], Curzio Ruegg[2], Adrian Merlo[3], Maddalena Lino[3], Ruth Chiquet-Ehrismann[1]
[1]*Growth Control Department, Friedrich Miescher Institute, Basel, Switzerland,* [2]*Division of Experimental Oncology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland,* [3]*Laboratory of Molecular Neuro-Oncology, University Hospital, Basel, Switzerland*

Aggressiveness of a tumor does not only reflect intrinsic features of cancer cells. The microenvironment hosting the tumor also actively participates in regulating tumor cell proliferation, migration, and invasion. Among the extracellular matrix proteins enriched in stroma of carcinomas are the tenascin family members tenascin-C and tenascin-W. Whereas tenascin-C overexpression in gliomas has been widely reported to correlate with adverse prognosis, the status of tenascin-W in brain tumors has not been investigated. We analyzed protein levels of tenascin-W in 38 human gliomas (29 glioblastomas, 5 astrocytomas, and 4 oligodendromas) and found expression of tenascin-W in more than 80% of all tumor samples, whereas no tenascin-W could be detected in control brain tissues. Immunohistochemical co-stainings of tenascin-W and von Willebrand factor revealed that tenascin-W is localized around blood vessels exclusively in tumor samples. To assess if tenascin-W influences the behavior of endothelial cells *in vitro,* Human Umbilical Vein Endothelial Cells (HUVEC) were seeded on a collagen substratum including tenascin-W. The presence of tenascin-W increased the proportion of elongated cells and augmented the mean speed of migration of the cell population. Furthermore, tenascin-W triggered sprouting of HUVEC spheroids to a similar extent as the pro-angiogenic factor tenascin-C. Our study thus identifies tenascin-W as a candidate biomarker for brain tumor angiogenesis that could be used as molecular target for therapy irrespective of the glioma subtype.

O26

***Protease activated receptor1,* PAR1 Acts via a Novel G_{a13}-DVL Axis to Stabilize b-catenin Levels**

Hagit Turm[1], Myriam Maoz[1], Stefan Offermanns[2], Rachel Bar-Shavit[1]
[1]*Oncology, Hadassah- Hebrew University Hospital, Jerusalem, Israel,* [2]*Institute of Pharmacology, University of Heidelberg, Heidelberg, Germany*

We have previously shown a novel link between human *protease-activated-receptor1* (*hPar1)* and b-catenin stabilization. The over-expression of *hPar1* leads to a striking stabilization of b-catenin, a well established core process of the Wnt signaling pathway. Here we elucidate the mechanism linking PAR1 to b-catenin oncogenicity. PAR1 is selectively associated with activated G_{a13}, recruiting next dishevelled (DVL), an upstream Wnt signaling protein. Using constructs exhibiting either individually distinct DVL domains (e.g., DIX, PDZ and DEP) or depleted DVL sites or a GST-DVL-DIX column, we showed that the DIX domain associates with G_{a13}. The impact of DVL involvement in PAR1 induced b-catenin stabilization is underscored by the marked reduction of PAR1-induced Matrigel invasion and the decrease in b-catenin level caused by DVL-*Si*RNA lentiviral construct. Activation of PAR1 also promotes the binding of b-arrestin 2 to DVL, playing a role in PAR1 induced DVL phosphorylation dynamics. While infection of *Si*RNA-LRP5/6 potently reduces Wnt3a mediated b-catenin expression, no effect is observed on PAR1 induced b-catenin stabilization. PAR1-induced b-catenin expression is also caused by the Wnt antagonists SFRP-2 or SFRP-5. Collectively, our data show that PAR1 mediates b-catenin stabilization independently of Wnts, Frizzled and the co-receptor LRP5/6. We hereby propose a novel path of PAR1 induced G_{a13}-DVL axis in cancer and b-catenin stabilization.

O27

Tumor-Mediated Suppression of Myeloid to Dendritic Cell (DC) Differentiation via Down Regulation of Protein Kinase C βII (PKCβII) Expression

Matthew Farren[1], Louise Carlson[1], Kelvin Lee[1]
[1]*Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY, USA*

Cancer induced immune suppression contributes to tumor outgrowth and immune escape and occurs, in part, due to tumor-mediated dysregulation of DC differentiation. This results in fewer dendritic cells and an accumulation of immature myeloid cells, themselves actively immunosuppressive. Tumors mediate impaired DC differentiation by secreting factors (e.g. VEGF) that hyperactivate Stat3 in DC progenitors, though the molecular mechanisms by which Stat3 signaling inhibits DC differentiation are poorly defined. We have previously shown that PKCβII is essential in myeloid progenitor to DC differentiation and that knock down or inhibition of PKCβII blocks DC differentiation. Here, we investigate the idea that tumors inhibit DC differentiation by down regulating PKCβII expression in myeloid progenitor cells via Stat3 hyperactivation.
Culture in human or murine tumor conditioned media (TCM) decreased PKCβII protein levels by 51% and 48% in a human myeloid progenitor cell line (KG1), respectively. Additionally, culture of KG1 in TCM significantly decreased PKCβII mRNA transcript levels (38-fold reduction, $p<0.01$). PKCβII down regulation was associated with decreased DC differentiation: culture of KG1 in TCM significantly reduced phorbol ester driven DC differentiation (assessed by T cell stimulatory ability, $p<0.01$). TCM significantly down regulated PKCβ promoter driven transcription in KG1, compared to cells grown in normal media (7-fold reduction, $p<0.01$). Importantly, TCM induced Stat3 phosphorylation in KG1. To test the role of Stat3 activity on PKCβII expression, we generated clones stably expressing wild type and constitutive active Stat3 constructs in a second myeloid progenitor cell line (K562). Compared to K562, PKCβII mRNA transcript levels were significantly down regulated (>10-fold) in clones stably expressing the constitutive active Stat3 construct ($p< 0.05$) while PKCβII protein levels were reduced 75–95%. Taken together, these observations argue that tumors inhibit DC differentiation by inducing down regulation of PKCβII expression in myeloid progenitor cells via Stat3 hyperactivation.

O28

Myeloma Cell Survival and Importance of Crosstalk between Notch1-Jagged2 and CD28-B7 Pathways in Dendritic Cells

Chandana Koorella[1], Jayakumar Nair[1], Sanjay Bansal[1], Louise Carlson[1], Pushpankur Ghoshal[2], Kelvin Lee[1]
[1]*Department Of Immunology, Roswell Park Cancer Institute, Buffalo, New York, USA,* [2]*Department Of Medicine, Roswell Park Cancer Institute, Buffalo, NY, USA*

Multiple myeloma is a neoplasm of bone marrow resident plasma cells characterized by a critical interaction between myeloma cells and bone marrow stromal cells, which produce IL-6, supporting myeloma cell survival. However, the molecular and cellular components involved in myeloma induced IL-6 production remain largely uncharacterized. At the cellular level, dendritic cells (DC) in the bone marrow microenvironment and at the molecular level the CD28-B7 and Notch1-Jagged2 pathways were separately implicated by us in myeloma induced IL-6 production. While Notch signaling leading to IL-6 production in DC is well understood, the mechanism of "backsignaling" via B7, a ligand with a short cytoplasmic tail, is largely uncharacterized. To gain insight into B7 signaling, DC were stimulated with CD28Ig in the presence or absence of an inhibitor of Notch signaling, gamma secretase inhibitor (GSI). DC treated with CD28Ig alone produced significantly higher levels of IL-6 when compared to DC treated with CD28Ig and GSI. GSI specifically targeted Notch signaling as observed by decreased expression of Notch gene targets: Hes1 and Deltex4. Also, decreased IL-6 levels in presence of GSI were not due to the decrease in B7 expression on DC. To specifically implicate the importance of Notch1 and Jagged2, we blocked them using antibodies and observed a similar decrease in IL-6 production upon blocking Notch1 signaling. Our results suggest that CD28 mediated IL-6 production is dependent on Notch1 signaling and crosstalk between the Notch1-Jagged2 and CD28-B7 pathways leads to IL-6 production by DC. We are examining a potential direct/ indirect mechanism of crosstalk in myeloma induced IL-6 production. Targeting IL-6 induced by crosstalk between these two pathways prompts not only clinical evaluation to improve MM patient outcome but also extends to advancing knowledge in T-cell biology.

O29

Interleukin-18-Dependent Genes of Highly Metastatic Human Melanoma

Olatz Crende[1], Marianna Sabatino[2], Maria Valcarcel[3], Ena Wang[2], Francesco M. Marincola[2], Fernando Vidal-Vanaclocha[1]
[1]*Department of Cell Biology and Histology, Basque Country University School of Medicine, Leioa, Bizkaia, Spain,* [2]*Department of Transfusion Medicine, Infectious Disease and Immunogenetics Section, National Institutes of Health, Bethesda, MD, USA,* [3]*Pharmakine SL, Bizkaia Technology Park, Derio, Bizkaia, Spain*

Because immune-stimulating effects of interleukin (IL)-18 have anti-neoplastic properties, IL-18 has been proposed as an adjuvant therapy against cancer. However, IL-18 increases in the blood of cancer patients and has been associated with metastatic recurrence in some cancer types. Melanoma cells heterogeneously express IL-18 receptors and consistent with its potential prometastatic action, we reported that experimental melanoma metastases are prevented in both ICE-deficient mice lacking secreted IL-18 and IL-18-binding protein-treated mice. Moreover, IL-18 promotes melanoma metastasis at multiple steps, including stimulating the capillary arrest of circulating tumor cells, immune escape, angiogenesis, and tumor cell proliferation. However, at the moment, the molecular mechanisms underlying IL-18-dependent melanoma me-

tastasis have not been elucidated. The aim of this study was to identify molecular mediators of IL-18 by exploring the melanoma cell gene display induced by this cytokine. We compared global gene expression between untreated and IL-18-treated melanomas using a high-throughput human 36 K cDNA microarray platform. Total RNA from four primary cultured human melanoma cell lines was used: two VLA-4-expressing highly-metastatic cell lines (A375 and 1182 melanoma), and two non-VLA-4 expressing low metastatic cell lines (526 and 624-28 melanoma). Gene profile was determined by cDNA microarray and real-time PCR. We found around 50 genes over-expressed (ANOVA, $p<0.05$) in IL-18-treated highly metastatic versus low-metastatic melanoma cells. Some of these genes were also co-expressed by effect of soluble VCAM-1 on highly metastatic but not on low-metastatic melanoma cells. None of these genes were expressed by melanoma patients that did not metastasize to distant sites within 4 years after diagnosis, while majority of them were expressed in melanomas associated with high risk of metastasis and death. In summary, we identified the biological and clinical relevance of IL-18-dependent genes for highly metastatic VLA-4-expressing human melanoma, and suggest molecular pathways relevant to melanoma metastasis in the inflammatory microenvironment of IL-18.

O30

Interleukin-8 Expression is Regulated by Histone Deacetylases through NF-kB Pathway in Breast Cancer

Carine Chavey[1], David Vindireux[1], Carine Bossard[1], Marcus Muehlbauer[2], Christian Jorgensen[1], Christian Jobin[2], **Gwendal Lazennec[1]**
[1]*U844, INSERM, Montpellier, France,* [2]*Department of Medicine, Pharmacology and Center for Gastrointestinal Biology and Disease, University of North Carolina, Chapel Hill, NC, USA*

We have recently reported that IL-8/CXCL8 was overexpressed in invasive estrogen receptor (ERalpha)-negative breast cancer cells, compared to ERa-positive breast cancer cells. We now demonstrate that histone deacetylases (HDAC) play an essential role in the regulation of IL-8 gene expression in ERalpha-positive MCF-7 breast cancer cells. Treatment of MCF-7 cells with the HDAC inhibitor trichostatin A (TSA) led to a strong up-regulation of IL-8 protein and RNA levels in MCF-7 cells. The up-regulation of IL-8 in MCF-7 cells was time - and concentration dependent. Moreover, run-on and transfection experiments demonstrated that IL-8 induction by HDAC inhibitors was transcriptional and involved mainly NF-kB site of IL-8 promoter. These observations are corroborated by an up-regulation of NF-kB activity in MCF-7 cells in the presence of TSA. In addition, blocking NF-kB pathway by adenoviral delivery of a dominant-negative IkB or IKK2 mutant abolished IL-8 gene induction by histone deacetylase inhibitors. HDAC inhibitors triggered IKK phosphorylation, up-regulated p65 nuclear translocation, while decreasing the protein levels of IkBalpha, which accounts for NF-kB activation. TSA increased the acetylation of Histone H3 on IL-8 promoter in a time-dependent manner. In summary, our results demonstrate that NF-kB pathway repression by HDAC is responsible for the low expression of IL-8 in ERalpha-positive breast cancer cells.

O31

Differential Expression of MicroRNA-17-3p Reverts Morphology of Prostate Cells in lrECM Gels, Reduces Tumor Growth *in vivo* and Correlates with Prostate Tumor Expression by LCM Analysis

Xueping Zhang[1], Amy Ladd[1], William Budd[1], Ema Dragoescu[1], Joy Ware[1], **Zendra Zehner[1]**
[1]*Departments of Biochemistry & Molecular Biology, Pathology and Center for Biological Complexity, Virginia Commonwealth University, Richmond, VA, USA*

MicroRNAs (miRs) are a novel class of RNAs with important roles in regulating gene expression at the level of protein synthesis. To identify miRs controlling prostate tumor progression, we utilized human prostate sublines derived from the immortalized P69 cell line, which differed in their tumorigenic properties *in vivo.* When grown embedded in lrECM gels (3D) these sublines displayed drastically different morphologies correlating with their behavior *in vivo.* The non-tumorigenic P69 subline grew as multiceullular acini with a defined lumen and basal/polar expression of relevant marker proteins. M12, a highly tumorigenic, metastatic derivative, grew as a disorganized mass of cells with no polarization, whereas the F6 subline, a weakly tumorigenic, non-metastatic M12 variant, reverted to organized acini. These sublines also differed in expression of vimentin, which was high in M12, but low in F6 and P69 sublines with E-cadherin exhibiting the opposite expression pattern. A miR array screen of M12 and F6 cell lines grown in 2D versus 3D revealed several miRs, which were differentially expressed. Of these miRs, miR-17-3p was found to target vimentin. Reduction of vimentin expression either by stable expression of a vimentin-specific siRNA or miR-17-3p in the M12 subline decreased vimentin levels and reverted growth to organized, polarized acini in lrECM gels. *In vitro* motility and invasion assays suggested a decrease in tumorigenic behaviour as confirmed by reduced tumor growth in male athymic, nude mice. qRT-PCR analysis of RNA extracted from laser capture microdissected (LCM) material of human prostatectomy specimens (FFPE) established that miR-17-3p levels were reduced 90% in tumor cells of Gleason pattern 3, 4 and 5 compared to hyperplastic glandular epithelium, normal glandular epithelium, hyperplastic stroma or normal stroma. Altogether these results suggest that miR-17-3p functions as a tumor suppressor, representing a novel, new target to block prostate tumor progression.

O32

Regulation of Colon Cancer Metastasis by Death Receptor-3 and E-selectin

Nicolas Porquet[1], Stéphanie Gout[1], Pierre-Luc Tremblay[1,2], Andrée Poirier[1], François Houle[1], François A. Auger[2], **Jacques Huot[1]**
[1]*Le Centre de Recherche en Cancérologie, Université Laval et CRCHUQ, Québec, QC, Canada,* [2]*Laboratoire d'Organogenèse Expérimentale, CHA de l'Université Laval, Québec, QC, Canada*

The adhesion of circulating cancer cells to endothelial cells (EC) is a prerequisite for their extravasation and metastatic dissemination. We have shown that E-selectin, a major endothelial adhesion receptor, interacts with Death Receptor-3 (DR3), present on colon carcinoma cells, to promote their adhesion to EC and to increase their motile and survival potentials (Gout et al. Cancer Res. 2006 and CEM, 2008). We also found that E-selectin and TL1A, the cognate ligand of DR3, trigger the tyrosine phosphorylation of DR3 in a Src family kinase (SFK)-dependent manner. Moreover, we obtained evidence indicating that interaction between DR3 and E-selectin or TL1A induces the activation of the PI3K/Akt pathway in HT-29 colon carcinoma cells. We further discovered that p65/RelA, the anti-apoptotic subunit of NFkB, is rapidly phosphorylated at Ser 536 in response to E-selectin or TL1A and found that the phosphorylation occurs downstream of PI3K/Akt. These findings suggest that E-selectin and TL1A induced-activation of DR3 confers a metastatic advantage to colon cancer cells by inducing SFK-dependent tyrosine phosphorylation of DR3 and by activating the pro-survival PI3K/Akt/NFkBp65 axis. Interestingly, the activation of E-selectin induces a remodeling of EC that is associated with disruption of the adherens junctions. This leads to increased interendothelial spaces enabling transendothelial migration (Tremblay et al Oncogene 2006). Using a laminar flow chamber, we identified three distinct mechanisms by which cancer cells interact with E-selectin to initiate their diapedesis: formation of a mosaic between cancer cells and EC, paracellular diapedesis at the junction of three EC, and transcellular diapedesis (Tremblay et al. Cancer Res. 2008). We conclude that E-selectin-mediated adhesion of colon cancer cells regulates metastasis by conferring inherent invasive potential to cancer cells following binding to DR3 and by remodeling the endothelium in a way that facilitates diapedesis. Supported by the Canadian Cancer Society and the Canadian Institutes for Health Research. NP, SG and PLT have equally contributed to this study.

O33

The Origin of Carcinoma-Associated Fibroblasts

Izhak Haviv[1,2,3], Adam Kowalczyk[6], Alex Boussioutas[2,3], Stephen Fox[2,3], Ian Campbell[2,3], Kornelia Polyak[4,5], Robin Anderson[2,3], David Bowtell[2,3]
[1]*The Blood and DNA Profiling Facility, Baker IDI Heart and Diabetes Institute, Prahran, Vic., Australia,* [2]*Biochemistry, School of Medicine, University of Melbourne, Melbourne, Vic., Australia,* [3]*Breast Cancer Metastasis Laboratory, Peter MacCallum Cancer Centre, Melbourne, Vic., Australia,* [4]*Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA,* [5]*Department of Medicine, Harvard Medical School, Boston, MA, USA,* [6]*NICTA VRL Laboratory, Department of Electrical and Electronic Engineering, University of Melbourne, Melbourne, Vic., Australia*

Recent evidence on the genomic integrity of non-malignant cells surrounding carcinoma cells has reinvigorated the discussion about the origin of the altered phenotype exhibited by carcinoma associated fibroblasts (CAF). Many hypotheses have been proposed for the origin of these altered cells, including standard connective tissue acute phase and stress response, fibroblast senescence, reciprocal interactions with the cancer cells, fibroblast specific somatic mutations, differentiation precursors and infiltrating mesenchymal stem cells. We have addressed each of those options experimentally and found evidence for reciprocal interaction between tumour associated macrophages and cancer associated fibroblasts are elevated in patients, with an associated poor outcome. This supports current understanding of cancer etiology, based on previous animal models, as well as offers novel avenues for therapy.

O34

VEGI, an Endogenous Antiangiogenic Cytokine, Inhibits Hematopoietic Stem Cell Differentiation into Endothelial Progenitor Cell

Lu-Yuan Li[1]
[1]*College of Pharmacy, Nankai University, Tianjin, China*

Endothelial progenitor cells (EPC) play a critical role in post-natal and tumor vasculogenesis. Vascular endothelial growth inhibitor (VEGI; TNFSF15) has been shown to inhibit endothelial cell proliferation by inducing apoptosis. We report here that VEGI inhibits the differentiation of EPC from mouse bone marrow-derived Sca1+ mononuclear cells. Analysis of EPC markers indicates a significant decline of the expression of endothelial cell markers, but not stem cell markers, on VEGI-treated cells. Consistently, the VEGI-treated cells exhibit a decreased capability to adhere, migrate and form capillary-like structures on Matrigel. In addition, VEGI induces apoptosis of differentiated EPC but not early stage EPC. When treated with VEGI, an increase of phospho-Erk and a decrease of phospho-Akt are detected in early stage EPC, while activation of NF-κB, JNK and caspase-3 are seen in differentiated EPC. Furthermore, VEGI induced apoptosis of differentiated EPC is, at least partly, mediated by death receptor-3 (DR3), which is detected on differentiated EPC only. VEGI induced apoptosis signals can be inhibited by neutralizing antibodies against DR3 or recombinant extracellular domain of DR3. These findings indicate that VEGI may participate in the modulation of post-natal vasculogenesis by inhibiting EPC differentiation.

O35

Colon Carcinoma Cell Interaction with Liver Sinusoidal Endothelium Inhibits Organ-Specific Anti-Tumor Immunity via Interleukin-1-Induced Mannose Receptor

Beatriz Arteta[1], Nerea Lasuen[1], Aritz Lopategi[1], Baldur Sveinbjörnsson[3], Bard Smedsrod[2], Fernando Vidal-Vanaclocha[1]
[1]*Department of Cell Biology and Histology, Basque Country University School of Medicine, Leioa, Bizkaia, Spain,* [2]*Department of Cell Biology and Histology, Tromso University, Tromso, Norway,* [3]*Childhood Cancer Research Unit, KarolinskaInstitutet, Stockholm, Sweden*

Mannose receptor (ManR)-mediated liver sinusoidal endothelial cell (LSEC) endocytosis plays a primary role in antigen presentation and innate immunity, but its role in hepatic metastasis is unknown. We studied ManR-mediated endocytosis during C26 colorectal cancer cell

interaction with LSEC and its immunological implications in the hepatic metastasis microenvironment. Labeled mannan or ovalbumin uptake and anti-mouse ManR immunohistochemistry were used to study ManR expression and endocytosis in vivo, in vitro, and by confocal microscopy. Several IL-1 inhibitors and cyclooxygenase (COX)-2 inhibitor Celecoxib were used to analyze the role of IL-1 and COX-2 in ManR regulation. Anti-mouse ManR antibodies and ManR knockout ($ManR^{-/-}$) mice were used to identify ManR-dependent mechanisms during anti-tumor immune response of liver sinusoidal lymphocytes (LSL) interacting with tumor-activated LSEC. Both ManR expression and endocytosis increased in tumor-activated LSEC through a two-step mechanism including: 1) Release of COX-2-dependent IL-1-stimulating factor(s) by LFA-1-expressing C26 cells in response to ICAM-1, which was over expressed and secreted by tumor-activated LSEC; and 2) wide-spread upregulation of ManR expression and endocytosis in LSEC by tumor-induced paracrine IL-1. In addition, LSL that had interacted with tumor-activated LSEC in vivo decreased their anti-tumor cytotoxicity and IFN-gamma secretion while increased IL-10 release to their supernatant ex vivo. IFN-gamma/IL-10 ratio also decreased in the hepatic blood from tumor-injected mice. Immune-suppressant effects of tumor-activated LSEC on LSL were abrogated in both LSEC from $ManR^{-/-}$ mice and tumor-activated LSEC given anti-mouse ManR antibodies. In summary, ICAM 1-induced tumor COX-2 led to regional anti-tumor immunity inhibition during hepatic colorectal metastasis via IL-1-induced ManR. ManR constituted a common mediator for prometastatic effects of IL-1, COX-2 and ICAM-1 in the liver. Rise of both hepatic IFNgamma:IL-10 ratio and anti-tumor cytotoxicity via ManR blockade is consistent with reported antimetastatic effects of IL-1, COX-2 and ICAM-1 inhibitors. These results support ManR as a molecular target for hepatic colorectal metastasis therapy.

O36

Reversal of the Transformed Phenotype and Normalisation of Oncogene-Regulated Genes through Contact with Normal Cells

David Allard[1], Ermanno Gherardi[2], David Tuveson[1]
[1]*CRUK Cambridge Research Institute, Cambridge, UK,* [2]*MRC Centre, Cambridge, UK*

During the initial growth of a tumour, interactions between the initiating cells which carry an oncogenic mutation and the immediate surrounding normal cells can suppress the growth of the oncogenic cells. This phenomenon of neighbour suppression has been studied in fibroblasts in vitro and we have extended the observation to epithelial cells and their transformed derivatives from a variety of tissues confirming a general relevance to cancer as >90% of cancers originate from epithelial cells. We confirm the contact-dependent nature of suppression in epithelial cells and determine the nature of the cell cycle arrest. To identify molecular effectors involved in this form of growth arrest, we studied pancreatic epithelial cells as >90% of pancreatic cancers carry a mutation in Kras as the initiating oncogenic event. To identify the molecular mechanism of neighbour suppression we analysed normal mouse pancreatic ductal epithelial cells, matched derivatives expressing physiological levels of oncogenic $Kras^{G12D}$ and co-cultures of these cells. Although expression of the oncogene, Kras, was not affected in co-cultures where the transformed growth was suppressed, gene expression profiling identified genes responsive to expression of $Kras^{G12D}$ along with a subset which were normalised upon co-culture with normal cells. Thus a subset of oncogene-responsive genes are normalised in conditions where the transformed phenotype is suppressed. Analysis of normal and tumourous human pancreatic tissue and mice with varying degrees of mosaicism for $Kras^{G12D}$ oncogene expression shows the importance of the phenomenon and this subset of oncogene-regulated and normalisation-competent genes in an in vivo setting.

O37

Fibroblast-Dependent Epithelial Cell Invasion in a Reconstruct Model for Esophageal Cancer

Claudia Andl[1]
[1]*Surgery and Cancer Biology, Vanderbilt University, Nashville, TN, USA*

The aim of our study is to analyze the effects of epithelial-mesenchymal crosstalk on cancer cell migration and invasion. Based on previous findings that 70% of esophageal tumors demonstrate the coordinated loss of E-cadherin and TGFβreceptorII (TβRII), we established an *in vitro* model using immortalized human esophageal keratinocytes, expressing dominant-negative mutants of E-cadherin and TβRII (ECdnT). To allow the analysis of epithelial and mesenchymal crosstalk, epithelial reconstructs were utilized by seeding ECdnT cells on an extracellular matrix with embedded fibroblast. We found that the ECdnT cells invade into the underlying collagen/matrigel matrix with embedded fibroblasts, but not in Boyden chamber invasion assays in the absence of fibroblasts. Crosstalk between the epithelial compartment and the surrounding microenvironment is essential for mediating invasion. Biochemical analysis of the fibroblasts in co-culture indicates an activated status, potentially through secretion of TGFβ1 by the ECdnT cells. While we observed these expression changes in the fibroblasts in response to the genotype of the epithelial cells, we also identified reciprocal changes in the epithelial cells themselves: Gene expression analysis of invasive and non-invasive areas of ECdnT cells in the organotypic epithelial reconstruct cultures identified cathepsin B and CD44 to be upregulated in invasive cells. The increase of cathepsin B expression in ECdnT cells appears to be an upstream event in the signaling cascade culminating in cell invasion, as cathepsin B can cleave and activate TGFβ1. CD44 activation is in part mediated through TGFβ1. We show then, that CD44 co-localizes with MMP-2 and MMP-9 to invasive areas and facilitates matrix degradation allowing for cell invasion into the underlying collagen/matrigel layer. In summary, we demonstrate here that the epithelial loss of E-cadherin and TβRII leads to an impaired balance of the epithelial-mesenychmal crosstalk resulting in the activation of fibroblasts and the induction of invasion through a fibroblast-secreted factor.

O38

Cancer-Associated Adipocytes: New Key Players in Breast Tumour Invasion

Béatrice Dirat[1,2], Ghislaine Escourrou[3], Stéphanie Dauvillier[1], Ludivine Bochet[1,2], Philippe Valet[2], **Catherine Muller[1]**
[1]*Microenvironment, Cancer and Adipocytes (MICA), IPBS-CNRS UMR 5089, Toulouse, France,* [2]*AdipOlab, INSERM U858- Team 3, I2MR, Toulouse, France,* [3]*Laboratoire d'Anatomie Pathologie et Histologie, Centre Hospitalier Universitaire Rangueil, Toulouse, France*

Most of the studies on epithelial-stroma interactions during breast cancer cell invasion have focused on fibroblasts, endothelial and inflammatory cells. Very little attention has been given to adipocytes, although it is obvious that in numerous organs including breast, early local tumour invasion results in immediate proximity of cancer cells to adipocytes. Until recently, adipocytes were considered as an energy storage depot, but there is now clear evidence that their ability to secrete many adipokines could potentially influence tumour behaviour. Using an original 2D co-culture system where adipocytes and tumour cell are separated by an insert, we show a crosstalk between the two cell types. Tumour co-cultivated during 3 to 5 days with adipocytes exhibit an increase in both migratory and invasive capacities and incomplete EMT. This pro-invasive effect was not recapitulated with "naïve" adipocyte-conditioned medium (Ad-CM), but was recapitulated when tumour cells were grown in the presence of Ad-CM obtained from adipocytes previously grown in the presence of cancer cells. In fact, adipocytes cultivated with cancer cells exhibit profound changes with delipidation and decreased of adipocyte markers associated to a concomitant expression of an activated phenotype marked by overexpression of proteases (including MMP-11) and pro-inflammatory cytokines (IL-6, Il-1β). Furthermore our results show that these two cytokines are involved in the observed pro-invasive effect. More importantly, these results have been confirmed in human breast tumours using both immunohistochemistry and qPCR (comparison of adipocytes isolated from tumorectomy or mammoplasty in a series of 28 patients). In conclusion, our data demonstrate for the first time that tumour-surrounding adipocytes cooperate with breast tumour cells to provide an invasive phenotype. These results might explain the poor prognosis of breast cancer in obese women that frequently exhibit extended tumour at diagnosis suggesting an effect of adipose tissue on early step of tumour invasion

O39

Paracrine Signaling by PDGF-CC Promotes Tumor Growth by Recruitment of Cancer-associated Fibroblasts Secreting Osteopontin

Charlotte Anderberg[1], Hong Li[1], Linda Fredriksson[2], Johanna Andrae[1], Christer Betsholtz[1], Xuri Li[3], Ulf Eriksson[1], Kristian Pietras[1]
[1]*Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden,* [2]*Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA,* [3]*Unit of Vascular Retinal Neurobiology Research, Porter Neuroscience Research Center, Bethesda, MD, USA*

Immunohistochemical staining for PDGF-CC has revealed prominent expression by tumor cells in different human skin cancers, including melanoma, but not in normal skin. To investigate the significance of PDGF-CC expression, we transfected B16 melanoma cells with PDGFC. The growth rate of B16 cells expressing PDGFC (B16PDGFC) was unaffected *in vitro*. However, tumors from B16PDGFC cells grew significantly faster compared to control tumors (B16ctrl). By injecting B16 tumors into PDGFRα/GFP reporter mice, we detected a thicker fibrous capsule surrounding B16PDGFC tumors and an increased number of infiltrating PDGFRα-expressing stromal cells. Stromal cells were analysed using coimmunostaining for PDGFRα and the fibroblast markers FSP-1 and α-SMA. Cells positively labeled for FSP1 were prevalent throughout the B16PDGFC tumors, while PDGFRα expression was restricted to cells at the edge of tumors. We demonstrated, by the use of antibody arrays, that B16PDGFC tumors contained increased levels of the extracellular matrix protein SPP1 (osteopontin), which was found to be expressed by fibroblasts. To investigate the effect of SPP1 *in vivo*, we coinjected B16 cells with mouse embryonic fibroblasts (MEFs) from wild type (wtMEF) or SPP1 knockout (KOMEF) mice. B16/wtMEF tumors exhibited a significant growth advantage compared to injection of B16 cells alone. In contrast, KOMEFs were not able to confer any growth advantage to B16 tumors. We conclude that expression of PDGFC in B16 melanoma cells results in increased tumor growth rate mediated by attraction of a PDGFRα-expressing population of cancer-associated fibroblasts, which secrete growth-promoting and proangiogenic factors such as SPP1. The results from the present study encourage therapeutic targeting of support functions performed by cell types populating the tumor stroma; a therapeutic strategy that may prove complementary to conventional treatments.

O40

Interplay between Stroma Chemokines and Endothelin-1 in Breast Cancer Cell Migration and Monocyte Recruitment

Muthulekha Swamydas[1], Adam Secrest[1], Ashley N. Jewell[1], Jill M. Hudak[1], **Didier Dréau[1]**
[1]*Department of Biology, UNC - Charlotte, Charlotte, NC, USA*

Stroma facilitates breast tumor cell migration, a key step in metastases by modulating the microenvironment. The different molecules including chemokines, cytokines and enzymes produced by stroma cells that remodel the extracellular environment of breast tumor have yet to be fully elucidated. Endothelin-1 has been shown to promote tumor growth, tumor inflammation and the development of metastases. Here, we present data demonstrating the role of chemical environment produced by stroma cells on tumor cell migration. In particular we show the indirect role of endothelin-1 in the recruitment of monocytes. 3D cultures using mammary epithelial cells (NMuMG) in combination with pre-adipocytes (D1) were grown in various extracellular matrix conditions. Following 5-day incubation, the number and area of structures were quantified. When co-cultured with D1 pre-adipocytes, D1 cells surrounded NMuMG epithelial cells and formed acinar structures with lumen formation. Both the number and area of acinar structures in cultures grown in Matrigel® and collagen in combination with agarose were higher than those observed in cultures grown in either agarose, Matrigel® or collagen alone ($p<0.05$). In 3D conditions, while NMuMG cells migrated towards conditioned media (CM) derived from NMuMG and D1 cells, 4 T1 cells migrated towards CM derived from MOVAS and NMuMG. In 2D conditions, D1 CM increased migration of NMuMG cells but not 4 T1 cells. Furthermore, 4T1CM and CM from 4 T1 cells stimulated with ET-1 but not ET-1 alone or CM from 4 T1 cells treated with an inhibitor of the endothelin converting enzymes inhibition promoted J774 monocyte chemotaxis and cell invasion ($p<0.05$). These results further underline the key role of the interplay of stroma and tumor cells secretion within the tumor microenvironment in the development of breast cancer metastases.

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O41

Autocrine Fibronectin is Essential for Matrix Assembly, Integrin Usage and Adherens Junction Formation in Endothelial Cells

Botond Cseh[1], Samantha Fernandez-Sauze[1], Dominique Grall[1], **Ellen Van Obberghen-Schilling[1]**
[1]*Centre A. Lacassagne, Institute of Developmental Biology and Cancer, CNRS UMR6543, Nice, France*

The importance of the extracellular matrix (ECM) in tumor development, progression and invasive behavior is becoming increasingly clear. Not only does the ECM provide an adhesive substrate for malignant and non-malignant cells, it initiates a host of cellular responses by activating surface receptors of the integrin family. Alternatively spliced fibronectin (FN) variants containing extra FN type 3 repeats, referred to as cellular or "oncofetal" FN, are major constituents of the extracellular matrix surrounding angiogenic blood vessels and carcinoma-activated fibroblasts. Whereas cellular FN is virtually absent from normal adult tissue, a massive upregulation is observed in highly angiogenic and invasive tumors, suggesting that cellular FN and signaling components that control its expression, assembly and rigidification may represent key targets for anti-tumoral therapies. We have examined the role and functional redundancy of cellular FN variants ED-B and ED-A (Extra Domains-B and -A) in vascular endothelial cells by isoform-selective RNA interference. FN-depleted cells fail to assemble a subendothelial matrix indicating that FN fibrillogenesis is a cell autonomous process in endothelial cells in which basal secretion of FN is tightly coupled to integrin-dependent assembly. Isoform-specific FN knock down alters integrin usage and impacts on downstream signaling events that regulate cytoskeletal organization, motility, cell-cell adhesion and capillary morphogenesis on a basement membrane matrix. In cells lacking FN, the integrin beta subunit partner ILK (Integrin-linked Kinase) shifts from alpha5beta1 fibrillar adhesions to alphavbeta3 adhesive structures. This integrin switch is accompanied by the disruption of adherens junctions and abrogation of loss of monolayer integrity. Altogether, these results highlight the importance of autocrine FN for angiogenic blood vessel remodeling and allow us to propose that cellular FN expression provides a spatially and temporally restricted control of vascular network formation and stability.

O42

Tumor-Derived, Low-Level TNFa Expression Augments the Formation of Tumor-Promoting Myeloid Subtype of Vascular Leukocytes through the Upregulation of Integrin a_5 and Enhanced Binding to Fibronectin

Bin Li[1], Alicia Vincent[1], **Pampee Young[1]**
[1]*Pathology and Medicine, Vanderbilt University Medical Center, Nashville, TN, USA*

Tumor associated myeloid cells are believed to promote tumor development by stimulating tumor growth, angiogenesis, invasion and metastasis. These tumor-associated myeloid cells are believed to be a heterogeneous population. There is growing data from multiple laboratories that tumor associated myeloid cells that co-express endothelial and myeloid markers represent a pro-angiogenic subtype of tumor associated myeloid cell known as vascular leukocytes. Recently, we demonstrated that tumor-derived TNFa promotes local tumor growth and vascularity, in part by increasing numbers of tumor-associated vascular leukocytes (*Can Res.* 2009; 69:338). We wished to explore the mechanism by which TNFa mediates endothelial differentiation of myeloid cells. Published studies have shown that fibronectin is a critical promoter of endothelial differentiation of blood mononuclear cells *in vitro*. We have found that TNFa treatment of monocytes significantly increased expres-

sion of a_5b_1 integrin, a major fibronectin receptor, leading to a consequent 4-fold increase in fibronectin adhesion. Furthermore, TNFa-treated monocytes upregulated expression of endothelial markers, VEGFR2 and VE-cadherin. Interestingly, a_5 subunit inhibitory antibodies blocked adhesion to fibronectin as well as blocked the consequent upregulation of VEGFR2 and VE-cadherin, implying a role for outside-in signaling by the a_5b_1 integrin after binding fibronectin. Finally, treatment of mouse tumors with anti-a_5 antibodies reduced accumulation of tumor vascular leukocytes and inhibited tumor growth. Our studies suggest that tumor-cell derived TNFa constitutes a tumor microenvironment signal that promotes differentiation of tumor-associated monocytes towards a proangiogenic/ provasculogenic myeloid-endothelial phenotype via upregulation of the fibronectin receptor a_5b_1.

O43

Overcoming Obstacles to Cancer Immunity at the T Cell - Tumor Microvascular Checkpoint

Sharon Evans[1], Daniel Fisher[1], Qing Chen[1], Jason Muhitch[1], Joseph Skitzki[1]
[1]*Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY, USA*

Trafficking of tumor-reactive T lymphocytes across microvascular barriers in tumor tissues is a critical juncture in the effector phase of T cell-mediated cancer immunity. While the multistep adhesion events directing lymphocyte trafficking to lymphoid organs and sites of inflammation are well defined, the mechanisms governing entry of blood-borne T cells into tumor tissues are largely unexplored. Here we demonstrate that steady-state homing of tumor-specific CD8 T cells across tumor vessels is limited by insufficient intravascular expression of the prototypical trafficking molecule, intercellular adhesion molecule-1 (ICAM-1). However, T cell trafficking to tumor sites could be substantially improved during systemic thermal therapy via a trans-signaling mechanism in which interleukin-6 (IL-6), together with a soluble form of the IL-6 receptor binding subunit, triggers ICAM-1 induction on tumor vessels. ICAM-1–dependent early entry of tumor-specific CD8 effector T cells is further shown to be causally linked to apoptosis of tumor cell targets. These findings indicate that therapeutic targeting of the tumor vasculature for T cell trafficking holds promise for improving cancer immunity and T cell-based tumor immunotherapy. This work is supported by grants from the NIH (R01 CA79765 and P01 CA094045), and the Roswell Park Alliance Foundation.

O44

Depletion of Treg Cells Enhances Inhibition of Tumour Growth by Cyclophosphamide Derivatives and IL-12-producing Cellular Vaccines

Jan Bubenik[1], Marie Indrova[1], Jana Simova[1], Milan Reinis[1]
[1]*Tumour Immunology, Institute of Molecular Genetics AS CR, Prague, Czech Republic*

Genetically modified cellular vaccines were found to be efficient against cancer both in experimental models (Bubenik, Curr. Cancer Drug Targets 8: 180–186, 2008) and in tumour-bearing patients (Russel et al., J.Immunother. 31: 812–819, 2008). It has been shown in various systems that the efficacy of conventional therapeutic modalities can be increased by their combination with relevant immunostimulatory vaccines as well as by depletion of immunosuppressive immunocytes (Zitvogel et al., Nature Rev. Immunology, 8: 59–73, 2008). The aim of this communication is to demonstrate that depletion of immunoregulatory immunocytes (T reg cells and immature myeloid cells) can enhance the efficacy of genetically (IL-12) modified cellular vaccines administered either alone or in combination with low doses of the cyclophosphamide derivative CBM-4A in the experimental model of HPV 16-induced murine tumours mimicking human HPV 16-associated neoplasms such as cervical carcinomas. The conclusion of this communication is that IL-12-producing cellular vaccines are good as adjuvant for CBM-4A treatment, since they can enhance the curative effect of the cyclophosphamide derivative and repair the CBM-4A produced defects in the immunocyte cytotoxicity and proliferative responses.

O45

Lymph Node Mimicry by Tumors Induces Immunological Tolerance

Jacqueline Shields[1], Iraklis Kourtis[1], Alice Tomei[1], **Melody Swartz[1]**
[1]*Bioengineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

Tumor manipulation of the host immune response is critical for invasion and metastasis. Here we introduce a mechanism by which tumors escape immune recognition by mimicking the natural tolerance-maintaining functions of the lymph node. We recently showed that some invasive human tumors secrete low levels of CCL21, which is known as a lymphoid chemokine because of its high expression in the lymph node and role in attracting antigen-presenting cells and naïve T cells to the node for T cell education. Here, we engineered three variants of the murine B16 melanoma: CCL21 knockdown, CCL21 overexpressing, and control-transfected. We found that control tumors – and CCL21-overexpressing but not knockdown variants – attracted lymphoid tissue inducers and developed lymphoid-like features including a reticular stromal network, complement-regulating protein Crry, and HEV-like vessels. Within this quasi-lymphoid environment, both the cytokine milieu and T cell populations were polarized towards a regulatory phenotype, while tumors lacking CCL21 induced tumor antigen-specific immunity. The CCL21 mediated immune tolerization was complement-dependent and systemic, with the presence of a control tumor protecting a distant CCL21-knockdown tumor from immune recognition. We suggest that "lymph node mimicry" gives tumors an advantage: by attracting naïve T cells and guiding their education in the immunosuppressive tumor environment, CCL21-secreting tumors can shift the host immune response from immunogenic to tolerogenic, facilitating growth and invasion.

O46

Role of P50 NF-kappaB in Dendritic Cell Functions

Paola Larghi[1], Chiara Porta[1,2], Monica Rimoldi[1], Alberto Mantovani[1,3], Antonio Sica[1,2]
[1]*Istituto Clinico Humanitas, Rozzano, Italy,* [2]*DISCAFF, University of Piemonte Orientale A. Avogadro, Novara, Italy,* [3]*University of Milan, Milano, Italy*

Tumor growth is supported by tumor stroma, which is made by matrix and infiltrating cells, such as tumor associated macrophages (TAM) and tumor associated dendritic cells (TADC). We have recently reported that TAM display massive nuclear localization of the p50 NF-kB inhibitory homodimer, which correlates with impaired inflammatory functions. The functional significance of this observation was demonstrated in p50 NF-kB deficient mice, which displayed tumor growth inhibition. More recently, in order to evaluate whether this tolerogenic mechanisms may target other compartments of the immune system, we characterized the role of p50 NF-kB in dendritic cell (DC) functions, during their differentiation and maturation.
Our data clearly show that p50 NF-kB plays a non redundant role in DC survival and APC functions. p50 NF-kB has pro-apoptotic functions in bone marrow derived DC, as its absence leads to a reduced rate of apoptosis/necrosis in DC activated for 48 h with LPS. Moreover, LPS-matured p50 -/- DC display higher expression of MHC molecules, as well as higher secretion of pro-inflammatory cytokines such as IL-1b, TNF-a and IL-18. This correlates with the enhanced capability of p50-/- DC to activate T cell responses, in vitro and in vivo.
Therefore, our data suggest that targeting p50 NF-kB activity may represent a strategy to enhance selective functions of DC, with potential application in anti-tumour vaccination strategies.

O47

JAM-B and JAM-C: Ying and Yang of Metastasis and Anti-Tumor Immune Response

Marie-Laure Arcangeli[1], Vincent Frontera[1], Florence Bardin[1], Elodie Obrados[1], Ralph H. Adams[2], Michel Aurrand-Lions[1]
[1]*Université de la Mediterrannée, Institut Paoli-Calmettes, CRCM INSERM U891, Marseille, France,* [2]*Department Tissue Morphogenesis, Max-Planck-Institute for Molecular Biomedicine, munster, Germany*

The adhesion molecules JamB and JamC belong to the Ig superfamily and have been shown to interact together. Through its expression on endothelial cells, JamC has been involved in the regulation of immune response, tumor growth and inflammation as demonstrated by several studies using blocking antibodies and transgenic mice[1 2 3]. Recently, high expression of JamC on fibrosarcoma has been correlated with increased metastatic potential of tumor cells. Whether this result simply reflects the adhesive property of JamC with JamB on endothelial cells or is due to a more complex regulation of inflammation and anti-tumor immune response remains to be established. Using B16F10 melanoma cells, which express JamC but not JamB, we show that silencing JamC in tumor cells inhibits proliferation, but that subcutaneous growth of B16F10 tumor is not affected in $JamB^{-/-}$ mice suggesting that JamC controls cell proliferation independently of JamB engagement. In contrast, pulmonary metastasis are greatly reduced in $JamB^{-/-}$ mice, indicating that JamC expressed on tumor cells interacts with JamB expressed on endothelial cells. However, we cannot exclude that the lack of JamB expression also favors a better control of metastasis by the immune system since our results show that metastasis of B16F10 expressing ovalbumin are totally cured by cytolytic T cells directed against ovalbumin without the need of priming. Ongoing experiments aim to define whether JamB and/or JamC are involved in cytolytic T cell recruitment and activation at metastatic sites. This will help to decipher if preventing metastasis with anti-JamC treatment will be counter-balanced by adverse effects on the immune system.

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O48

Epstein Barr Virus Infection in Hodgkin's Lymphoma: A Mechanism Facilitating Induced Regulatory T Cells Recruitment

Violaine Francois[1], Olivier Morales[1], Céline Miroux[1], Stéphane Depil[1], Anne-Valérie Decouvelaere[2], Pauline Lionne-Huyghe[3], Hervé Groux[4], Claude Auriault[4], Yvan De Launoit[1], Véronique Pancre[1], **Nadira Delhem[1]**
[1]*CNRS, UMR 8161, Institut de Biologie de Lille, Lille, France,* [2]*Service d'Anatomo-Pathologie, Pôle Biologie Pathologie, Eura-santé, Lille, France,* [3]*Service des Maladies du sang, CHRU, Lille, France,* [4]*UMR 6097, IPMC, Nice, France*

Purpose:
$CD4^+$ helper and regulatory T cells play important but opposing roles in regulating host immune responses against Hodgkin's Lymphoma (HL). In 20–40% of patients with HL, Epstein Barr Virus (EBV) is present in the neoplastic cells, however very little is known about regulatory mechanisms induced in presence of EBV. Here, we described associations of regulatory T cells (Treg) with EBV-positive and EBV-negative Hodgkin's lymphoma.
Methods:
In a retrospective, population-based study, patients with Hodgkin's lymphoma were reclassified according to the WHO classification, and EBV status was assessed by *in-situ* hybridisation of EBV-encoded small RNAs. Using quantitative real time PCR, we first analyzed gene expression of chemokines, immunosuppressive cytokines and regulatory T cells markers on RNA isolated from nodes of 20 EBV-positive HL patients and from 20 EBV-negative HL patients. We also investigated presence of regulatory T cell markers in PBMCs and sequential tonsil biopsies of HL patients.
Results:
We described in nodes of EBV-positive HL patients, a significant increase of gene expression for the major immunosuppressive cytokine: IL-10 which was correlated with an increased gene

expression of several markers of regulatory T cells (CD4+CD25+, Fox P3,CTLA4, GITR). This increase was confirmed by immunohistochemical on frozen nodes biopsies and by flow cytometry on PBMCs of HL patients. Moreover, we also described an overexpression of CCL17 and CCL22 which attract Th2 and regulatory T cells and may evade immune surveillance by Th1 cells.

Conclusion:

This study suggests direct evidence of regulatory T cells particularly in EBV positive Hodgkin's Lymphoma and a pivotal role of these cells in controlling the immune response in the context of viral infection. These results will provide fundamental insights into the mechanisms of tumor immune surveillance and escape, and yield novel approaches to therapy of cancer.

O49

CT-011, a Humanized Monoclonal Antibody, Interacts with the PD-1 Receptor and Modulates Survival and Trafficking Signals in Effector/memory T Lymphocytes

Rinat Rotem-Yehudar[1], Galina Rodionov[1], Shimon Landes[1]
[1]*CureTech Ltd., Yavne, Israel*

Introduction:

PD-1 (Program Death-1), an immune inhibitory receptor and its ligands PD-L1 and PD-L2, participate in peripheral tolerance and play key role in immune suppression and evasion mechanisms in a variety of human malignancies. PD-1 inhibits activation signals and functions as a pro-apoptotic receptor in effector lymphocytes. CT-011 is a humanized monoclonal antibody that interacts with PD-1 and modulates the immune response eliciting effective activities of T and NK cells against experimental targets in cultures and in animal tumor models. CT-011 completed a Phase I single dose, dose escalation clinical study in patients with advanced stage hematological malignancies demonstrating acceptable safety and tolerability at all tested dosage levels and clinical beneficial responses in 33% of the patients including 1 pt with CR, 4 pts with DS and 1 pt with MR.

Results:

Here we demonstrate that CT-011 binds a conserved epitope on the PD-1 receptor and blocks its function. CT-011 (1ug/ml) inhibits spontaneous or FAS-mediated cell death processes and enhances the survival of human antigen- challenged effector/memory CD4+CD45RO +lymphocytes via the PI3K pathway. Consistent with its enhancing effect on lymphocyte survival, the antibody increases the intracellular levels of BclXL, a survival protein and reduces the levels of activated caspase 8 in CD4+CD45RO+ but not in CD4+CD45RO- suggesting that it modulates two apparently separated apoptotic pathways in specific subsets of T lymphocytes. Furthermore, antigen- challenged CD4+CD45RO+lymphocytes incubated in the presence of CT-011 (1ug/ml) have shown increased trafficking in SDF-1 gradient in a chemotaxis test, noted even at high concentration levels of SDF-1 (500 ng/ml).

Conclusions:

CT-011 binds a unique conserved epitope on the PD-1 receptor and blocks its activity. This specific interaction results in intracellular signaling affecting the survival and trafficking properties of antigen-challenged effector/memory CD4+CD45RO+ lymphocytes. The function of PD-1 and PD-L1 has been demonstrated to be one of the leading causes of immune suppression in cancer patients. Accordingly, CT-011 is being studied in several malignancies, including, Phase II clinical studies in diffuse large B cell lymphoma and metastatic colorectal cancer.

O50

Tumor Cell Plasticity under Immune Micro-Environment Pressure

Joelle Riond[1], Bent Rubin[1], Stéphane Rodriguez[1], Laetitia Courtiade[1], Nicolas Roullet[1], William Riquet[1], **Jean Edouard Gairin[1]**
[1]*UMR2587 CNRS - Pierre Fabre, Toulouse, France*

The role of immune cellular and molecular effectors in the detection and elimination of tumor cells is clearly established. However, the high incidence of cancer in humans shows the inefficacy of the immune system to control this process. Indeed, the immune system not only stimulates neoplasia by triggering inflammation, but also seems to participate to the escape or resistance of tumor cells to innate and / or adaptive immunity. Melanoma, refractory to most chemotherapies and immunotherapeutic strategies, represents a clinical and experimental model of choice to develop innovative approaches integrating both chemo and immuno-therapeutic knowledges. One mechanism used by tumor cells to escape to immune recognition is down-regulation of the antigen-presenting machinery. Many tumor cells have low or absent expression of major histocompatibility complex class I (MHC-I) molecules. Exploring the role of the immune system in the modulation of tumor cells phenotype, we discovered that MHC-I^{low} tumor cells re-expressed MHC-I molecules in presence of syngeneic spleen cells (NSC). Cell-cell contact between tumor cells and NSC was necessary and resulted in IFNg production and a consequent increased MHC-I expression. The effector cells responsible for the increased IFN-g production were identified as CD4$^+$ CD1d-independent NKT, NK1.1$^+$ NK cells and CD4$^+$ CD11c$^+$DCs. We used a model of murine melanoma graft (B16F10) and showed that MHC-I induction occurs also *in vivo* and coincides with recruitment of lymphoid cells. gdT cells and NK cells contributed to the induction of the expression of MHC-I molecules on B16F10 tumor cells. Our results show the plasticity of a tumor cell under the influence of immune microenvironment. Deciphering the role of early interactions between tumor and immune cells in term of tumor phenotype modification may allow innovative pharmacological strategies to interfere with this regulation.

O51

Macrophages, IL-15, and Follicular Lymphoma: Towards a Better Understanding of the Interface Between Tumor B Cells and their Microenvironment

Guerric Epron[1], Thierry Fest[1], Thierry Lamy[1], Patricia Ame-Thomas[1], Karin Tarte[1]
[1]*INSERM U917, Rennes, France*

Follicular lymphoma (FL), the most common indolent B-cell lymphoma, involves an initial t(14;18) translocation leading to Bcl-2 anti-apoptotic protein overexpression. Additional genetic events could lead to its transformation into an aggressive lymphoma. However, clinical behavior in FL is essentially determined by the gene expression profile of the microenvironment rather than by inherent properties of the tumor cells themselves. In agreement, an increased number of macrophages is associated with a poor prognosis in FL whereas they support the growth of DLBCL cells *in vitro*. We thus decided to unravel the role of macrophages in FL with a specific focus on interleukin-15 (IL-15), a cytokine mainly expressed by macrophages, dendritic cells, and stromal cells that is trans-presented on cell membranes associated with IL-15RA high-affinity receptor, and is a well-described T and NK cell growth factor. We demonstrated that whereas exogenous IL-15 promoted the survival of unpurified normal B cells, resting purified B cells could not respond to this cytokine. Nonetheless, after CD40-triggering or coculture with autologous T cells, normal and FL-derived B cells became responsive to IL-15 that enhanced their proliferation, in association with a phosphorylation of STAT5. Normal and FL B-cell growth was also increased when cocultured with monocytes and this feeder effect was reinforced by IL-15. Furthermore, targeting IL15 and IL15RA in monocytes by siRNA decreased monocyte-mediated B-cell growth. Specific depletion of $CD14^{pos}$ cells among tonsil cells decreased normal B-cell growth in presence or not of IL-15, confirming the essential role played by myeloid cells in this context. Finally, confocal microscopy revealed the presence of IL-15RA at the cell interface between monocytes and B cells. Collectively, these data depict for the first time IL-15 as a B-cell growth factor within normal and FL B-cell niches and describe a potent new therapeutic target.

O52

Anti-Tumor Treatment of Tumor-Bearing Immunocompetent Mice with Anti-CD20 mAb Induces an Adaptive Immune Response that can be Strengthened by IL-2 Infusion

Riad Abes[1,2], Emmanuelle Gelize[2], Jean-Luc Teillaud[2]
[1]*Laboratoire Français du Fractionnement et des Biotechnologies (LFB), Paris, France,* [2]*Team 14 Antibody Technology, INSERM U872 / Cordeliers Research Center; Pierre & Marie Curie University, UMR S 872; Paris Descartes University, UMR S 872, Paris, France*

The long-lasting responses observed in some lymphoma patients treated with rituximab suggests that this antibody induces an anti-tumor immune response. We have investigated whether anti-CD20 treatment of $CD20^{+}$ tumor bearing mice can trigger a adaptive immune response and whether it is possible to potentiate it by subsequent IL-2 infusion. C57Bl/6 mice were i.v. injected with EL4 tumor cells expressing human CD20 and treated with i.p. injections of the anti-CD20 mouse mAb CAT-13. Whereas all untreated animals died before Day 35, about 60–70% of CAT-13-treated mice survived. The surviving mice were then challenged at Day 70 by a new i.v. injection of either EL4-huCD20 or EL4 cells without any mAb treatment. All EL4-challenged-mice died before Day 26, while about 50–60% of EL4-huCD20-challenged mice were still alive at Day 70. Furthermore, a single i.v. injection of spleen cells isolated from these surviving animals into naive recipients injected with EL4-huCD20 cells 24 h later was sufficient to protect the latter animals.These data suggest that anti-CD20 mAb treatment induces a long-lasting adaptive immune response. Since IL-2 can exert an anti-tumor effect through the activation of T cells in some cancers, we injected IL-2 to the surviving CAT-13-treated mice just after having challenged them with EL4-huCD20 cells. After 70 days, an increase of the survival rate of IL-2 infused animals was observed as compared to animals challenged with EL4-huCD20 cells only. Thus, IL-2 injection at distance from mAb treatment may strengthen the immune response against EL4-huCD20 tumor cells induced by this treatment. In conclusion, our work shows that an anti-CD20 mAb treatment can induce a long-lasting adaptive immune response that can be manipulated with IL-2.

O53

Hypoxia-Regulated MicroRNAs, New Players in Tumorigenesis

Mircea Ivan[1], Meredith Crosby[2], Cecilia Devlin[1], Peter Glazer[2], Adrian Harris[3], Robert McCormick[3]
[1]*Medicine, Indiana University, Indianapolis, Indiana, USA,* [2]*Therapeutic Radiology, Yale University, New Haven, CT, USA,* [3]*Weatherall Institute of Molecular Medicine, Oxford University, Oxford, UK*

Adaptation to decreased oxygen tension is critical for the tumorigenic process and involves a complex network of genes. Our recent studies revealed that the hypoxic response is not restricted to expressed genes. Several microRNAs, including miR-210 and miR-373, represent direct targets of HIF and preliminary data indicate that they play important roles in the response to extended hypoxic stress. miR-210 is upregulated in a variety of solid tumors, it is positively correlated with a hypoxia signature in vivo, and confers a negative prognosis in breast cancer. Therefore this miR may represent a key component for cancer cell adaptation to the tumor microenvironment.
Clonogenic assays in a variety of cancer cell backgrounds demonstrate that miR-210 supports cell survival and proliferation during hypoxic stress and we are studying critical target genes that contribute to this effect. The impact of miR-210 manipulation on hypoxic expression profiles reveals for the first time pathways that are regulated via miR-dependent mechanisms and of relevance for tumor biology, such as mitochondrial ROS generation (the *iron-sulfur cluster scaffold homolog* ISCU). Additionally, miR-210 and 373 directly target DNA repair genes such as Rad52 and Rad23b, potentially contributing to the well-established correlation between hypoxia and DNA damage. We developed models for addressing the role of miR-210 in tumorigenesis, using stable miR-overexpressing breast cancer cells xenografts, and by performing in vivo miR inactivation using locked nucleic acids probes (LNAs). These strategies are aimed to interfere with the ability of cells to survive and proliferate in a hypoxic microenviroment, and could provide the starting point for miR-based therapeutic developments.

O54

Role of Lactate as a Fuel in a Unique Microenvironmentally Controlled Metabolic Symbiont

Pierre Sonveaux[1,2], Frédérique Végran[1], Thies Schroeder[2], Olivier Feron[1], Mark W. Dewhirst[2]
[1]*Unit of Pharmacology & Therapeutics, University of Louvain (UCL) Medical School, Brussels, Belgium,* [2]*Department of Radiation Oncology, Duke University Medical Center, Durham, NC, USA*

The glycolytic activity of hypoxic cells creates a gradient of lactate that mirrors the gradient of oxygen in tumors. In human tumors, high levels of lactate predict the likelihood of tumor recurrence, metastasis, and poor survival. We recently addressed the intrinsic contribution of the lactate anion to tumor growth and report that lactate is key for a metabolic symbiosis in tumors. The symbiosis involves the recycling of lactate, released by glycolytic tumor cells, as an oxidative fuel for oxygenated tumor cells. The preferential use of lactate over glucose to fuel tumor cell respiration renders glucose available to fuel the glycolytic metabolism of hypoxic tumor cells. We further identified monocarboxylate transporter 1 (MCT1), selectively expressed at the plasma membrane of oxygenated tumor cells, as the prominent path for lactate uptake. We successfully disrupted the metabolic symbiosis by inhibiting MCT1 with a specific siRNA or with the selective inhibitor α-cyano-4- hydroxycinnamate (CHC), causing a switch from lactate-fueled respiration to glycolysis in oxygenated tumor cells. As a consequence, CHC delivery to tumor-bearing mice causes hypoxic/glycolytic tumor cell death by virtue of glucose starvation and the remaining oxygenated tumor cells may be targeted by radiotherapy. Validation of this new therapeutic strategy using three different tumor models and MCT1 expression in an array of primary human tumors provide clinical significance to anticancer MCT1 inhibition.
Reference: Sonveaux P. *et al.* Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J. Clin. Invest. 2008;118:3930–42.

O55

Hypoxia Tolerance and Breast Cancer Metastasis

Elizabeth Louie[1], Juei-Sue Chen[1], Sara Nik[1], Jillian Cypser[1], **Emily Chen[1]**
[1]*Department of Pharmacological Sciences, Stony Brook University, Stony Brook, NY, USA*

The tumor microenvironment, particularly hypoxia, has been demonstrated to have tremendous impact on tumor progression and patient prognosis. In patients, hypoxic tumors tend to be more aggressive, resistant to radiation therapy, and therefore likely to recur locally or metastasize. Although the development of hypoxia tolerance in tumors seems to predict poor prognosis, mechanisms contributing to hypoxia tolerance remain to be elucidated. To study hypoxia tolerance in breast cancer progression, we isolated sub-populations of breast cancer cells that survived under severe hypoxic conditions. Particularly, we identified a novel sub-population of breast cancer cells that exhibited more aggressive and invasive phenotypes after exposure to repetitive cycles of hypoxia and reoxygenation. We also observed that tumor cells isolated from 3D selection (grown as spheres) are more resistant to hypoxia stress than 2D selection (grown as monolayer). Therefore, the hypoxia culture microenvironment appears to be able to drive the selection of an aggressive subpopulation of breast cancer cells. In addition to increased aggressive phenotypes, we found that regulation of mTOR signaling is critical to the survival of the non-adherent breast cancer sub-population under hypoxia. This aggressive sub-population showed increasing sensitivity to rapamycin compared to the total breast cancer cell population. Furthermore, augmented Akt and mTOR signaling were found in the non-adherent breast cancer sub-population even when they are grown under normal growth condition. Such aggressive cancer cells are difficult to target by chemotherapy and are likely to repopulate the tumor after cytotoxic treatment. Therefore, we anticipate that improved anti-cancer treatment could be achieved if methods were identified to target this sub-population. Our ultimate goal is to understand the heterogencity of hypoxia responses in breast cancer sub-populations, and their role in breast tumor progression and metastasis. We will also examine collaborations of signaling pathways essential to confer hypoxia tolerance in sub-populations of breast cancer cells.

O56

Silencing Hypoxia Mediated Expression of Carbonic Anhydrase IX Induces Regression of Primary Breast Tumor Growth and Metastasis

Shoukat Dedhar[1], Paul McDonald[1], Yuan-Mei Lou[1], Arusha Oloumi[1], Stephen Chia[1]
[1]*Department of Cancer Genetics, BC Cancer Research Centre, Vancouver, BC, Canada*

Mortality from cancer is primarily due to the formation of distant metastases. However, the molecular properties of primary tumours that dictate metastatic potential are poorly understood. Here we show that spontaneously metastasizing breast tumors are distinguished by the expression of a group of hypoxia inducible genes that include carbonic anhydrases (CA) IX and XII and vascular endothelial growth factor C (VEGF-C). Primary tumors with high metastatic potential are distinguished by large areas of hypoxia and necrosis, higher numbers of apoptotic cells, high CAIX expression, and well formed intratumoral lymphatic vessels relative to non-metastatic tumors which are highly vascularized, and do not have intratumoral lymphatic vessels. The metastatic, but not the non-metastatic cells can induce CAIX and regulate extracellular acidification under hypoxia. Gene silencing of CAIX expression in the metastatic cells resulted in increased cell death in hypoxia *in vitro* and in dramatic regression of primary tumor growth *in vivo* and complete inhibition of formation of spontaneous metastases. Examination of CAIX expression in *3,630* primary human breast cancers with long term follow-up revealed CAIX to be an independent poor prognostic biomarker for distant metastases and for overall survival. Our findings strongly implicate hypoxic tumor microenvironments and lymphangiogenesis as drivers of metastatic potential. We have also identified CAIX as a targetable biomarker for breast cancer

metastatic potential, allowing for the identification and selection of patients for treatment with CAIX inhibitors.

O57

Specific Sulfonamide Inhibitors of CA IX are able to Image Hypoxia Response and Enhance the *in vivo* Therapeutic Effect of Conventional Cancer Treatments

Ludwig Dubois[1], Natasja G. Lieuwes[1], Anne Thiry[1,2], Jean-Michel Dogné[2], Claudiu T. Supuran[3], Bradly G. Wouters[1,4], Bernard Masereel[2], Philippe Lambin[1]
[1]*Maastricht Radiation Oncology (MaastRO) Lab, GROW - School for Oncology and Developmental Biology, University Maastricht, Maastricht, The Netherlands,* [2]*Department of Pharmacy, Drug Design and Discovery Center, FUNDP, University of Namur, Namur, Belgium,* [3]*Laboratory of Bioinorganic Chemistry, Università degli Studi di Firenze, Florence, Italy,* [4]*Ontario Cancer Institute/Princess Margaret Hospital, University Health Network, Toronto, ON, Canada*

Background and Purpose:
Hypoxia is an important micro-environmental parameter that influences tumor progression and treatment efficacy. The hypoxia target carbonic anhydrase IX (CA IX) is associated with poor prognosis and therapy resistance and is an important regulator of tumor pH. Several studies suggest it may be a potential imaging and therapeutic target. Recently, sulfonamide inhibitors (CAI) that bind and inhibit CA IX only during hypoxia have been developed. The aim of this study was to investigate the *in vivo* CAI binding properties using fluorescent imaging and the possible therapeutic gain of combining specific CAI with irradiation.
Material and Methods:
NMRI-*nu* mice were inoculated subcutaneously into the lateral flank with HT-29 colorectal carcinoma cells. Non-invasive imaging was performed at several time points after CAI#1 (fluorescein-thioureido-homosulfanilamide) injection with our without modifying the tumor oxygen concentration levels. Tumor growth and potential treatment toxicity was monitored after injection of CAI#2 (indanesulfonamide) combined with irradiation (single tumor dose 10 Gy).
Results:
In vivo fluorescence imaging revealed for the first time specific CAI#1 accumulation (P=0.008 compared with controls) in delineated tumor areas dependent on the oxygen concentration. Treatment of animals with CAI#2 alone resulted in a significant growth delay (P=0.024). Single irradiation treatment also demonstrated an increased specific doubling time evaluated at 4 times the starting tumor volume (P< 0.001). The specific doubling time was further increased by combining CAI#2 with irradiation (P=0.016). No significant toxicity was observed, neither for the single, neither for the combined treatment schedules.
Conclusions:
These *in vivo* results confirm previous data showing that *in vitro* CAI binding occurs only under hypoxia. Furthermore, CAI as a single treatment is able to significantly reduce tumor growth, which was further enhanced by combining with irradiation, promising for further clinical testing.

O58

Targeting Hypoxic Microenvironment in Acute Lymphocytic Leukemia (ALL)

Marina Konopleva[1,2], Olga Frolova[2], Sergej Konoplev[3], Juliana Benito[2], Yuexi Shi[2], Rodrigo Jacamo[2], Hongbo Lu[3], Carlos Bueso-Ramos[3], Deborah A. Thomas[1], Michael Andreeff[1,2]
[1]*Leukemia, M.D. Anderson Cancer Center, Houston, TX, USA,* [2]*Section of Molecular Hematology and Therapy, Department of Stem Cell Transplantation and Cellular Therapies, M.D. Anderson Cancer Center, Houston, TX, USA,* [3]*Hematopathology, M.D. Anderson Cancer Center, Houston, TX, USA*

The main therapeutic challenge in the treatment of acute lymphocytic leukemia is the development of strategies aimed at overcoming resistance to chemotherapy. While intensive chemotherapy nduce remissions in 90% patients, there has been little improvement in reducing the risk of leukemia relapse. Recent studies indicate that interactions between leukemia cells and bone marrow (BM) microenvironment promote leukemia cell survival and confer resistance to drugs commonly used to treat ALL. We have focused on the role of hypoxia as a natural physiologic component of BM microenvironment. Our data using the metabolic marker pimonidazole suggest that the hypoxic BM niche in leukemias is greatly expanded, contrary to the discrete, subendosteal or perivascular niches found in normal hematopoiesis. BM hypoxia promotes a switch to glycolytic metabolism and contributes to the resistance of leukemic cells in BM niches. These events are at least in part mediated via transcription factor HIF-1α. Expression of HIF-1α and its target gene CAIX was detected in 68% of primary ALL samples (n=53), while it was sparingly expressed in few hematopoietic cells in normal BM, and inversely associated with patients' survival (p=0.023). HIF-1α is induced under hypoxic conditions in co-cultures with bone marrow-derived stromal cells (MSC) through mTOR and MAPK pathways. Silencing of HIF-1α with siRNA, or blockade of mTOR signaling with rapamycin derivatives reduced expression of the glucose transporter Glut-1 and diminished glucose flux, decreased glycolytic rate and ATP production and sensitized leukemic cells to pro-apopotic effects of chemotherapeutic agents under hypoxic conditions. In further support of the role of hypoxia, utilization of the hypoxia-activated pro-drug (PR-104) resulted in cures of a proportion of NOD/Scid/IL2Rg-KO mice transplanted with primary human leukemia. Altogether, these findings strongly support a role for hypoxic BM microenvironment in the chemoresistance of ALL cells and provide a mechanism-based rationale for eliminating resistant ALL progenitor cells.

O59

Mitochondrial VDAC3 Splice Variant is Induced in Hypoxia and Protects from Apoptosis

Nathalie M. Mazure[1], Johanna Chiche[1], Matthieu Rouleau[3], Pierre Gounon[2], M. Christiane Brahimi[1], Jacques Pouysségur[1]
[1]*University of Nice CNRS-UMR 6543, Institute of Developmental Biology and Cancer Research, Nice, France,* [2] *University of Nice, Centre Commun de Microscopie Appliquée, Nice, France,* [3]*University of Nice - Faculty of Medicine, INSERM U898, Nice, France*

It is well-established that cells exposed to the limiting oxygen microenvironment (hypoxia) of tumors acquire resistance to chemotherapy, through mechanisms not fully understood. We noted that numerous cell lines showed protection from apoptotic stimuli, staurosporine or etoposide, when exposed to long-term hypoxia (72 hours). In addition, these cells had unusually enlarged mitochondria. Here we reveal that mitochondria of hypoxia-induced chemotherapy-resistant cells undergo a hypoxia-inducible factor-dependent and mitofusin 1-mediated change in morphology from a tubular network to an enlarged phenotype. An imbalance in mitochondrial fusion/fission occurs since silencing of the mitochondrial fusion protein mitofusin 1 reestablished a tubular morphology. Enlarged mitochondria conserved their transmembrane potential and ATP production, and contained an as yet undetected short isoform of the voltage-dependent anion channel VDAC3. Hypoxic cells were insensitive to staurosporine- and etoposide-induced cell death, but the silencing of VDAC3 restored sensitivity. Our results demonstrate that hypoxia, by inducing mitochondrial fusion, confers selective protection from apoptosis through expression of a short isoform of VDAC3 that allows maintenance of ATP and cell survival in hypoxia.

O60

Biomechanical Model of Stress-Dependent Formation of Tissue Organizing Structures (TOS) Associated with Solid Tumor Formation, Invasion and Metastasis

Sarah Crawford[1]
[1]*Cancer Biology Research Laboratory, Department of Biology, Southern Connecticut State University, New Haven, CT, USA*

Research studies on early stage solid tumor formation in our laboratory led to the identification of a novel class of cell derived vesicles released by cell budding or fission that play a critical role in this process, termed "tissue organizing structures" (TOS). These trypsin-resistant, membrane-delimited particles, approximately 2 micron diameter, are produced by diverse cell types, both normal and malignant, and contain genetic material. Documented activities include a critical role in orchestrating solid tumor formation *in vitro* and the induction of cell morphogenesis following fusion with neighboring cells. Proposed mechanisms of cell transformation include horizontal gene transfer and a novel mechanism termed "insertional membrane editing". Recent studies in this laboratory have focused on the biophysical components of the cell microenvironment that may contribute to the formation of these novel structures. This research extends previously elaborated biomechanical models of malignant transformation by implicating a specific biological/structural response with direct physiological consequences to biophysical forces initiated by tissue structure interactions. In this model, TOS formation is initiated by the stress-dependent restructuring of the cytoarchitecture initiated by the change in cell division parameters of individual cells that produce a tissue-encompassing regional integrated biomechanical response intrinsically linked to the formation of cell structures (TOS). The formation of TOS tubules and the migration of these entities drive the organization of the local cell population to generate a new architectural entity, the solid tumor. This is the first biomechanical/structural model of solid tumor formation, invasion and metastasis that integrates current biophysical theories of solid tumor formation with the formation of specific biological cell structures responsible for many of the genetic, physiological and biochemical parameters that characterize malignant transformation.

O61

EGFR Signaling Mediates Metabolism-Dependent Epigenetic Control in a Model of Human Breast Cancer. CPT1A is a Novel Partner of Histone Deacetylase 1 in Cell Death Escaping Mechanisms

Paola Mazzarelli[1], Sabina Pucci[1], Maria J. Zonetti[1], Luigi G. Spagnoli[1]
[1]*Department of Biopathology, University of Rome Tor Vergata, Rome, Italy*

The altered metabolism of tumor cells may be a potential means by which these cells evade programmed cell death, favouring survival and tumoral growth. In particular, lipid metabolism is markedly altered in the tumoral context. Neoplastic cells use endogenously synthesized fatty acids to satisfy their metabolic necessities and fatty acids synthase (FASN), the major enzyme required for the synthesis of fatty acids, is up-regulated in a wide array of solid tumors. Experiments of RNA interference-knockdown have confirmed its role as *metabolic oncogene*. ErbB2 receptor, amplified in 25% of breast cancers, has been recognized as activator of FASN promoter. Thus, Epidermal growth factor receptor (EGFR) family system, activated in tumor microenviroment, could influence FASN activity via Her2 activation.
We previously studied human breast carcinomas and breast cancer cell lines (SK-BR3, BT474, MCF-7) with or without Her2 gene amplification confirming that FASN was over-expressed in a high percent of cases and that FASN expression levels could be indicators of Her2 transduction activity (unpublished data). On the other hand, we found an inhibition of fatty-acids b-oxidation in the tumoral context. In particular carnitine palmitoyl transferase I (CPT I), the rate-limiting enzyme in the transport of long-chain fatty acids for b-oxidation, was significantly decreased in the mitochondria and it strikingly localized in the nuclei of tumoral samples, where it could be implicated in the epigenetic regulation of transcription by its link to HDAC1.
Here we report that the silencing of CPT1A nuclear expression by small interfering RNAs is a sufficient condition to induce apoptosis in MCF-7 breast cancer cells. The apoptosis triggered by RNA interference correlates with reduction of HDAC activity and hyper-acetylation of histone- and non histone-proteins, involved in cancer-relevant death pathways. Moreover, the CPT1A knockdown induces downstream effects on pro-apoptotic genes (up-regulation) and invasion and metastasis related genes (down-modulation), as shown by microarray analysis. In a breast cancer model, these results provide evidence of a mechanism linking the increased biosynthesis of fatty acids induced by Her2/Neu signaling to the down-regulation of mitochondrial CPT1A. This enzyme can shuttle into the nucleus regulating at epigenetic level pro-survival and cell-death escape genes.

O62

The GCN2-ATF4 Pathway is a Key Determinant of Tumor Cell Survival and Proliferation in Response to Amino Acid and Glucose Deprivation

Constantinos Koumenis[1], Jiangbin Ye[1], Monika Kumanova[1], Haiyan Zhang[1], Kelly Sloane[1]
[1]*Radiation Oncology, University of Pennsylvania, Philadelphia, PA, USA*

The basic leucine-zipper (bZip) transcription factor ATF4 has been shown to regulate the expression of mRNAs involved in amino acid metabolism, cellular redox homeostasis and anti-stress responses. It is translationally upregulated upon phosphorylation of the translation factor eIF2a by cytoplasmic kinase GCN2 under amino acid starvation and the endoplasmic reticulum (ER) kinase PERK under ER stress and hypoxia. ATF4 is overexpressed in clinical samples of human tumors and co-localizes with hypoxic regions, suggesting that it may play an important role in tumor progression. Here we report that knockdown of ATF4 in tumor cells results in significant inhibition of survival and proliferation, despite an initial activation of an autophagic response and that this inhibition was more pronounced under hypoxic stress. These effects are ameliorated by supplementation of tumor cells with non-essential amino acids (NEAA), but not with antioxidants. Asparagine, but not any other NEAA, is sufficient to recapitulate this rescue effect. Knockdown of ATF4 significantly reduces the levels of asparagine synthetase (ASNS) and overexpression of ASNS reverses the proliferation block and increases survival of ATF4 knockdown cells. Both amino acid and glucose deprivation activate the upstream eIF2a kinase GCN2 to upregulate ATF4 and target genes involved in amino acid transport and synthesis. Abrogation of ATF4 or GCN2 levels significantly inhibits transformed cell proliferation and tumor growth *in vivo*. Since the GCN2-eIF2a-ATF4 pathway is critical for maintaining amino acid homeostasis under different stresses, targeting this pathway represents a novel anti-tumor approach.

O63

Epigenetic Regulation of SPARC in Tumor Microenvironment Stromal Cells is Associated with Vascular Status of Early Stage Colon Cancer

Dave Hoon[1], Tetsunori Yoshimura[1]
[1]*Department of Molecular Oncology, John Wayne Cancer Institute, Santa Monica, CA, USA*

Stromal cells are integral components of the tumor microenvironment (TM) in early stage colon cancer progression. An important protein that is activated and secreted by both tumor and stromal cells during tumor progression is SPARC (secreted protein acidic and rich in cysteine). The relation of SPARC expressed by tumors and adjacent TM stromal cells is poorly understood. SPARC is secreted in the extracellular matrix of tumors and has many cellular regulatory functions including angiogenesis. The objective was to determine SPARC activity in TM stromal cells in relation to lymphovascular invasion(LVI) activity of the primary tumor. To assess SPARC role in the TM of primary colon cancer we examined patients whose tumors were histopathology grouped based on LVI. Immunohistochemistry (IHC) analysis with anti-SPARC of 82 primary colon tumors had no significant differences of SPARC regardless of LVI status. Examination of adjacent stromal cells in the TM SPARC expression levels varied considerably. In further analysis of LVI(-)($n=35$) and LVI(+)($n=37$) colon tumors, it was demonstrated in the former group TM stromal cells had significantly ($p<0.0001$) elevated SPARC. Epigenetic regulation of SPARC gene was then assessed in the stromal cells using microdissected archival paraffin-embedded tissues through assessment of SPARC gene CpG island region methylation status in the promoter region by MassARRAY quantitative sequencing. The analysis demonstrated concurrent activity of hypermethylation of specific CpG islands that were significantly ($p<0.0001$) correlated to LVI status and SPARC expression. The methylation sequencing analysis showed significant hypermethylation of specific CpG islands correlated to SPARC downregulation. Analysis of angiogenesis activity was carried out by assessment of stromal cells with anti-VEGF-A Ab. VEGF-A levels in the stromal cells were inversely correlated ($p=0.005$) with SPARC protein levels. The studies demonstrate SPARC activity of TM stromal is epigenetically regulated and significantly correlated with LVI activity of colon primary tumors.

O64

The New Identity of L1: from a Neural Adhesion Molecule to a Central Modulator of Tumor/Microenvironment Crosstalk?

Luigi Maddaluno[1], Chiara Martinoli[2], Maria Rescigno[2], **Ugo Cavallaro[1]**
[1]*IFOM, The FIRC Institute of Molecular Oncology, Milan, Italy,* [2]*Department of Experimental Oncology, European Institute of Oncology, Milan, Italy*

The immunoglobulin-like cell adhesion molecule L1 is a cell surface molecule that mediates various essential processes in the nervous system, as demonstrated by the broad spectrum of neurological defects in mice and humans carrying deletions or mutations in the L1 gene. L1 is also expressed in several non-neural cell types where, however, its function has remained elusive. In particular L1 is aberrantly expressed in various tumor types, and its expression often correlates with poor prognosis. We have focused on epithelial ovarian carcinoma (EOC), one of the most fatal malignancies in which many of the pathobiological mechanisms have not been elucidated yet. L1 exhibits a peculiar expression pattern in EOC lesions, and exerts a cell context-dependent role, with a clear pro-malignant function. Moreover, L1 appears as a hallmark of pathological vessels, as we found the molecule to be expressed in the vasculature associated to both neoplastic and inflammatory vessels, and to be induced in endothelial cells by inflammatory and angiogenic stimuli. Our data pointed to L1 also as a marker of certain hematopoietic cell lineages. The functional relevance of these observations was tested in a conditional knockout mouse model, which revealed the causal

role of L1 in the transendothelial migration of immune cells and in their trafficking in vivo, two processes strictly related to cancer progression. Hence, L1 is present in invasive tumor cells, in cancer-associated vasculature and in inflammatory cells, and in all these cell types its function is consistent with a pro-malignant role through the modulation of tumor-host interactions. These observations provide the rationale to explore L1 targeting as a strategy to interfere with the tumor-promoting action of some microenvironment components.

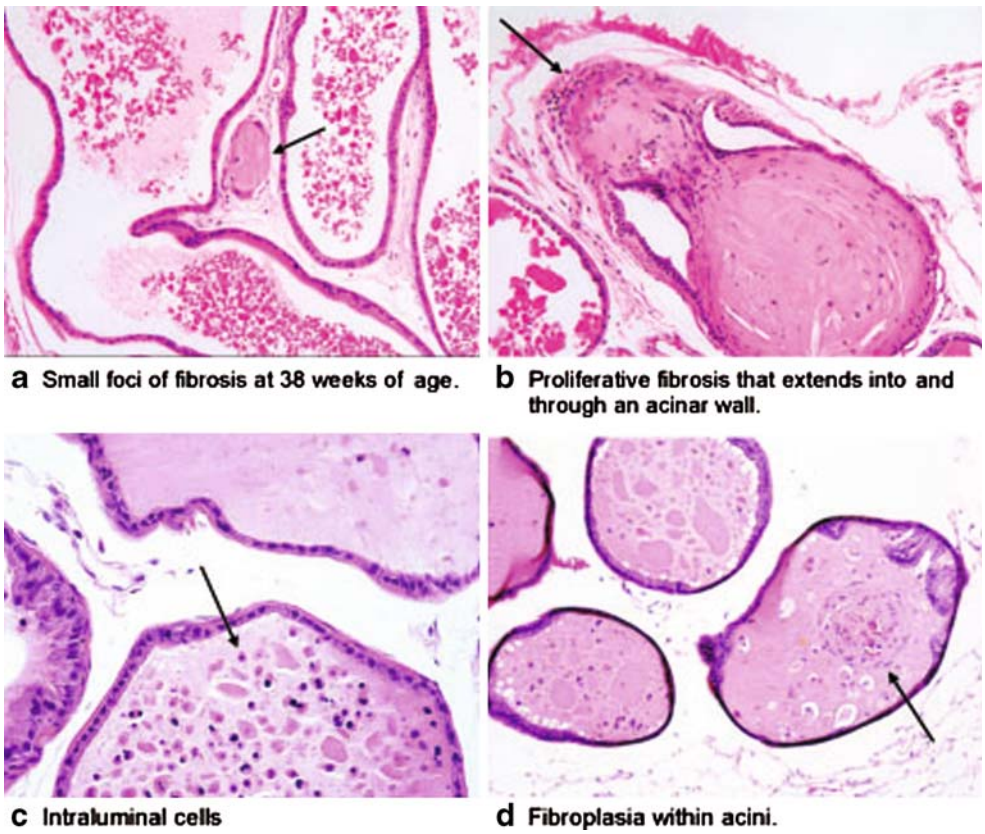
O65

Further Defining Reactive Stroma in Prostate Cancer

David Barron[1], Douglas Strand[2], Isaiah Schauer[3], Steven Ressler[1], Truong Dang[1], David Rowley[1]
[1]*Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA,* [2]*Vanderbilt Prostate Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA,* [3]*Department of Pathology, MD Anderson Cancer Center, Houston, TX, USA*

Myofibroblasts make up reactive stroma associated with prostate, mammary, lung, colon, and stomach carcinoma, suggesting that this cell type plays a critical role in a generalized response to injury. Our lab has shown a direct correlation of degree of reactive stroma with both severity and biochemical recurrence of human prostate cancer. The precise origin of myofibroblasts and their mechanism of recruitment in cancer are unknown. Recent studies in wound repair suggest that at sites of reactive stroma they originate from fibrocytes derived from circulating CD34+ hematopoietic progenitor cells. TGF-β has emerged as a key factor in mediating the recruitment and differentiation of fibrocytes to sites of wounding, however its corresponding role in cancer has not been examined.

To further understand the role of reactive stroma in adenocarcinoma, we analyzed several tissue microarrays containing patient matched normal and cancer regions that were subjected to a dual labeling immunohistochemistry approach. Recent data suggest that prostate cancer reactive stroma originates from vimentin+/CD34+/CD14+ progenitor cells that are juxtaposed to the sub-basal lamina surface at the stromal-epithelial junction. Moreover, xenograft modeling studies suggest that reactive stroma originates from bone marrow derived cells that may be of the monocyte series. Mechanistic studies examining TGF-β overexpression *in vivo* demonstrate age-dependent changes that mimic human reactive stroma. Transgenic mice exhibited focal collagenous micronodules that appear to correlated with TGF-β1 expression. Intraluminal fibroplasia with influx of inflammatory cells was also present in various regions of transgenic prostate. We propose a model of reactive stroma potential, affected by activation of local progenitors and subsequent recruitment of circulating progenitors to initiate and sustain a reactive microenvironment adjacent to cancer foci. We propose that this microenvironment is selective for more aggressive cancer phenotypes and is therefore a potential target for more advanced prognostics and novel therapeutics.



O66

Newly Characterised *ex vivo* Colospheres as a Three-Dimensional Colon Cancer Cell Model of Tumour Aggressiveness

Louis-Bastien Weiswald[1], Sophie Richon[1], Pierre Validire[2], Marianne Briffod[3], René Lai-Kuen[4], Fabrice P. Cordelières[5], Françoise Bertrand[3], Gerald Massonnet[1], Elisabetta Marangoni[6], Marc Pocard[7,8], Ivan Bieche[9], Marie-France Poupon[6], Dominique Bellet[1], **Virginie Dangles-Marie[1]**

[1]*IFR 71 Sciences du Médicament, Faculté des Sciences Phamraceutiques et Biologiques Paris Descartes, Paris, France*, [2]*Département d'Anatomie Pathologique, Institut Mutualiste Montsouris, Paris, France,* [3]*Service d'Anatomie et de Cytologie Pathologiques, Centre René Huguenin, Saint Cloud, France,* [4]*Plateforme d'Imagerie Cellulaire et Moléculaire, IFR71 Sciences du Médicament, Faculté des Sciences Pharmaceutiques et Biologiques Paris Descartes, Paris, France,* [5]*Plateforme Imagerie Cellulaire et Tissulaire, Research Center, Institut Curie, Orsay, France,* [6]*Département du Transfert, Hôpital Institut Curie, Paris, France,* [7]*Département Médico-Chirurgical de Pathologie Digestive Chirurgie, Hôpital Lariboisière, Paris, France,* [8]*UMR U965 INSERM/ Paris7 Université Paris Diderot, Hôpital Lariboisière, Paris, France,* [9]*UMR745 INSERM, Faculté des Sciences Pharmaceutiques et Biologiques Paris Descartes, Paris, France*

New models continue to be required to improve our understanding of colorectal cancer progression. The impact of microenvironment - like cell-cell interactions, extracellular matrix- on cell phenotype is now well described and multicellular three-dimensional tumour spheroids have been shown to closely mimic phenotype characteristics of *in vivo* solid tumours. In this context, we characterized here a three-dimensional multicellular tumour model we named colospheres, directly obtained from mechanically dissociated colonic primary tumours and correlated with metastatic potential.

Colorectal primary tumours (n=203) and 120 paired non-tumoral colon mucosa were mechanically disaggregated into small fragments for short-term cultures. Colospheres, exclusively formed by viable cancer cells, were obtained in only one day from 98 tumours (47%). Inversely, non-tumoral colonic mucosa never generated colospheres. The colosphere forming capacity was statistically significantly associated to tumour aggressiveness, according to AJCC stage analysis. Further characterization was performed using colospheres, generated from a human colon cancer xenograft, and spheroids, formed on agarose by the paired cancer cell line.

Despite close morphology, colospheres displayed higher invasivity than spheroids. Spheroids and colospheres migrated into Matrigel but MMP-2 and MMP-9 activity was detected only in colospheres. Mouse subrenal capsule assay revealed the unique tumorigenic and metastatic phenotype of colospheres. Besides, colospheres and parental xenograft reproduced similar CD44 and CD133 expression in which $CD44^+$ cells represented a minority subset of the $CD133^+$ population. Different growth conditions (*ex vivo versus in vitro*) involve distinct microenvironments, which consequently could participate in explaining these differences.

The present colospheres provide an *ex vivo* three-dimensional model, potentially useful for studying metastatic process, and underline the interest of studying different 3D microtumours with a different microenvironment origin.

O67

Adipocytes Protect Acute Lymphoblastic Leukemia Cells from Chemotherapy

James Behan[1], Ehsan Ehsanipour[1], Anna Arutyunyan[1], Anna Butturini[2,3], **Steven Mittelman[1,3,4]**

[1]*Division of Endocrinology, Childrens Hospital Los Angeles, Los Angeles, CA, USA,* [2]*Division of Hematology & Oncology, Childrens Hospital Los Angeles, Los Angeles, CA, USA,* [3]*Department of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA,* [4]*Department of Physiology & Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA*

We have previously shown that obesity is an independent predictor of leukemia (ALL) relapse. We have also found that obese mice transplanted with syngeneic ALL have poorer survival after treatment with vincristine, Nilotinib, or L-asparaginase, even when these agents are dosed proportional to body weight. Since ALL cells were found in the fat pads of relapsed mice, and adipocytes are a significant component of the bone marrow microenvironment, we investigated the role of adipocytes in ALL drug resistance.

We developed an in vitro co-culture system in which human or murine ALL cells were cultured together with adipocytes (differentiated 3 T3 L1s). Undifferentiated 3 T3-L1 fibroblasts were used as a control. Adipocytes protected murine preB ALL cells ("8093") from the anti-leukemic effects of all chemotherapeuties tested (vincristine, dexamethasone, nilotinib, daunorubicin, and L-asparaginase). This occurred independent of cell contact. Most significant was the protection by adipocytes against daunorubicin; after a 3-day exposure to 35 nM daunorubicin, there were 3.2 ± 0.3 vs. $0.4\pm0.1 \times 10^5$ viable cells in transwells over adipocytes vs. fibroblasts ($p<0.005$). This protection was also observed with murine bone marrow derived adipocytes (OP9), human immortalized adipocytes (Chub S7s), and human SD-1, RCH ACV, and BV-173 leukemia cells.

Further experiments demonstrated that media conditioned by adipocytes did not protect ALL cells from daunorubicin. However, media conditioned by the presence of both adipocytes and ALL cells simultaneously conferred a high degree of resistance to the leukemia cells ($1.3\pm0.4 \times 10^5$ viable cells, vs. $<0.1\times10^5$ in all other media types, $p<0.05$).

In summary, adipocytes protect ALL cells from multiple chemotherapies in vitro. Adipocytes take part in a two-way communication with ALL cells which leads to the secretion of factor(s) that confer resistance to daunorubicin. Adipose tissue may contribute to increased ALL relapse in obese patients.

O68

Human Lung Fibroblasts Prematurely Senescent after Exposure to Ionizing Radiation Enhance the Growth of Malignant Epithelial Cells *in vitro* and *in vivo*

Adamantia Papadopoulou[1], **Dimitris Kletsas[1]**
[1]*Institute of Biology, NCSR "Demokritos, Ag. Paraskevi, Athens, Greece*

Cellular senescence is considered to be a potent anticancer mechanism. However, it has been proposed that senescent stroma cells may enhance the growth of adjacent malignant epithelial cells. Exposure of tumours to repeated low doses of γ-irradiation is a common treatment regime in several tissues. However, the effect of this stress to the neighboring stromal cells and the interaction of the latter with cancer cells have not been adequately investigated. In this study, we have exposed confluent cultures of human lung fibroblasts, derived from normal or cancer-associated regions, to repeated subcytotoxic doses of 4 Gy of γ-irradiation. We have found that a single dose immediately activates a DNA damage response, as shown by the activation of the ATM/Chk2/p53/$p21^{WAF1}$ axis, leading to an intense cell cycle arrest. After a series of doses (total dose approx. 50 Gy), followed by cell subculturing, cellular senescence was accelerated, as shown by morphological alterations, growth arrest, $p21^{WAF1}$ and $p16^{INK4a}$ upregulation and senescence-associated β galactosidase staining. This process was found to be p53-dependent. Next, we studied the effect of these prematurely senescent cells on the growth of human malignant lung cell lines (A549 and H1299). Medium conditioned by young and prematurely senescent cells has no major effect on the proliferation of all three cell lines. However, in co-culture studies we have found that the growth of cancer cells was strongly enhanced when cultured on senescent cells. In addition, in immunocompromised (SCID) mice γ-irradiation-induced senescent cells, similarly to replicative senescent fibroblasts, intensely promoted A549 cells to form tumours; this process was partly dependent on the upregulation of matrix metalloproteases in senescent cells. These findings support the idea that replicative- or stress-induced-senescence may contribute to tumourigenesis.
This work has been partly supported by KEΣΥ.

O69

Cancer-Associated Fibroblasts Protect Head and Neck Squamous Cell Carcinoma Cells from Cetuximab-Induced Cytotoxicity

Ann-Charlotte Johansson[1,2], Arne Östman[2], Karin Roberg[1]
[1]*Division of Oto-Rhino-Laryngology, Linköping University Hospital, Linköping, Sweden,* [2]*Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden*

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with 650 000 new cases worldwide every year. The epidermal growth factor receptor (EGFR) is frequently overexpressed in HNSCC, thus, the anti-EGFR antibody cetuximab (Erbitux®) has been introduced in the treatment of this disease. Cancer-associated fibroblasts (CAFs), which are the major component of the stromal compartment, are known to support tumor growth and progression. It has also been suggested that CAFs could reduce the sensitivity of tumor cells to certain anti-cancer treatments. Therefore, their effect on cetuximab response in HNSCC cell lines was investigated.
CAFs, isolated from HNSCC biopsies from 7 patients, were found to stimulate HNSCC tumor cell proliferation. Interestingly, CAFs also reduced the sensitivity of 5 tested tumor cell lines to the growth-inhibitory effect of cetuximab. The effects were particularly prominent in the UT-SCC-9 cell line. In this cell line cetuximab caused a 40% reduction in cell number in the absence of CAFs. However, in co-culture with fibroblasts cetuximab instead stimulated tumor cell proliferation. Fibroblast conditioned media gave similar results, indicating that the CAF-derived protective effect is mediated by soluble factors.
The mechanism by which CAF-derived soluble factors reduce cetuximab-induced growth inhibition will be further characterized. According to preliminary data, fibroblast conditioned media prevented the cetuximab-induced reduction in EGFR phosphorylation. Thus, fibroblast-derived factors appear to interfere with the proximal effects of cetuximab on receptor activity. These results thus identify a previously unrecognized CAF-dependent modulation of cetuximab-sensitivity, and also present preliminary data on the underlying mechanism. In a longer perspective these results should aid in selection of HNSCC patients for cetuximab treatment. Finally, they suggest targeting of CAF-derived factors, yet to be identified, as a novel strategy to improve the effects of cetuximab.

O70

RCAS1 Protein Involvement in Creation of Suppressive Tumor Microenvironment in Salivary Gland Adenocarcinoma

Magdalena Dutsch-Wicherek[1], Agata Lazar[2], Romana Tomaszewska[3]
[1]*Department of Otolaryngology, Jagiellonian University, Krakow, Poland,* [2]*Department of Pathology, Jagiellonian University, Krakow, Poland,* [3]*Department of Pathology, Jagiellonian University, Krakow, Poland*

Introduction:
It has been established that tumor microenvironment inhibits the infiltration and activity of T lymphocytes and creates the local immunosuppression. However, it still remains unknown which component of tumor microenvironment is really responsible for tumor immunopathgenity. RCAS1 (receptor cancer binding antigen expressed on SiSo cells) is a protein expressed by various cancer cells responsible for the inhibition of activated immune cells such as T, B lymphocytes and NK cells and induction of their apoptosis, participating in the tumor escape from host immunological surveillance and the creation of immune tolerance for tumor cells. Tumor-associated macrophages might promote tumor growth and metastases.
Materials and Methods:
RCAS1 and CD68 antigens immunoreactivity was determined in 50 tissue samples of salivary gland adenocarcinomas and in 50 tissue samples of their stroma and 30 tissue samples of healthy control (palatine tonsils) by immunohistochemistry method in the Department of Pathology.

Results:
RCAS1 immunoreactivity was identified in both adenocarcinoma and healthy stromal samples. Significantly higher RCAS1 immunoreactivity was shown in the cancer samples than in stromal samples. RCAS1 immunoreactivity in stromal samples was significantly higher in patients with the presence of lymph node metastases in comparison to patients without metastases. We also observed significantly higher number of CD68 positive cells (macrophages) in adenocarcinoma samples and in stromal samples than in the control group. Moreover, the number of CD68 positive cells in adenocarcinoma and stroma were higher in patients with lymph node metastases in comparison to patients without metastases. Additionally, in our study macrophages were identified to possess the immunoreactivity of RCAS1, RCAS1 expressing macrophages were observed in the mucous.
Conclusion:
In the present study we have demonstrated that RCAS1 expression by the tumor cells, tumor microenvironment and tumor associated macrophages participate in creating the immunosuppressive microenvironment in salivary adenocarcinomas.

O71

Tumor Microenvironment Induced Drug and Radio Resistance in Invasive Breast Cancer Cells

Sumanta Goswami[1,2]
[1]*Anatomy and Structural Biology, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, USA,* [2]*Department of Biology, Yeshiva University, New York, NY, USA*

Metastasis, drug and radio resistance continue to cause significant morbidity and patient mortality. This is in spite of recent introduction of a number of different chemotherapy agents and newer radiotherapy protocols. Using unique animal models and cell separation techniques coupled with sensitive assays we have recently discovered that the invasive breast cancer cells are hypoproliferative and antiapoptotic. Since the invasive cells have shut down their cell cycle and have become dormant they continue to resist cytotoxic drugs and ionizing radiation. We have used cells isolated from the primary tumor, invasive cells, circulating tumor cells and lung metastasis to identify the underlying molecular mechanism for drug and radio resistance. We used a combination of cytotoxic and cytostatic drugs along with molecular pathway directed drugs to target the invasive, drug and radio resistant breast cancer cells. Secondly using both classical gene expression studies as well as by the identification of different invasion and resistance specific splice variants we have identified a genetic signature which will predict potentially invasive, chemo and radio resistant cancers. Third we have evaluated the *in vitro* and *in vivo* efficacy of a protein phosphatase inhibitor that pushes the dormant cells into the cell cycle at both G0/G1 and G2/M stages, thereby converting the resistant cells to chemo and radio sensitive. There are two very important clinical advantages of this research program; first we can predict which patient will respond to which drug depending on the genetic signature of their cancer, second we are able to target the dormant cells by reverting them to become chemo and radiosensitive. In summary we conclude that the tumor microenvironment renders the invasive cells chemo and radio resistant and thereby protecting them from the initial chemo and radio therapy. This probably causes a relapse of the disease after a period of apparent remission.

O72

Immunosuppressive Tumor Microenvironment in *ret* Transgenic Mouse Melanoma Model

Viktor Umansky[1], Fang Zhao[1], Christiane Meyer[1], Silvia Kimpfler[1], Dirk Schadendorf[1]
[1]*Skin Cancer Unit, German Cancer Research Center (DKFZ), Heidelberg, Germany*

Melanoma is known for its poor response to current immunotherapies due to immunosuppressive cells and factors in the tumor microenvironment, which inhibit antitumor immune responses. We use a recently developed *ret* transgenic mouse skin melanoma model, which closely resemble human melanoma with respect to genetics, histopathology and clinical features. After a short latency (20–70 days), around 25% of mice spontaneously develop melanoma metastasizing to lymph nodes, liver and lungs.
We demonstrated a tumor infiltration with immature dendritic cells (DCs) that secreted more interleukin (IL)-10 and less IL-12p70 and showed a decreased capacity to activate T cells compared to DCs from normal animals. Observed dysfunction was linked to p38 MAPK activation. Inhibition of its activity led to normalization of cytokine secretion pattern and T-cell stimulation capacity of DCs from tumor bearing mice. TCR zeta-chain expression in lymphoid organs and tumors was down-regulated, which was associated with an increase in Gr1+CD11b+ myeloid derived suppressor cells (MDSC) in these mice. Co-culture of normal T cells with MDSCs from tumor bearing mice led to the down-regulation of zeta-expression. Oral application of an inhibitor of phosphodiesterase-5 sildenafil (Viagra) resulted in a retardation of melanoma progression associated with an increase in tumor-infiltrating $CD8^+$ and $CD4^+$ T cells and in their zeta-chain expression. Higher numbers of regulatory T cells (Treg) were found at early stages of melanoma progression compared to more advanced tumors. These data inversely correlated with Treg amounts in the bone marrow suggesting a possible Treg recruitment to primary tumors. Although anti-CD25 antibody injections resulted in the efficient Treg depletion from lymphoid organs, melanoma development was not delayed indicating that in the autochthonous melanoma genesis, other immunosuppressive cells could play replace tumor promoting Treg functions. We suggest that effective melanoma immunotherapy should include the neutralization of tolerogenic DCs, MDCS and Treg in the tumor microenvironment.

O73

Mechanisms of Tumor-escape from the Immune System: Adenosine-producing Treg, Exosomes and Tumor-associated TLRs

Theresa L. Whiteside[1], Marta Szajnik[1], Miroslaw J. Szczepanski[1], Magis Mandapathil[1,3], Margareta Czystowska[1], Edwin K. Jackson[2], Stephan Lang[3], Elieser Gorelik[1]
[1]*Departments of Pathology, University of Pittsburgh, Pittsburgh, PA, USA,* [2]*Department of Pharmacology, University of Pittsburgh, Pittsburgh, PA, USA,* [3]*Department of Otorhinolaryngology, University of Duisburg-Essen, Essen, Germany*

Human solid tumors have evolved numerous strategies for escape from the host immune system. Recently, it has been shown that regulatory T cells (Treg) accumulate in blood and tissues of patients with cancer influencing prognosis. One mechanism for Treg-mediated suppression of anti-tumor immunity involves ectonucleotidases CD39 and CD73 overexpressed on $CD4^+CD25^{high}FOXP3^+$ cells. These enzymes sequentially convert ATP into AMP and adenosine, which binds to A_{2a} receptors ($A_{2a}R$) on effector cells, suppressing their functions. Treg express low levels of adenosine deaminase (ADA) responsible for adenosine breakdown and of CD26, a surface-bound glycoprotein associated with ADA. Inhibitors of ectonucleotidases or antagonists of the $A_{2a}R$ block Treg-mediated suppression. The increased frequency and suppressor activity of Treg in patients with cancer are in part regulated by the presence in body fluids of tumor-derived microvesicles (TMV) also referred to as exosomes. When isolated and purified from tumor cell supernatants or sera of patients with cancer, TMV induced conversion of $CD4^+CD25^{neg}$ into $CD4^+CD25^{high}FOXP3^+$ Treg and enhanced Treg proliferation ($p<0.001$) as well as suppressor functions ($p<0.01$). These changes in Treg were associated with increased expression of phosphorylated STAT3 and resistance of Treg to TMV-mediated apoptosis. TMV were positive for TGF-β1 and IL-10 and their suppressor functions were in part abrogated by neutralizing antibodies to these cytokines. In addition to producing adenosine and releasing TMV, human tumors were found to express TLR4. Triggering of this receptor by its ligands, LPS or paclitaxel (PTX), promoted tumor cell proliferation, activated the P13K pathway up-regulated Akt phosphorylation and NF-κB translocation to the nucleus, increased resistance of the tumor to apoptosis and protected the tumor from NK-cell mediated lysis. Further, TLR4 triggering on tumors was associated with the up-regulation of IRAK-4 expression, and increased production of IL-6, IL-8, GM-CSF and VEGF. IL-4 ligation on tumor cells also protected them from effects of chemotherapy. In aggregate, our data suggest that the elimination of tumor immune escape will require combination strategies designed to target several distinct molecular mechanisms.

O74

Radiation-Induced Modifications of the Tumor Microenvironment Promote Metastasis

Curzio Rüegg[1,2,3,5], Natsuko Imaizumi[1,2,3,5], François Kuonen[1,2,5], Julien Laurent[1,2,5], Girieca Lorusso[1,2,5], Yan Monnier[4,5]
[1]*Division of Experimental Oncology, Multidisciplinary Oncology Center (CePO), Lausanne, Switzerland,* [2]*University Medical Center (CHUV), Lausanne, Switzerland,* [3]*National Center for Competence in Research, Molecular Oncology, ISREC-EPFL, Lausanne, Switzerland,* [4]*ENT Department, University Medical Center (CHUV), Lausanne, Switzerland,* [5]*University of Lausanne (UNIL), Lausanne, Switzerland*

Radiotherapy is successfully used to treat human cancer. Emerging evidence suggests that radiation-induced modifications of the tumor microenvironment may contribute to the therapeutic effects of radiotherapy. Recurrence after radiotherapy, however, is associated with increased local invasion, metastatic spreading and poor prognosis. We are investigating whether radiation-modified tumor microenvironment may possibly contribute to the increased aggressiveness of relapsing tumors. Irradiation of the prospective tumor bed results in a sustained impairment of growth factor-driven and tumor angiogenesis without disrupting the preexistent vasculature, through sustained inhibition of proliferation, induction of senescence and inhibition of migration and sprouting of endothelial cells. Using xenografts tumor models and an orthotopic model of murine breast cancer, we observed that tumors growing within a preirradiated stroma have reduced growth while they display increased hypoxia, necrosis, local invasion and lung metastasis. Mechanisms of progression involve adaptation of tumor cells to local hypoxic conditions as well as the selection of escape variantsretaining an invasive and metastatic phenotype upon returning to normoxia. Though gene expression analysis experiments, we have identified the matricellular protein CYR61 and αVβ5 integrin as molecules that cooperate to mediate lung metastasis, as well as a gene expression signature associated with tumor hypoxia and predictive for a shorter relapse-free survival after adjuvant radiochemotherapy in human breast cancer. The αV integrin small molecular inhibitor Cilengitide prevented lung metastasis formation without impinging on primary tumor growth. Radiotherapy also modify the recruitment of bone marrow derived / immune cells known to contribute to tumor angiogenesis and metastasis. Taken together these results demonstrate the impact of radiotherapy-induced modifications of the tumor microenvironment in determining tumor evolution and identify candidate therapeutic targets. We are currently investigating additional cellular and molecular determinants of tumor escape and progression after radiotherapy, and at this conference we will present the latest results.

O75

The Microenvironment Adjacent to Prostate Cancer Exhibits Numerous Differential Expression Changes that are Useful for Diagnosis without Tumor Cells

Dan Mercola[1], Yipeng Wang[1,2], Zhenyu Jia[1], Steve Goodison[3], Anne Sawyers[1,2], Huazhen Yao[1,2], Anne Simoneau[4]
[1]*Pathology and Laboratory Medicine, University of California at Irvine, Irvine, CA, USA,* [2]*Vaccine Research Institute of San Diego, San Diego, CA, USA,* [3]*M.D. Anderson Cancer Center, Orlando, FL, USA,* [4]*University of California at Irvine, Irvine, CA, USA*

We have developed a linear model of prostate tissue that describes gene expression changes as a sum of contributions of four major cell types in tumor enriched samples including tumor cells, stroma cells, epithelial cells of BPH, and dilated cystic glands. When combined with knowledge of the cell type distribution as estimated by pathologists, the model provides estimates of gene expression for each cell type (1). By comparing the expression of stroma cells in low (<15%) tumor samples with normal volunteer biopsy samples, we derived 417 significant gene expression differences which were further filtered to remove genes with significant expression in tumor cells. The resulting 17 genes, which appeared to have high expression in stroma only when in the presence of tumor, were applied to a training set of 18 PCa cases and 17 noncancer tissues of the same cases all measured on U133plus2 Affymetrix arrays. The program PAM yielded 97% accuracy for discriminating tumor cases vs. non tumor cases. The classifier was then tested on multiple independent prostate samples including 65 tumor cases and a separate 79 case set both measured on U133A arrays and both publically available, and 55 independent cases measured on U133plus2 arrays in house which yielded an accuracy of 96–100% for the three sets. To exclude performance that may be based on recognition of tumor cells, we tested the classifier on 9 additional independent normal volunteer biopsy cases and 7 normal rapid autopsy cases that were histologically confirmed to be tumor free which yielded 100% accuracy as nontumor cases for both series. Thus a classifier based on tumor-adjacent stroma is highly accurate for discrimination of tumor and nontumor. A significant number of the million prostate biopsies in the U.S. per year have equivocal pathological readings, therefore, methods for augmenting diagnostic accuracy based on stroma may be helpful.

1.Stuart et al. PNAS 2004;101:615–20.

O76

Bone Marrow Endothelial Progenitor Cells are Systemic Sensors of Breast Cancer

Robert Suriano [1], Andrea George[1], Shilpi Rajoria [1], Erin Lambers [2], Raj Kishore [2], **Raj Tiwari [1]**
[1]*Department of Microbiology and Immunology, New York Medical College, Valhalla, NY, USA,* [2]*Feinberg Cardiovascular Institute, Northwestern University, Chicago, IL, USA*

Circulating bone marrow derived endothelial progenitor cells (BM-EPC) have been observed to contribute to neo-vascularization of breast cancers and the identification of its systemic mediators will impact clinical care. We discovered a crucial role for BM-EPCs in breast cancer progression with estradiol (E2) as a major modulator. We utilized TEK2/GFP-Balb/c ± ovariectomized ± estrogen supplementation as our experimental mouse model. These mice were transplanted with bone marrow (BMT) derived from TEK 2/GFP mice that were used as donors. Tumors were induced in these mice by surgical implantation of TG1 or 4T1 murine mammary adenocarcinoma cells (derived from syngeneic BALB/c mice; 2×10^6 cells/0.3 ml PBS) into the fourth inguinal mammary gland after clearing the fat pad region of BMT mice. BM-EPC mobilization at the tumor site was measured and correlated with capillary density. We observed the concomitant mobilization of GFP and CD133 (marker of EPC) double-positive cells at the tumor site with high levels in the blood prior to migration at the tumor site. Comparison of estrogen supplemented and non-supplemented group, revealed that estradiol supplementation enhances both mobilization of GFP-CD133+ EPCs in the tumors as well facilitate EPCs to physically integrate into neo-vasculature resulting in significantly higher capillary density. The contribution of estrogen in angiogenesis and tissue remodeling, which are two processes indispensable for tumor growth, was also examined by Q-RT-PCR experiments on excised tumor-inoculated mammary tissues, in which the transcripts of various angiogenic cytokines were significantly increased. E2 stimulated EPCs were also observed to secrete paracrine factors which increased the proliferation and migration of 4T1 tumor cells. These *in vivo* studies were recapitulated in an *in vitro* model of tubulogenesis. Our studies define BM-EPCs as possible prognostic sensors and key determinants in vasculogenic remodeling necessary for breast cancer progression.

O77

Stabilization of the Breast Tumor Microenvironment Using Hox Genes

Ileana Cuevas[1], Amy Chen[1], Mina Bissell[3], Lisa M. Coussens[2], **Nancy Boudreau[1]**
[1]*Surgery, University of California San Francisco, San Francisco, CA, USA,* [2]*Pathology, Univeristy of California San Francisco, San Francisco, CA, USA,*
[3]*Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA, USA*

Breast cancer development is accompanied by progressive loss of epithelial cell polarity and growth control, infiltration of macrophages and activation of angiogenesis. Understanding how epithelial and stromal cell behavior and/or phenotype is coordinately dysregulated in breast cancer, enables identification of molecules that coordinately control not only normal cellular interactions in the breast, but also tumor-associated interactions that promote breast cancer progression. To this end we have been investigating a role for the Homeobox (Hox) family of master morphoregulatory genes.

HoxD10 and HoxA5 are highly expressed in normal breast epithelial cells and in quiescent vascular endothelium and fibroblasts and contribute to establishment of functional differentiated breast tissue. However, invasive breast tumors progressively lose HoxD10 and HoxA5 expression in both the epithelial and endothelial cells. Significantly, restoration of HoxD10 in metastatic breast epithelial cells induces a phenoytpic reversion and restores polarity, reduces invasion and redcues tumor growth in vivo and restoring HoxA5 leads to growth arrest and apoptosis of tumor epithelial cells. Restoring epithelial HoxD10 also reduces VEGF expression and restoring either HoxA5 or HoxD10 in epithelial cells also suppresses expression of several chemokines including CCL-2 and CxCL12 that in turn decrease recruitment of immune cells to tumors. In addition directly restoring expression of either HoxD10 or HoxA5 in angiogenic endothelial cells directly attenuates angiogenesis by reducing endothelial cell invasion and stabilization of vascular structures. Thus, both HoxD10 and HoxA5 are potent breast tumor suppressors that coordinately stabilize the breast tumor microenvironment by inhibiting epithelial cell growth and invasion, directly impairing angiogenesis and suppressing leukocyte infiltration (inflammation). We are currently developing targeted approaches to restore expression of HoxD10 and/or HoxA5 to cells within mammary tumor tissues in vivo.

O78

Macrophages are an Important Component of Myeloma Microenvironment and Protect Myeloma Cells from Chemotherapy Drug-Induced Apoptosis

Jing Yang[1], Qing Yi[1]
[1]*Department of Lymphoma and Myeloma, MD Anderson Cancer Center, Houston, TX, USA*

Multiple myeloma is a B-cell malignancy characterized by proliferation of plasma cells in the bone marrow. It is the second most common hematological malignancy and is still largely incurable. One of the major problems is that myeloma cells develop drug resistance upon interaction with bone marrow stromal cells. To understand the importance of different stromal cell components in the bone marrow microenvironment, we examined the effects of macrophages on myeloma cell survival and response to chemotherapy. We report here that macrophages, in particular tumor-associated macrophages obtained by culturing macrophages with myeloma cell culture supernatants, are a protector of myeloma cells. Macrophages protected both myeloma cell lines and primary myeloma cells, isolated from patients from spontaneous and chemotherapy drug-induced apoptosis via attenuating the activation and cleavage of caspase-dependent apoptotic signaling. The protective effect was dependent on direct contact between macrophages and myeloma cells. However, the reduced numbers of apoptotic tumor cells in the cocultures were not the result of macrophage-uptake of apoptotic cells, because macrophages with or without the capacity to phagocytose apoptotic cells provide similar protection to myeloma cells against chemotherapy-induced apoptosis. Although tumor-associated macrophages secreted large amounts of IL-6, which is the most important survival factor for myeloma cells, our results show that IL-6 neutralizing antibodies failed to significantly affect the protective effects of tumor-associated macrophages, suggesting that other cytokines may be involved. These findings are clinically relevant, because we examined bone marrow biopsies of patients by immunochemistry analysis and found that $CD68^{+}$ macrophages are heavily infiltrated in the bone marrow of patients with myeloma but not control patients. Thus, our results indicate that macrophages are an important component of the bone marrow stromal cells and may contribute to myeloma cell survival and resistance to chemotherapeutic treatment in vivo.

O79

Blockade of TNFα Signaling in Tumor-associated Macrophages: a New Radiosensitizing Strategy

Yuru Meng[1], Michael A. Beckett[1], Hua Liang[1], Nico van Rooijen[2], Helena J. Mauceri[1], Kenneth Cohen[3], **Ralph R. Weichselbaum[1]**
[1]*Department of Radiation and Cellular Oncology, The University of Chicago Medical Center, Chicago, IL, USA,* [2]*Department of Molecular Cell Biology, Vrije Universiteit, VUMC, Amsterdam, Netherlands,* [3]*Department of Medicine, Section of Hematology/Oncology, The University of Chicago Medical Center, Chicago, IL, USA*

Radiotherapy is an important anti-cancer treatment and approximately 60% of all cancer patients receive radiotherapy during the course of their disease. However, improvements in the therapeutic index of radiation therapy have been mostly based on physical improvements in radiation delivery. Radiosensitizer development targeting tumor cells has not yielded effective agents. Recent investigations in several laboratories have focused on the tumor stroma as a potential target for radiosensitization. Here we report that depletion of tumor associated macrophages prior to radiotherapy increases the anti-tumor effects of ionizing radiation (IR) following both systemic and local injection of macrophage depleting Liposomal Clodronate (Lip-Clod). These anti-tumor effects were noted following large single dose (20 Gy) and low dose (2 Gy) fractionated radiation. Co-implantation of tumor cells with BM-derived macrophages (BMDMϕ) resulted in increased tumor resistance to IR. Experiments using animals with germ line deletions of TNF receptors 1,2 (TNFR1,2-/-) or TNFα (TNF-/-) demonstrated that the radioprotective effect of BMDMϕ required intact TNFα signaling. The radioprotective effect of TNFα was mediated by the upregulation of VEGF production in tumor associated macrophages (TAMϕ). Treatment of experimental tumors with a neutralizing antibody to TNFα ($Enbrel^{R}$) improved tumor regression with IR compared to IR alone without an increase in host toxicity. These data provide a mechanistic basis for targeting macrophage populations generally and TNFα induced macrophage VEGF specifically to improve radiotherapy outcomes.
Y.M., M.A.B., and R.R.W. contributed equally to this work.

O80

The Role of Microenvironment on the Regulation of Epstein-Barr Virus Latent Gene Expression

Eva Klein[1], Lorand L. Kis[1], Daniel Salamon[1]
[1]*Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Stockholm, Sweden*

Depending on the differentiation of EBV-carrying cells, the virally encoded proteins are expressed in various combinations. These determine the fate of the viral genome harbouring cells. Virus transformed B lymphocytes - lymphoblastoid cell lines- LCL- express six virally encoded nuclear and three surface localised proteins. This phenotype is encountered only in B lymphocytes and induces their proliferation. It is usually referred to as Type III EBV expression or growth transformation program. Such cells are readily recognized by the immune response.
The presence of the EBV genome in lymphocytes with a restricted viral protein expression, as it occurs in Hodgkin's and nasal NK lymphomas, that lacks the nuclear protein EBNA-2, does not induce proliferation. However it modifies the behaviour of the cell. Such cells can avoid apoptosis, and induce an enrichment of inflammatory cells in the microenvironment environment. Intercellular contacts and /or cytokines induce their proliferation.
We studied the details of IL21 imposed modification of EBV gene expression: We found that in Type III cells IL-21, enhanced the LMP-1 promoter and silenced the C promoter with the consequence that 5 of the 6 EBNAs disappeared. EBNA-1 that can be transcribed from its own specific promoter, Qp, was maintained. Thus the cells switched to the Type IIa (EBNA-1 and LMP-1) pattern with elevated expression of the LMP-1 protein. Exposure of Type I (only EBNA-1 expressed) BL cells to IL-21, activatied the LMP-1p and thus resultsd also in a Type IIa pattern because the cells maintained the Qp deriven EBNA-1 expression. We could show that IL21 has a direct effect on the LMP-1p. We postulate that silencing of the Cp occurs through the activation of a suppressor protein

O81

Adhesive Interactions Regulate Transcriptional Diversity in Malignant B-cells

Ben-Zion Katz[1,4], Liat Nadav[1,2], Tal Shay[3], Eytan Domany[3], Elizabeth Naparstek[1,4], Benjamin Geiger[2]
[1]*The Hematology Institute, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel,* [2]*Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel,* [3]*Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot, Israel,* [4]*Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel*

The genetic profiling of B-cell malignancies is rapidly expanding, providing important information on the tumorigenic potential, response to treatment, and clinical outcome of these diseases. However, the relative contributions of inherent gene expression vs. microenvironmental effects are poorly understood. The regulation of gene expression programs by means of adhesive interactions was studied in ARH-77 human malignant B-cell variants, derived from the same cell line by selective adhesion to a fibronectin matrix. The populations included cells that adhere to fibronectin and are highly tumorigenic (designated "Type-A" cells), and cells that fail to adhere to fibronectin, and fail to develop tumors *in vivo* ("Type-F" cells). To identify genes directly affected by cell adhesion to fibronectin, Type-A cells deprived of an adhesive substrate (designated "AF cells") were also examined. Bioinformatic analyses revealed a remarkable correlation between cell adhesion, and both B-cell differentiation state and the expression of multiple myeloma-associated genes. The highly adherent Type-A cells expressed higher levels of NF*k*B-regulated genes, many of them known to be associated with multiple myeloma. Moreover, we found that the transcription of several multiple myeloma-related proto-oncogenes is stimulated by adhesion to fibronectin (i.e., expressed in "A-cells, but not in "AF"). In contrast, Type-F cells, which display poor adhesive and tumorigenic properties, expressed genes associated with higher levels of B-cell differentiation. Our findings indicate that B-cell differentiation, as manifested by gene expression profiles, is attenuated by cell adhesion to fibronectin, leading to up-regulation of specific genes known to be associated with the pathogenesis of multiple myeloma.

O82

Changes in Epigenetic Expression Patterns of Tumour Associated Fibroblasts (TAF)

Kerstin Junker[1], Astrid Enkelmann[1], Joana Heinzelmann[1], Daniel Steinbach[2], Michaela Weidig[3], Heiko Wunderlich[1]
[1]*Department of Urology, University Hospitals Jena, Jena, Germany,* [2]*Department of Gynaecology and Obstetrics, University Hospitals Jena, Jena, Germany,* [3]*Department of Pathology, University Hospitals Jena, Jena, Germany*

Background:
Interaction of tumour cells and tumour stroma has a high impact on tumour growth and progression due to different mechanisms in which they are involved, e.g. cell proliferation and invasion. These processes are normally regulated but in case of tumour growth several cell regulation mechanisms are defective. DNA methylation of CpG sites in promoter region of genes is known to be involved in regulation of tumour suppressor genes. Furthermore microRNAs (miRNA) are known to be crucial for negative regulation of translational gene expression. Purposes of this work are isolation and epigenetic characterisation of TAF from primary urinary bladder carcinoma.
Material and Methods:
TAF were isolated from cultured urinary bladder tumour specimen by treatment with EDTA and differential trypsinisation. Non-tumour fibroblasts were isolated from foreskin and normal urinary bladder tissue. Furthermore total RNA was isolated from TAF and non-tumour fibroblasts to analyse the miRNA expression profile by miRNA array. DNA isolation was performed to determine the methylation pattern of CpG sites in promoter region of selected oncogenes in TAF and non-tumour fibroblasts.
Results:
We developed a cell culture routine to isolate and subsequently cultivate TAF from primary material of urinary bladder carcinoma.

Microarray analyses indicated a significant down regulation of expression levels of several miRNAs in TAF in comparison to non-tumour fibroblasts. Determining the methylation level of CpG sites of selected oncogene promoter regions revealed a specific methylation pattern of TAF and non-tumour fibroblasts.

Conclusion:

The ability to obtain TAF from primary tumour material leads us to analyse epigenetic characteristics of TAF. Thereby a specific miRNA expression profile was observed in comparison to non-tumour fibroblasts. Furthermore a specific methylation pattern of CpG sites of several selected oncogenes was determined in TAF. These results facilitate to understand the specific regulation of gene expression in TAF.

O83

Cancer Cell-adipocyte Cross-talk: Role of Matrix Metalloproteinase-11/stromelysin-3

Marie-Christine Rio[1], **Emilie Buache[1]**

[1]*Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Department of Cancer Biology, CNRS UMR 7104, INSERM U964, Université De Strasbourg, Illkirch, France*

High matrix metalloproteinase 11/stromelysin-3 (MMP11/ST3) expression in primary tumors is associated with cancer aggressiveness as well as with poor patient clinical outcome (Basset et al., Nature 1990, 348:699). Mouse tumor models show that MMP11 acts very early subsequent to cancer cell invasion. The invasive processes lead to the proximity of cancer cells and cells of mesenchymal origin. In this context, most studies focusing on cancer cell-connective cell interactions have emphasized the role of fibroblasts, endothelial and inflammatory cells in fully-constituted tumor stroma that contains very few, if any, adipocytes. We have demonstrated the MMP11 involvement in cancer cell-adipocyte cross-talk. We showed that cancer cells induce MMP11 expression by adjacent adipocytes/pre-adipocytes at the human breast tumor invasive front.

These data point to the essential role of adipocytes in invasive steps and highlights the MMP11 participation. The origin of peritumoral fibroblasts, known to favor tumor progression, remains debated. Our results support the concept that pioneer invading cancer cells that induce the MMP11 production by proximal adipocytes/preadipocytes, initiate a vicious cycle leading to a default of adipocyte differentiation and the accumulation/maintenance of peritumoral fibroblast-like cells. Accordingly, recombinant MMP11 reverts chemically-induced adipocyte differentiation of MMP11-deficient mouse embryonic fibroblasts (MEF) (Andarawewa et al., Cancer Res 2005, 65:19862) (Motrescu and Rio, Biol Chem 2008, 389:1037). Finally, MMP11 exhibits collagenolytic activity against the native alpha 3 chain of collagen VI, a collagen required for correct fat tissue cohesion and adipocyte function (Motrescu et al. Oncogene 2008, 27:6347). Interestingly, collagen VI has been reported to be involved in breast cancers (Iyengar et al., J Clin Invest 2005, 115:1163).

Collectively, our data constitute the first evidence implicating an MMP in cancer cell-adipocyte cross-talk, and are of particular interest since epidemiological studies identify obesity as a major risk and/or a poor prognosis factor for cancer.

O84

Forced Hemidesmosome Assembly as a Novel Mechanism for Somatostatin Receptor sst2 Tumor Suppressive Activity in Pancreatic Cancer

Séverine Laval[1], Hanane Laklai[1], Marjorie Fanjul[1], Jean-Pierre Estève[1], Mélanie Pucelle[1], Christiane Susini[1], Stéphane Pyronnet[1], **Corinne Bousquet[1]**

[1]*INSERM U858, Département Cancer, Institut de Médecine Moléculaire de Rangueil, Toulouse, France*

We have introduced the proof of concept that by introducing sst2, whose expression is lost in 90% of pancreatic cancers, into human pancreatic cancer cells, *in vitro* and *in vivo* cell proliferation, tumor progression and metastasis are decreased. Sst2 tumor suppressor activity relies on an autocrine loop whereby its natural ligand somatostatin is secreted by sst2-expressing cells resulting in constitutive sst2 activation. However, molecular mechanisms responsible for sst2-dependent inhibition of invasiveness are unknown.

The a6b4 integrin plays a critical role in epithelia integrity: its presence in hemidesmosal structures (HDs) at the basal cell surface links the intracellular intermediate filament network to the extracellular laminins of the basement membrane. Interestingly, HDs are frequently absent in cancer cells, whereas the a6b4 integrin (mostly its β4 subunit) is overexpressed in several cancers, including pancreatic, and contributes to carcinoma invasiveness by stimulating cell migration. This is partly achieved through a6b4 integrin delocalization into lamellipodia and filopodia.

We have demonstrated that somatostatin, by acting through sst2, can revert a6b4 integrin delocalization to migration structures, an hallmark of epithelial cancer cells, by forcing its relocalization to HDs, thereby stabilizing epithelial cell anchorage to basement membrane and inhibiting cell migration. Underlying molecular mechanisms are here shown to rely on a sst2-dependent up-regulation of HDs protein expression, including BP180.

Strikingly, knocking-down BP180 expression (siRNA) impairs somatostatin-induced HDs assembly in sst2-expressing cells. Interestingly, BP180 siRNA partially reverts sst2 inhibitory role on *in vitro* and *in vivo* cell migration and invasion, as demonstrated using the chick chorioallatoic membrane model whereby tumor progression of pancreatic cancer cell xenografts is monitored.

We have identified an original mechanism for sst2 to revert cancer cell pro-migratory phenotype by relocalizing the a6b4 integrin to HDs thereby facilitating hemidesmosome assembly and cancer cell anchorage to basement membrane.

O85

Anti-JAM-C Tumor Growth Inhibition Occurs through Modulation of Thrombomodulin Expressing Stromal Cells

Vincent Frontera[1], Marie-Laure Arcangeli[1], Claudia Zimmerli[2], Florence Bardin[1], Elodie Obrados[1], Stephane Audebert[1], Beat Imhof[2], Jean Paul Borg[1], Michel Aurrand-Lions[1]

[1]*Université de la méditerrannée institut poali calmettes, Inserm U891, Marseille, France,* [2]*Department of Pathology and Immunology, Centre Médical Universitaire, Geneva, Switzerland*

The Junctional Adhesion Molecule-C (JAM-C) has been identified as an adhesion molecule highly expressed by lymphatic sinuses of lymph nodes, mesenchymal and endothelial cells [1]. Functionally, antibodies directed against JAM-C decrease tumor growth and modulate the adaptative immune response [2 3]. The aim of the present study is to better characterize the cellular compartment, which is targeted by anti-JAM-C *in vivo*: lymphatic, mesenchymal or endothelial. We have generated a new monoclonal antibody against a mouse lymphatic cell line (JAM-C^{high}), which does not recognize a brain endothelial cell line (JAM-C^{low}). This antibody is directed against thrombomodulin, initially described as a vascular specific protein. We show here that thrombomodulin is co-expressed with JAM-C on lymphatic sinuses and fibroblastic reticular cells of lymph nodes and on tumoral vessels, whereas it is not expressed on specialized vascular beds such as high endothelial venules. This suggests that the role of thrombomodulin largely exceed its reported function of a vascular specific protein involved in coagulation and inflammation. We further demonstrate that anti-JAM-C treatment specifically decreases the lymph node fibroblastic reticular compartment expressing PDGRFa and thrombomodulin. Similarly, thrombomodulin expression associated with tumoral vessels is reduced in anti-JAM-C treated mice, indicating that inhibition of tumor growth by anti-JAM-C treatment may rely on the killing of a stromal compartment present in tumor and lymph nodes. Whether this cellular compartment is mandatory for tumor growth and plays a role in tumor metastasis to lymph nodes is currently addressed.

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O86

Identification of Glucocorticoid-Induced Leucine Zipper as a Key Regulator of Tumor Cell Proliferation in Epithelial Ovarian Cancer

Nassima Redjimi[1], Françoise Gaudin[1], Cyril Touboul[1], Karl Balabanian[1], Marc Pallardy[3], Armelle Biola-Vidamment[3], Hervé Fernandez[2], Sophie Prevot[2], Dominique Emilie[1,2], **Véronique Machelon[1]**
[1]*UMRS 764, Université Paris-Sud 11, Inserm, Clamart, France,* [2]*Service de Microbiologie-Immunologie Bioogique, Service d'Anatomie et Cytologie Pathologiques, Service de Gynécologie Obstétrique et de Médecine de la Reproduction, Assistance Publique-Hôpitaux de Paris, Hôpital Antoine Béclère, Clamart, France,* [3]*UMR-S 749, Faculté de Pharmacie, Chatenay-Malabry, France*

Little is known about the molecules that contribute to tumor growth of epithelial ovarian cancer (EOC) that remains the most lethal gynecological neoplasm in women. Glucocorticoid-Induced Leucine Zipper (GILZ) is frequently detected in epithelial tissues and controls key signaling pathways. We investigated its expression by immunohistochemistry in tumor specimens from 50 patients surgically treated for diagnosis of epithelial ovarian cancer. GILZ was detected in the cytoplasm of tumor cells of all the well-defined histological types. GILZ immunostaining scores were positively correlated to the proliferation marker Ki-67 ($P < 0.00001$). They were also higher in tumor cells containing high amount of phosphorylated protein kinase B (p-AKT) ($P < 0.01$). To further investigate the apparent dependency of proliferation and AKT activation upon GILZ expression, we used BG-1 cell line derived from tumor cells as a cellular model, either overexpressing GILZ by stable transfection or bringing down GILZ by the use of small interfering (si) RNA targeting GILZ. Modulation of GILZ expression directly controlled cell proliferation, phospho-AKT cellular content and AKT kinase activity. It also changed the expression level of p21 and cyclin D1, two proteins known to control cell cycle progression. Our findings demonstrate the emerging role of GILZ, an intracellular factor not identified before in EOC, in the control of cell proliferation and AKT activity in ovarian epithelial tumor cells.

O87

Correlated Expression Analysis of VEGF Family Members and Lipid Inflammatory Mediators in Human Colon Polyps and Carcinomas and Liver Metastases

Sarah Pringels[1], Nancy Van Damme[2], Marc Peeters[2], Johan Grooten[1]
[1]*Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium,* [2]*Department of Gastroenterology, Ghent University Hospital, Ghent, Belgium*

Inflammatory mediators, such as prostaglandin E_2 (PGE_2), and responsive angiogenic factors, mainly vascular endothelial growth factor A (VEGFA), have emerged as pathways driving neo-angiogenesis and supporting the progression and metastasis of solid tumors.
To understand the relation in human solid tumors between COX and LOX-derived eicosanoids and expression of VEGF family members ($VEGF^F$) (VEGFA, -B, -C, -D and PlGF), we performed a RT-qPCR comparative expression analysis of colon carcinoma samples.
Up to now, tumor samples and matched normal colon tissues from 52 patients were analyzed. The results showed a complex and diversified expression phenotype. 88% of the tumor samples showed increased expression of at least one VEGF family member. In a considerable proportion of samples multiple VEGF family members were overexpressed with a predominance of VEGFA and especially PlGF. Correlating the $VEGF^F$ and eicosanoid enzymes gene expression profiles not only revealed a clear linkage between both signaling pathways but also a clear association of 5-LOX with VEGFB and COX2 with PlGF.
A similar analysis was performed on 23 colon polyps and 30 liver metastases. Strikingly, already in polyps a pronounced inflammatory expression profile with increased expression of COX

enzymes was apparent. This was accompanied by an increased expression of mainly VEGFA and PlGF. Also in liver metastases, an inflammatory signature accompanied by $VEGF^F$ expression was apparent. Yet, the profiles observed in liver metastases diverged from those in colon polyps and carcinomas. This divergence may be due to the different tumor microenvironment. The results from this correlated expression analysis of VEGF family members and genes involved in eicosanoid biosynthesis are promising for the diagnosis and prediction of treatment outcome of colon cancer patients. In addition, our results clearly indicate that the perception of a $COX2/PGE_2$-driven VEGFA expression, sustaining neo-angiogenesis, is an oversimplification.

O88

Tenascin-C in the Tumor Microenvironment Triggers Oncogenic Signaling

Yundan Jia[1,2], Isabelle Gasser[1], Caroline Spenle[1], Martial Kammerer[1], Falk Saupe[1], Marija Marko[1], Jessica Kant[1], Valentin Djonov[3], Klaus-Peter Janssen[4], Anne-Catherine Feutz[2], Michael van der Heyden[1], Wentao Huang[2], Patricia Simon-Assmann[1], Michèle Kedinger[1], Agnes Neuville-Mechine[5], Gerhard Christofori[2], **Gertraud Orend[1,2]**
[1]*Inserm U682, Tissue Homeostasis in Inflammation and Cancer, Faculté de Médicine, Université de Strasbourg, Strasbourg, France,* [2]*Institute of Biochemistry and Genetics, Center for Biomedicine, University of Basel, Basel, Switzerland,* [3]*Department of Medicine, Gross Anatomy and Vascular Biology, University of Fribourg, Fribourg, Switzerland,* [4]*Department of Surgery, Technische Universitaet Muenchen, Muenchen, Germany,* [5]*Hospital Hautepierre, Faculté de Médicine, Université de Strasbourg, Strasbourg, France*

The extracellular matrix molecule tenascin-C (TNC) is highly expressed in most cancers which correlates with a bad survival prognosis and tamoxifen resistance. TNC plays a role in promoting tumor cell proliferation, angiogenesis, invasion and metastasis but the molecular mechanisms are poorly understood (1). We showed that TNC induces cell rounding by two mechanisms: it counteracts cell adhesion to fibronectin by blocking the syndecan-4 / integrin alpha 5 beta 1 complex (2). This stimulates tumor cell proliferation (3) by activation of oncogenic Wnt (through repression of DKK1) and MAPkinase signaling (4). TNC also stimulates endothelin receptor type A (EDNRA) expression which maintains cell rounding (5). FAK, paxillin, RhoA and Tropomyosin-1 are critical targets of TNC downstream of syndecan-4 and EDNRA (5, 6). TNC also triggers cell migration in combination with LPA/PDGF (6).
Results on the tumorigenesis-promoting effects of TNC in our newly established transgenic mice that ectopically express TNC in the pancreatic islets of insulinoma-prone SV40 Tag-expressing RipTag2 mice (RT2/TNC) will be presented.
(Supported by INSERM, INCa, Bourse Region Alsace, Oncosuisse, SNF, ARC, AICR, Krebsliga Beider Basel)
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O89

WWOX Expression Suppresses Tumorigenecity by Inducing Apoptosis and Attenuating Migration of Metastatic Cells

Suhaib Abdin[1], Sara Del Mare[1], Mohammed Abu Odeh[1], Zaidoun Salah[1], **Rami Aqeilan[1]**
[1]*Lautenberg Center for Immunology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel*

The WW domain containing oxidoreductase (*WWOX*) spans one of the most active common fragile sites, FRA16D, involved in cancer. *WWOX* encodes a 46-kDa protein that contains two N-terminal WW domains and a central short-chain dehydrogenase/reductase (SDR) domain. Through its WW domain, the Wwox protein interacts with its partners and modulates their functions. Wwox suppresses the transactivation functions of several transcription factors implied in cancer by sequestering them in the cytoplasm. Targeted deletion of the *Wwox* gene in mice causes increased spontaneous tumor incidence confirming that *WWOX* is a *bona fide* tumor suppressor. Wwox expression is absent or reduced in most cancer cell lines and its ectopic overexpression induces apoptosis *in vitro* and suppresses tumorigenecity *in vivo*. Furthermore, Wwox attenuates the migration and invasion ability of MDA-MB-231 breast carcinoma metastatic cells. Additionally, its restoration results in reduced attachment and migration on fibronectin. By contrast, knocking down endogenous Wwox increases adhesion to fibronectin. Therefore, Wwox acts as a tumor suppressor not only by inducing apoptosis mediated by caspase activation but also through modulating the interaction between tumor cells and the extracellular matrix.

O90

Oncogenes do not Fully Override the Cellular Programme: Pronounced Impact of Cellular Microenvironment

Jozefa Wesierska-Gadek[1], Eva Walzi[1], Iva Doleckova[1], Gerald Schmid[1]
[1]*Dept. of Medicine 1, Div.; Inst. of Cancer Research, Medical University of Vienna, Vienna, Austria*

Data on the biological effects of some overexpressed oncogenes and their cooperation with cellular factors are, at least partially, contradictory. A strong G_1 arrest or high rate of apoptosis was reported in transformed cells overexpressing temperature-sensitive (ts) $p53^{135val}$ when maintained at permissive temperature. Comparison of the experimental protocols reveals that cells used for transfection strongly differ. Therefore, we decided to explore the

impact of primary cells used for generation of cell clones on the biological effects evoked by p53 and c-Ha-Ras.
We used primary rat cells (RECs) isolated from rat embryos of different age: at 13.5 gd (y) and 15.5 gd (o). We immortalized rat cells using ts $p53^{135val}$ mutant and additionally generated transformed cells after co-transfection with oncogenic c-Ha-Ras[1]. The ts $p53^{135Val}$ mutant, switching between wild-type and mutant conformation, offers the possibility to study the escape from p53-mediated cell cycle control in a model of malignant transformation in cells with the same genetic background. Surprisingly, the kinetics of cell proliferation at non-permissive temperature and that of cell cycle arrested at 32°C strongly differed between cell clones established from yRECs and oRECs[2]. Furthermore, the kinetics of the re-enter of G1-arrested cells in the active cell cycle largely differed between distinct cell clones. Finally, the susceptibility of immortalized and transformed cells to growth-inhibitory and apoptosis-promoting drugs was evaluated. Inhibition of cellular CDKs by purine analogues revealed that y and o transformed cells differentially respond to the pharmacological CDK inhibitors thereby indicating that overexpression of genes such as $p53^{135Val}$ mutant and oncogenic-Ha-Ras is not able to fully override the intrinsic cellular programme.
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O91

The Role of Myeloma-Derived Chemokine CCL27 on Tumor Progression and Immune Escape

Karin Joehrer[1], Angelika Olivier[1], Philipp Ofer[1], Daniel Neureiter[2], Richard Greil[1,3]
[1]*Tyrolean Cancer Research Institute, Innsbruck, Austria,* [2]*Institute of Pathology at the Private Medical University Hospital, Salzburg, Austria,* [3]*Laboratory for Immunological and Molecular Cancer Research and IIIrd Medical Department, University Hospital, Salzburg, Austria*

Multiple myeloma is a still incurable plasma cell tumor and considerable efforts are undertaken to establish new immunotherapeutic strategies to target this B- cell neoplasm. Chemokines are major players in shaping the tumor microenvironment and can contribute to immune escape of the malignant cells. In the search for important actors of the chemokine network in multiple myeloma we found CCL27, which has so far only been correlated with skin diseases such as atopic dermatitis, consistently upregulated in all cell lines investigated. In bone marrow supernatants of tumor patients CCL27 expression correlated with the severity of disease. Myeloma cells were found to express CCR10, the respective receptor, and to be able to utilize the ligand-receptor interaction as an autocrine proliferation loop. Additionally, transendothelial migration of myeloma cells in response to CCL27 was enhanced whereas migration over fibronectin was not affected. We further investigated the impact of CCL27 on immune cells such as T cells and dendritic cells. Dendritic cells differentiated and matured in the presence of CCL27 exhibited a reduced capacity to activate T cells in allogeneic mixed leukocyte reactions. T cell proliferation as well as cytokine production was impaired. Treated dendritic cells showed normal expression of costimulatory molecules but impaired spontaneous migration as well as cytokine production which might explain the impaired T cell function. In coculture experiments with myeloma cell lines, however, these dendritic cells induced enhanced growth of the malignant plasma cells.
In summary, we found that CCL27 can modify migration of malignant plasma cells and immune cells. In addition, this chemokine modulates dendritic cells by impairing their potential to activate T cells but, at the same setting, enhances their potential to induce tumor cell growth. Targeting CCL27 therefore could constitute an essential additional component in myeloma therapy.

O92

Reconstitution of PTEN Activity and Inhibition of the PI3-K/Akt Signaling Prevent the Pro-Survival Effect of Bone Marrow Microenvironment and Induce Apoptosis in CLL Cells

Medhat Shehata[1,2,3], Susanne Schnabl[1,2], Dita Demirtas[1,2], Martin Hilgarth[1,2], Rainer Hubmann[1,3], Elena Ponath[1,2], Sigrun Badrnya[1,2], Josef Schwarzmeier[1,2], Peter Valent[1,3], Christoph Zielinski[1,2,4], Ulrich Jaeger[1,2]
[1]*Department of Internal Medicine I, Hematology/Hemostaseology Division, Medical University of Vienna, Vienna, Austria,* [2]*Karl Landsteiner Institute for Cytokines and Tumor Microenvironment, Medical University of Vienna,Department of Internal Medicine I, Vienna, Austria,* [3]*Ludwig Boltzmann Institute- Cluster Oncology, Medical University of Vienna, Department of Internal Medicine I, Vienna, Austria,* [4]*Center of Excellence for Clinical and Experimental Oncology (CLEXO), Medical University of Vienna, Department of Internal Medicine I, Vienna, Austria*

Stromal cells represent a central component of the lymphoid microenvironment and are involved in B cell lymphopoiesis and leukemogenesis. Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of clonal B cells due to inhibition of apoptosis and expansion of the malignant clone in the lymphoid organs. However, CLL cells die rapidly by spontaneous apoptosis in vitro. The aim of this study is to elucidate the role of lymphoid microenvironment in the activation of the anti-apoptotic PI3-K/Akt pathway and prolonging survival of CLL cells. Early passages of primary human non-transformed bone marrow stromal cells (BMSC) served as an in vitro model for lymphoid microenvironment. Co-cultures of CLL cells with BMSC inhibited spontaneous apoptosis of the leukemic cells. PI3-K inhibitors (wortmannin or LY294002) or siRNAs against PI3-K-p110 and Akt1 prevented the pro-survival effect of BMSC and led to apoptosis of the leukemic cells. Apoptosis was associated with a decrease in the PIP3, PDK1 and Akt1 and de-phosphorylation of the tumour suppressor PTEN. Western blotting demonstrated a high expression of phosphorylated PTEN and casein kinase 2 (CK2) in CLL

cells. Since CK2 is involved in the phosphorylation of PTEN, CLL cells were treated with CK2 inhibitors (apigenin, TBB, DRB and DMAT) and cell viability was assessed by MTT assays and FACS analysis. CK2 inhibitors decreased the phosphorylation of PTEN and Akt1, induced apoptosis and overcame the pro-survival effect of BMSC. Combination of inhibitors of CK2 and PI3-K demonstrated an additive effect and significantly increased the rate of apoptosis in CLL cells. In conclusion, the interaction between CLL cells and the lymphoid microenvironment leads to the activation of the anti-apoptotic PI3-K/Akt pathway, inactivation of PTEN and prolongation of the survival of CLL cells. The inhibition of PI3-K/Akt and recovery of PTEN activity by CK2 inhibitors may represent a promising therapeutic concept for CLL.

O93

Interactions of Microenvironment with Carcinomas Depend on Tumor Type, Grade and Stage

Harry L. Ioachim[1]
[1]*Department of Pathology, Columbia University and Lenox Hill Hospital, New York, NY, USA*

The interaction with carcinoma (Ca) of lymphocytes (Ly) and intercellular matrix (Ma) were investigated comparatively in 22 gastric, 26 pulmonary and 28 breast Ca. Ly are constant companions of tumor cells which they may infiltrate and/or destroy.Their amounts vary with the types of tumors.In the lung B-and T-cell Ly are abundant in non-small cell Ca and plasma cells in squamous cell Ca but are almost absent in carcinoids and small cell Ca.In the breast Ly are more abundant in e-cadherin + duct Ca than in the e-cadherin- lobular Ca.In the stomach B-and T-cells are numerous in intestinal type and rare in diffuse type. In all these Ca,well differentiated tumors are accompanied by more Ly than poorly differentiated The Ma appears normally loose in the former and collagenized, desmoplastic in the latter.The kinds, amounts and distribution of Ly also vary with the stage of Ca being more abundant in early stages and rare, replaced by desmoplasia in late stages.In the breast,aggregates of Ly are next to the precancerous lesions and Ca in situ far more than in late stages of infiltrating Ca.FAS receptor was >than FAS-L ligand in mammary tumors and in their infiltrating Ly while their ratios were reversed in their lymph node metastases. In bronchi,Ly accumulate next to dysplastic changes. In the stomach, B-cells form barrier bands and reactive follicles in the mucosa around. atypical cells while T-cells,mainly CD8+ infiltrate the Ca cells. These observations indicate that the Ly and Ma reactions to Ca are not uniform but correlated with the tumor type, grade and stage.

O94

Role of the Tumor Suppressor p16 Protein in Tumor-Stromal Interactions in Breast Cancer

Maysoon Al-Ansari[1], Siti-Faujiah Hendrayani[1], **Abdelilah Aboussekhra**[1]
[1]*Department of Biological and Medical research, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia*

Carcinoma-associated fibroblasts (CAFs) play important roles in the genesis and thrive of various types of epithelial cancers, including breast carcinomas. Indeed, various genetic and epigenetic variations have been identified in stromal fibroblasts, and we have recently shown that CAFs as well as their corresponding counterparts (TCF) display neoplastic-specific changes (Hawsawi et al., 2008). In the present study we have shown that the level of p16 protein is lower in 80% of CAFs as compared to their corresponding TCFs. This decrease resulted from lower stability of the p16 mRNA owing to an increase in the level of the mRNA binding and destabilizing protein AUF1 in CAF cells. Furthermore, using specific p16-siRNA we have shown that p16 negatively controls the expression of various proteins involved in the stromal-epithelial interactions. These include the stromal cell-derived factor 1 (SDF1), the vascular endothelial growth factor (VEGF) and the matrix metalloproteinase-2 (MMP2). In addition, ELISA and immunoblotting assays were used to show that the level of these proteins was higher in the conditioned media from stromal fibroblasts expressing specific p16siRNA as compared to control cells. Importantly, conditioned media from p16-defective cells stimulated the invasion and the migration of cultured human epithelial cells. These results clearly show the role of the breast stromal fibroblast p16 protein in suppressing tumoregenesis. Moreover, we have shown that curcumin can normalize p16 expression and therefore reduces the expression and the secretion of these cancer promoting factors. This indicates that curcumin has potential use as stromal fibroblast normalizing factor that can be utilized for the inhibition of both cancer initiation and recurrence.

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O95

Role of Heparanase in Colitis Associated Cancer

Immanuel Lerner[1], Eyal Zcharia[1], Esther Bensoussan[1], Dina Rodkin[1], Yoav Sherman[2], Israel Vlodavsky[3], **Michael Elkin**[1]
[1]*Department of Oncology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel,* [2]*Department of Pathology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel,* [3]*Cancer and Vascular Biology Center, The Rappaport Faculty of Medicine, Haifa, Israel*

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that is closely associated with colon cancer. Here we report that heparanase enzyme acts as an important mediator of colitis-associated tumorigenesis. Heparanase is an only known mammalian enzyme that cleaves heparan sulfate, the major polysaccharide of the extracellular matrix, and plays multiple roles in inflammation and cancer progression. Applying histological specimens from UC patients and a mouse model of dextran sulfate sodium (DSS)-induced colitis, we found that heparanase is constantly overexpressed and activated during the course of the disease, both in the active and inactive phases of inflammation. Employing heparanase-overexpressing transgenic mice in the model of colitis-associated cancer, induced by carcinogen azoxymethane followed

by repeated DSS administration, we demonstrated that heparanase overexpression markedly increased the incidence and severity of colitis-associated colonic tumors, enabling faster tumor take, angiogenic switch and enhanced tumor progression. Notably, DSS-induced colitis alone (without azoxymethane pretreatment) lead to formation of colonic tumors in heparanase-transgenic, but not wild type mice, positioning heparanase as important physiological determinant in inflammation-driven colon carcinoma, replacing the need for carcinogen. Investigating molecular mechanisms underlying heparanase induction in colitis, we found that TNFalfa is responsible for continuous overexpression of heparanase by chronically-inflamed colonic epithelium. Moreover, our results suggest the occurrence of heparanase-driven vicious cycle that powers colitis and associated tumorigenesis: heparanase activity in inflamed colon, acting synergistically with the local cytokine milieu, stimulates macrophage activation, and the activated macrophages secrete TNFalfa which stimulate further production of heparanase by colonic epithelium. In addition, activated macrophages secrete cathepsin L – a cysteine protease responsible for proteolytic activation of latent heparanase enzyme. Altogether, our results identify heparanase as a key factor in pathogenesis of colitis-associated cancer and attest the inhibition of heparanase as a promising mean to disrupt the vicious cycle that fuels chronic colitis and the associated tumorigenesis.

O96

The Role of Heparanase in Promoting Multistage Pancreatic Islet Tumorigenesis

Karen Hunter[1], Carmela Palermo[1], Karoline Dubin[1], Israel Vlodavsky[2], Johanna Joyce[1]
[1]*Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [2]*The Cancer and Vascular Biology Research Center, Technion - Israel Institute of Technology, Haifa, Israel*

Heparanase is a matrix-degrading enzyme whose increased expression is significantly associated with malignant progression in many human cancers. We have previously shown that heparanase expression increases during tumorigenesis in the RIP1-Tag2 (RT2) transgenic mouse model of pancreatic islet carcinogenesis. Moreover, we have found that heparanase is expressed in human pancreatic neuroendocrine tumors and its increased expression is correlated with metastases. However, the exact molecular and cellular mechanisms by which this enzyme functions in pancreatic tumorigenesis remain to be elucidated. To study the role of heparanase in RT2 tumorigenesis, we crossed transgenic mice that constitutively overexpress *heparanase* (*hpa*-Tg) to RT2 mice to generate the *hpa*-Tg RT2 line. *Hpa*-Tg RT2 mice exhibit increased tumor invasion, angiogenesis and lymphangiogenesis. To further investigate heparanase function in RT2 tumorigenesis, heparanase knockout mice have been crossed to RT2 mice. These mice are currently being analyzed for multiple parameters of tumorigenesis.
Additionally, a heparanase-overexpressing β-tumor cell line (Hpa-βTC) was derived from a *hpa*-Tg RT2 tumor and utilized in in vitro approaches to dissect the mechanisms by which heparanase promotes tumor progression. The Hpa-βTC line was found to be highly invasive in Matrigel invasion assays when compared to a wildtype βTC line (WT-βTC). Furthermore increased heparanase expression renders the Hpa-βTC line highly motile when tested in cell migration assays. Interestingly, the WT βTC line has a very low intrinsic migration ability that can be significantly enhanced by factors secreted by the Hpa-βTC line in co-culture assays. Efforts are currently underway to identify the precise factors that are secreted by heparanase overexpressing cells in the tumor microenvironment to promote malignant tumor progression.

O97

Maspin Restores Redifferentiation of Prostate Cancer Cells in Collagen I

Margarida Bernardo[1,2], Shijie Sheng[1,2]
[1]*Department of Pathology, Wayne State University, Detroit, MI, USA,* [2]*Barbara Ann Karmanos Cancer Institute, Detroit, MI, USA*

Maspin belongs to the serine protease inhibitor (SERPIN) superfamily. Several molecular partners of maspin have been identified to date, including the pro-form of urokinase-type plasminogen activator (pro-uPA) and collagen I (Col I), the most abundant protein in the bone matrix. Maspin is a tumor supressor gene, since its expression inversely correlates with malignancy in human breast and prostate cancer (PC) progression. Both tumors metastasize to bone. In a murine model, maspin inhibited PC bone growth, osteolysis and angiogenesis, and in so doing, increased fibrosis and produced hollow lumen acini.
We investigated herein the effect of maspin in PC cell growth and morphology on top of a layer of polymerized Col I (2D) or embedded in the collagen matrix (3D). To this end, three different clones of DU145 cells stable transfected with maspin (M3, M7 and M10) and cells transfected with empty vector (Neo) were used. In 2D, the maspin transfectants spread uniformly on Col I whereas the Neo cells form disconnected patches. Reaction with overlaid fluorescein labeled Col I (DQ-collagen) revealed that the Neo cells exhibit more collagenolytic activity per cell than the maspin transfectants. In 3D, however, the Neo cells spread whereas the M7 cells, which were shown to express the most maspin, formed spheroid structures of compact polarized cells in a cobblestone-like formation. Cell polarization was ascertained by functional visualization of collagenolytic activity and by b1-integrin immunostaining using a Zeiss LSM 510 confocal microscope. DQ-collagen cleavage was detected in the periphery of the spheroids, whereas the core was devoid of collagenolytic activity. The b1-integrin was also found predominantly localized at the basal cell-matrix interface. Hoechst nuclear staining revealed hollow lumens. The M3 and M10 cells, which express lower levels of maspin, formed less compact spheroids. This maspin-induced cell redifferentiation appears to be specific for fibrillar Col I, since in the basement membrane-like Matrigel, containing nonfibrillar collagen IV, acinus formation was not detected. In sum, this investigation shows that maspin can restore the redifferentiation of PC cells in the bone microenviron-

ment, thus recapitulating the *in vivo* observations, with important consequences for therapeutic intervention in PC metastatic progression to bone.

O98

Osteoblastic Maturation-dependent Microenvironment Mediated by Retinoid Signaling Inhibits Proliferation and Induces Terminal Differentiation of Leukemia Cells

Lingtao Wu[1], Qiaojun He[2], Peihua Luo[3], Parvesh Chaudhry[3], Anxun Wang[3], Meidan Ying[3], Xiaochun Yang[3]
[1]*Department of Pathology, CHLA/USC Keck School of Medicine, Los Angeles, CA, USA,* [2]*Department of Pharmacology and Toxicology, Zhejiang University, Hangzhou, Zhejiang, China,* [3]*Department of Pathology, Childrens Hospital Los Angeles Saban Research Institute, Los Angeles, CA, USA*

Retinoic acid (RA) is a potent agent that coordinates inhibition of proliferation with differentiation of many cell types. Although the striking success of epigenetic reversion of genetic malignant-phenotype, as exemplified by RA-induced differentiation of acute promyelocytic leukemia cells, has directed attention to bone-lining osteoblasts that form the specialized microenvironment required for development of human hematopoietic stem cells (HSC), there is remarkably little data on the role of these epigenetic processes mediated by RA signaling in coordinating osteoblastic differentiation with hematopoietic development. We reported here that either RA-induced lose of retinoic acid receptor alpha (RARa) phosphorylation or mimicked RARa hypophosphorylation by expression of RARa phosphorylation-defective mutant RARaS77A mediates human osteosarcoma U2OS cell differentiation. Gene expression analysis showed that either RA or RARaS77A induces many same differentiation response molecules/pathways mediating osteoblastic differentiation and hematopoietic development. Importantly, overexpression of FGF8f in U2OS cells, a secreted growth factor and one of the targets of both RA and RARaS77A, not only induced expression of osteoblastic differentiation response genes, but also inhibited proliferation of both human lymphocytic and myeloid leukemia cells treated with U2OS conditional medium or co-cultured with differentiating U2OS cells. In addition, granulocytic differentiation of normal primitive human CD34+ cells and myeloid leukemia cells was induced by co-culture or conditional medium. Moreover, overexpression of FGF8f in U2OS cells and human mesenchymal stem cells (hMSC) mimicked RA-modulated induction of osteoblastic differentiation, while U2OS cells expressing RARaS77A inhibited osteosarcoma formation in nude mice. These findings strongly suggest a novel bi-directional RARa-FGF8f signaling pathway that within the bone marrow hematopoietic niche, coordinates osteoblastic maturation with differentiation of both normal and malignant hematopoietic precursors through RARa-modulated osteoblastic cell secretion of FGF8f.

O99

VE-cadherin Regulates Philadelphia Chromosome Positive (Ph+) Acute Lymphoblastic Leukemia (ALL) Sensitivity to Apoptosis

Laura Gibson[1,2], Stephen Akers[3], Debbie Piktel[1], James Fortney[1], Karen Martin[1,2], Michael Craig[1], Heather O'Leary[3]
[1]*Mary Babb Randolph Cancer Center, West Virginia Universiy, Morgantown, WV, USA,* [2]*Department of Microbiology, Immunology and Cell Biology, West Virginia University, Morgantown, WV, USA,* [3]*Cancer Cell Biology Program, West Virginia University, Morgantown, WV, USA*

Expression of the Philadelphia chromosome (Ph+) translocation is clinically important in acute lymphoblastic leukemia (ALL) and is correlated with high risk of relapse and poor prognosis.[1] Our laboratory has identified a subpopulation of Ph+ leukemic cells which constitutively express VE-cadherin, interact with bone marrow stromal cells (BMSC), express stem cell markers, and are resistant to chemotherapy.[2] Tumor cell expression of VE-cadherin has been associated with aggressive phenotype and poor prognosis in other tumor models, but has not been investigated in hematopoietic malignancies.[3] Therefore, we investigated the regulation of VE-cadherin by BMSC and its contribution to Ph+ ALL therapeutic response. We determined that Ph+ ALL cell lines, as well as primary patient cells, express VE-cadherin. Exposure of Ph+ cells to Imatinib diminished VE-cadherin mRNA, which is blunted by Ph+ ALL contact with BMSC. Knockdown of VE-cadherin expression by siRNA rendered Ph+ ALL cells more susceptible to chemotherapy, even in the presence of BMSC. Additionally, pre-treatment of Ph+ ALL cells with ADH100191, a VE-cadherin antagonist, resulted in elevated Ser/Thr phosphorylation of beta-catenin and increased apoptosis during treatment. In contrast, lentiviral mediated expression of VE-cadherin in Ph- ALL cells resulted in increased resistance to treatment-induced apoptosis. These observations suggest a therapeutic role for VE-cadherin in modulation of chemoresistance in Ph+ ALL and demonstrate the importance of cues from the microenvironment in regulating tumor cell response to treatment.

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3) Hendrix MJ, *et al.* Expression and functional significance of VE-cadherin in aggressive human melanoma cells: role in vasculogenic mimicry. Proc Natl Acad Sci U S A 2001 Jul 3;98 (14):8018–23.

O100

Galectin-3 Binding Protein Produced by Neuroblastoma Cells Stimulates the Expression of Interleukin-6 in the Tumor Microenvironment

Ayaka M. Silverman[1], Yasushi Fukaya[1], Leonid S. Metelitsa[1], Robert C. Seeger[1], Hiroyuki Shimada[2], Ebrahim Zandi[3], Yves A. DeClerck[1]
[1]*Department of Pediatrics, The Saban Research Institute of Childrens Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, USA,* [2]*Department of Pathology, The Saban Research Institute of Childrens Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, USA,* [3]*Department of Molecular Microbiology and Immunology, University of Southern California Keck School of Medicine, Los Angeles, CA, USA*

There is recent evidence that mesenchymal cells derived from the bone marrow play an important role in bone metastasis in several cancers, including myeloma and neuroblastoma. We previously reported that contact-independent interaction between neuroblastoma cells and bone marrow stromal cells (BMSC) stimulates the production of interleukin-6 (IL-6) by BMSC which promotes the growth and the survival of tumor cells (Ara *et al.*, *Cancer Research* 2009). Here, we have identified Galectin-3 binding protein (Gal-3BP) as a soluble factor produced by neuroblastoma cells that upregulates IL-6. We observed that several neuroblastoma cell lines express and secrete Gal-3BP, and that expression correlates with the ability of these cells to induce the production of IL-6 by BMSC. Expression of IL-6 by Gal-3BP seems to be mediated by Gal-3, a multifunctional glycoprotein that binds Gal-3BP and is present in BMSC. Signaling involves activation of the Raf-1/MEK/ERK1/2 pathway and can be blocked in the presence of the MEK inhibitor PD 98059 or in the presence of an anti Gal-3 antibody. We also observed that Gal-3BP can upregulate IL-6 in peripheral blood monocytes suggesting that it may contribute to tumor-associated inflammation. In primary neuroblastoma tumors, Gal-3BP is present in tumor cells and in the surrounding extracellular matrix, whereas IL-6 is present in stromal and inflammatory cells. Preliminary studies also suggest that higher levels of Gal-3BP are present in neuroblastoma tumors with an unfavorable histology and more severe clinical outcome. Thus the data provide a novel function for Gal-3BP in the tumor microenvironment and cancer progression.

O101

Tumor-Derived IL-4 Upregulates Cathepsin Activity in Tumor-Associated Macrophages to Promote Cancer Development and Progression

Hao-Wei Wang[1,2], Vasilena Gocheva[1,2], Bedrick Gadea[1], Tanaya Shree[1], Johanna Joyce[1]
[1]*Cancer Biology & Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [2]*Weill Cornell Graduate School of Medical Sciences, Cornell University, New York, NY, USA*

While macrophages are a fundamental component of the host innate immune system, their presence within the tumor microenvironment has been found to facilitate tumor initiation and progression. Previously, we have shown that cysteine cathepsin proteases are upregulated as tumors develop in the RIP1-Tag2 (RT2) mouse model of pancreatic islet carcinogenesis and that tumor-associated macrophages (TAMs) are the major source of cathepsin activity in tumors. Using pharmacological inhibition and genetic ablation, we have further shown that specific cathepsins are critical in several steps of tumor progression, including tumor cell proliferation, angiogenesis and tumor invasion. Therefore, we set out to investigate the mechanisms whereby cathepsin activity is upregulated in TAMs.
Using an activity-based probe for cathepsin proteases and a novel cell-based system, we have shown that tumor cell-conditioned media (TCM) upregulates cathepsin activity in bone marrow-derived macrophages. Cytokine protein expression arrays revealed enrichment of several candidate cytokines and growth factors in TCM. Through experiments using recombinant proteins and neutralizing antibodies, we have identified interleukin (IL)-4, a Th2 cytokine, as the tumor cell-secreted factor that is responsible for the upregulation of cathepsin activity in TAMs. To validate our in vitro findings, we have generated *Il4* null RT2 mice, and shown that the cathepsin activity in TAMs was significantly reduced in *Il4* knockout animals. Taken together, our results indicate that tumor cell-derived IL-4 is a principal activator of TAM phenotype through upregulation of cathepsin activity in TAMs.

O102

Chronic Inflammation-Induced Immunosuppression: Micro and Macro Environmental Factors and Implications for Cancer Therapy

Ilan Vaknin[1], Moshe SadeFelman[1], Aya Eisenberg[1], Inna Varfolomeev[1], Eliran Ish Shalom[1], **Michal Baniyash[1]**
[1]*The Lautenberg Center for General and Tumor Immunology, The Hebrew University, Hadassah Medical School, Jerusalem, Israel*

A substantial body of evidence supports the notion that chronic inflammation and cancer are associated. This association is apparent under two circumstances: 1) Chronic inflammation can predispose an individual to cancer and 2) Developing tumors induce a micro and/or macro chronic inflammatory environment associated with enhanced tumor development and metastasis. Under both circumstances the generation of an immunosuppressive environment is evident, enabling escape of the tumor from immune surveillance. Based on our studies on mouse model systems that mimic the immunosuppressive conditions generated in tumor-bearing hosts, we proved chronic inflammation and associated myeloid derived suppressor cells (MDSCs) as the causative link for the induced immunosuppressive environment. This leads to T and NK cells immune dysfunction associated with zeta chain downregulation, as described in a large number of various tumors. Moreover, we demonstrate that such a harmful environment suppresses not only the host's immune system but

also inhibits newly administered T lymphocytes, which is most likely the limiting factor for the success of currently used cancer immunotherapies based on vaccination and T cell transfer. Our current studies focus on an in depth characterization of the chronic inflammation induced immunosuppressive environment and its impact on tumor development and spreading aiming at the discovery of blockers neutralizing the immunosuppressive environment. In parallel, we are in a process of establishing a high-fidelity detection system for monitoring the existence of an immunosuppressive environment. This novel approach will enable a better understanding of tumor-associated immunosuppression and facilitate the design of innovative strategies for cancer immunotherapy that will be combined with monitoring the patient's immune status prior to a given immunotherapy. If immunosuppression is detected, specific inhibitors for the immunosuppressive environment will be applied prior to a given immunotherapy, thus enabling the establishment of a successful personalized cancer therapy.

O103

Functional Studies on Toll-Like Receptor Expression on Cell Lines of Laryngeal Carcinoma

Jan Żeromski[1], Jan Sikora[1], Magdalena Frydrychowicz[1], Bartosz Brzezicha[1], Grzegorz Dworacki[1], Mirosław Szczepański[1]
[1]*Poznan University, of Medical Sciences, Poznan, Poland*

Toll-like receptors (TLRs) have been shown to play crucial role in the recognition of unicellular pathogens and mount protective immune response. They create link between innate and adaptive immunity. TLRs are abundant on cells of the immune system but have been also demonstrated on cells of other origin such as various epithelia. We were able to show the expression of three TLRs (TLR2, 3 and 4) on tumor cells of human laryngeal carcinoma by means of immunohistochemistry. This study was followed by the demonstration of most TLRs on cell lines of this cancer both, on protein and molecular level. On the current study we wished to see the impact of respective TLR ligands on TLR expression in the cells mentioned.

Six larynx carcinoma cell lines obtained from Pittsburgh Cancer Institute, USA, (courtesy of prof. Theresa Whiteside), have been used. They were cultured for 24 hrs in the presence of respective TLR ligands. Following culture cells were harvested and subjected to flow cytometry both on intact and permeabilized cells, using fluorochrome labelled anti TLR1-10 monoclonal Moabs. The cells were evaluated in FacsCanto (BD) flow cytometer for mean fluorescence intensity (MFI) of membrane and cytoplasmic cell staining, using FacsDiva software.

Results: Each cell line exhibited distinct pattern of expression of individual TLRs following interaction with respective ligand. Unexpectedly, cell culture with ligand resulted in the decrease of TLR expression in some cell lines. Cytoplasmic TLR staining had usually higher MFI value than membrane one. TLRs 5, 7 and 9 showed the highest expression in the majority of tumor cells tested. In general, cytoplasmic TLR protein product formation seems to exceed cell membrane expression. In conclusion, culture of TLR expressing tumor cells with respective ligand has ambiguous effect on TLR expression but points out for potential reactivity of tumor cells with TLR agonists.

O104

Functional Assessment of the Inflammatory Tumor Microenvironment during Spontaneous Breast Cancer Progression and Metastasis Formation

Metamia Ciampricotti[1], Tisee Hau[1], Ewoud Speksnijder[1], Jos Jonkers[1], **Karin de Visser[1]**
[1]*Department of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands*

Interactions between cancer cells and normal, healthy cells present cells are one of the most abundant cell types recruited to the microenvironment of many tumors. The role of the immune system during tumorigenesis is rather controversial; both tumor-protective and tumor-promoting properties of the immune system have been described. It is currently unclear which tumor types and which tumor stages are either positively or negatively regulated by specific components of the immune system. The overall goal of our research is to address the role and underlying pathways of the adaptive and innate immune system during spontaneous breast cancer progression and metastasis formation. We utilize a mouse tumor model that faithfully recapitulates human invasive and metastatic lobular carcinoma, e.g. a conditional mouse breast cancer model based on mammary epithelium-specific deletion of p53 and E-cadherin. Like human breast cancers, mammary carcinomas arising in this mouse model are characterized by abundant presence of innate immune cells, including degranulating mast cells and macrophages, T and B lymphocytes, antibody depositions and increased levels of pro-inflammatory mediators. Suppression of chronic inflammation attenuates premalignant progression and tumor formation. Preliminary data suggest a critical role of adaptive immune cells in outgrowth of metastases. By genetic elimination and pharmacological inhibition of specific subsets of the adaptive and innate immune system, we are currently investigating their functional significance in a tumor-stage specific manner. Ultimately, the outcome of these studies may shift therapeutic focus from a cancer cell intrinsic point of view towards a more combined cancer cell intrinsic and extrinsic point of view (Research supported by the Dutch Cancer Society, NKB 2006–3715 and NWO/VIDI 91796307).

O105

A Novel Tumor-Derived Inflammatory Myeloid Suppressor Cell Subset Inhibits Anti-Tumor Activity of T and NK Cells

Moshe Elkabets[1,2], Christian A. Vossherich[2], Shahar Dotan[1], Vera G. Rinerio[2], Elena Voronov[1], Charles A. Dinarello[3], Sussan Ostrand-Rosenberg[4], James Di Santo[2], Ron N. Apte[1]
[1]*The Shraga Segal Department of Microbiology and Immunology, The Cancer Research Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel,* [2]*Unité des Cytokines et Développement Lymphoide, Institut Pasteur, Paris, France,* [3]*Department of Medicine, University of Colorado Health Sciences Center, Denver, CO, USA,* [4]*Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD, USA*

Chronic inflammation is associated with the promotion and enhancement of malignancy and tumor growth. Many tumors enhance the accumulation of myeloid derived suppressor cells (MDSC), which contribute to tumor progression and escape from the immune system, by inducing tolerance of suppression. Previously, we have shown that tumor-derived IL-1β secreted into the tumor microenvironment can induce a massive accumulation of MDSC in the spleen of tumor bearing mice and induce T cell suppression. In this work, we describe a novel polymorphonuclear MDSC subpopulation characterized by the phenotype: $Gr1^{+}CD11b^{+}IL\text{-}4Ra^{+}CD115^{high}\text{-}Ly6C^{low/-}$ SSC^{high} which we termed– Inflammatory MDSC (Inf-MDSC). This population accumulates in the BM and spleen of mice bearing 4T1 breast cancer tumors of cells which over-expressing IL-1β (4T1/IL-1β) in IL-1β dependent manner. We showed the involvement of other inflammatory mediators such as IL-6 and PGE2, in Inf-MDSC accumulation and differentiation. Unlike to the MDSC from 4T1 tumor bearing mice, the expression of T cell mediates suppression; INOS2 and Arginase1 by Inf-MDSC are not dependent on IFNg. Inf-MDSC are able to suppress NK cell activity *in vivo* via reduction of the NK activating receptor NKG2D. *In vitro* this suppressive activity is dependent on cell-to-cell contact. The inflammatory signal (IL-1b) up-regulates IL-4Ra expression of MDSC, which correlates with enhanced tumor growth and suppression of cytotoxic activity of NK cell. Our data suggest that tumor derived inflammation enhances the development of a specific MDSC subset that has the ability to suppress T and NK cells, and therefore, can serve as a new target for chemotherapy.

O106

Triggering of TLR7 and 8 on Human Lung Cancer Induces Cell Survival and Chemoresistance

Julien Cherfils-Vicini[1], Sophia Platonova[1], Pierre Validire[1], Fathia Mami-Chouaib[2], Marie-Caroline Dieu-Nosjean[1], Wolf Herman Fridman[1], Christos Chouaid[3], Diane Damotte[1], Catherine Sautès-Fridman[1], **Isabelle Cremer[1]**
[1]*Team 13: Immune microenvironment and tumors, U872 INSERM, Paris, France,* [2]*Institut Gustave Roussy, U753 INSERM, Villejuif, France,* [3]*Service de pneumologie, AP-HP Hôpital St Antoine, Paris, France*

Lung tumor prognosis is very bad, with a survival rate being 20 to 30% five years after surgery. In general, patients relapse into three years because they develop metastasis. It is thus crucial to identify novel therapies or combinatory therapies to improve the prognosis of the disease. To date, the proposed therapies for NSCLC patients consists in surgery associated with neo-adjuvant or adjuvant polychemotherapy. Novel cancer immunotherapies using TLR7 or 8 agonists are being developed, which are based on the amplification of immune responses. However, recent studies implicate some TLRs in tumor development based on their ability to facilitate tumor growth, but TLR7 and 8 have not yet been implicated. We hypothesized that TLR7 and 8 are expressed by lung tumor cells, and their signaling could interfere with chemotherapy-induced cell death.
We demonstrate for the first time that TLR7 and TLR8 are highly expressed by primary human lung tumor cells in NSCLC. We show TLR7 ligation with Loxoribine or TLR8 ligation with Poly U results in activation of NF-kB and upregulation of Bcl-2 expression. This is associated with increased tumor cell survival and a strong resistance to apoptosis induced by chemotherapeutic agents that are currently used to treat patients. Finally, transcriptional analysis revealed a gene expression signature that suggests chronic stimulation of tumor cells by TLR7 and 8 ligands in situ.
TLR7 or 8 expression by lung tumor cells in patients could predict bad responders to standard chemotherapies and could allow to adapt the new therapeutic protocols. We propose that anticancer immunotherapies using TLR7 or 8 adjuvants should take into account the expression of these TLRs on tumor cells. Indeed, such adjuvants could be a double edge sword by acting on cells of the immune system or on tumor cells or both, with opposite effects.

O107

Tumor-Specific CD4CD8ab T Cells Infiltrating Human Colorectal Tumors

Murielle Corvaisier[1], **Guillaume Sarrabayrouse[1],** Laure-Hélène Ouisse[1], Céline Bossard[1], Bernard Le Mével[2], Elisabeth Diez[1], Lucien Potiron[3], Nadine Gervois[1], Agnès Moreau-Aubry[1], Francine Jotereau[1]
[1]*INSERM U892, Nantes, France,* [2]*Centre Régional de lutte contre le cancer, Nantes, France,* [3]*Service de Chirurgie digestive, Clinique Jules Verne, Nantes, France*

Despite the demonstration that high T cell infiltration of Colorectal tumors (CRC) is of good prognosis, few is known about the tumor reactivity of CRC infiltrating lymphocytes (TIL). The presence in CRC, and phenotype of tumor reactive TIL was addressed. We obtained ex-vivo TIL and TIL lines, by enzymatic digestion or culture respectively, from primary, and metastatic CRC samples (n=4), and tumor cell lines from four of these. TIL reactivity to tumor cells was analyzed by intracellular cytokine secretion. In two patients tumor-reactive T cells were detected among a subset of TCRab CD8ab+CD4+ double positive (DP) TIL. Using a DP TIL clone tumor reactivity was shown to be HLA-A2 restricted and directed against a large panel of carcinoma but not EBV-B or normal-cell lines. We then documented the presence of DP T cells in human CRC and healthy colon mucosa, and showed that these cells produced higher levels of IL-4 and IL-13 than CD4+ or CD8 + SP T cells. These findings demonstrate the presence of DP T cells in human normal colon mucosa and colonic tumor samples, and show a major contribution of this subset to CRC TIL reactivity. Their high capacity to secrete IL-4 and Il-13 suggests that colon DP T cells are likely involved in colonic mucosa homeostasis and in the immunity to human CRC.

O108

The Signaling Pathway PAR1-PAFR-MUC18 Links Inflammation with Melanoma Metastasis

Vladislava O. Melnikova[1], Gabriel J. Villares[1], Andrey S. Dobroff[1], Maya Zigler[1], Krishnakumar Balasubramaniam[1], Hua Wang[1], Victor Prieto[1], Menashe Bar-Eli[1]
[1]*Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA*

The cellular and molecular pathways that regulate platelet activation, blood coagulation, and inflammation are emerging as critical players in cancer progression and metastasis. We previously demonstrated that the pro-inflammatory Protease-Activated Receptor a (PAR1, thrombin receptor) is overexpressed in metastatic melanoma, where it modulates the expression of IL-8, MMP-2, VEGF, PDGF, and integrins. Most recently, we demonstrated that antagonists of the pro-inflammatory Platelet-Activating Factor receptor (PAFR) abrogate experimental human melanoma lung metastasis. We found that PAF activates p38 MAPK/CREB-mediated expression of MMP2 and MT1-MMP. Here, we demonstrate that in metastatic melanoma cells, PAR1 and PAFR are constitutively active, linked together and regulate gene expression. Indeed, in A375SM and C8161-c9 metastatic melanoma cells, silencing of PAR1 with small hairpin RNA resulted in downregulation of expression of PAFR and decrease in production of PAF. Silencing of either PAR1 or PAFR expression abrogated expression of MUC18, a critical marker of homo- and heterotypic adhesion in melanoma. Overexpression of PAFR led to restoration of MUC18 expression in PAR1shRNA cells, suggesting that PAFR acts downstream of PAR1. We found that PAR1-PAFR-MUC18 signaling mechanism mediates melanoma cells' adhesion to microvascular endothelial cells, transendothelial migration, and metastatic retention in the lungs. Rescuing PAFR expression in PAR1-silenced cells restores metastatic phenotype of melanoma, indicating that PAFR plays critical role in the molecular mechanism of PAR1 action. Correlating with our previous findings on PAR1, tissue microarray analysis revealed elevated PAFR expression in primary human melanomas with subsequent metastasis. Finally, we demonstrate that PAFR knockout mice have delayed B16F10 mouse melanoma tumor growth and lower B16F10 tumor incidence as compared to wild-type C57Bl/6 counterparts. Together, our results link the two pro-inflammatory G-protein coupled receptors, PAR1 and PAFR, with the metastatic dissemination of melanoma and suggest that functional PAFR is essential for pro-tumorigenic influence of the tumor microenvironment. Our findings suggest that PAR1, PAFR and MUC18 are attractive therapeutic targets for preventing melanoma metastasis.

O109

Extensive Upregulation of Proinflammatory Cytokines in the Gastric Mucosa of Stomach Cancer Patients

Jenni Adamsson[1], Shugui Wang[2], Bert Kindlund[1], Åsa Sjöling[1], Henrik Sjövall[4], Lars-Erik Hansson[3], Sven Pettersson[2], Ann-Mari Svennerholm[1], **Samuel Lundin[1]**
[1]*Department of Microbiology and Immunology, University of Gothenburg, Gothenburg, Sweden,* [2]*Genome institute of Singapore, Singapore, Singapore,* [3]*Department of Surgery, University of Gothenburg, Gothenburg, Sweden,* [4]*Department of Internal Medicine, University of Gothenburg, Gothenburg, Sweden*

In patients with gastric cancer, as well as other epithelial cancers, there is an over-expression of proinflammatory cytokines. This is accompanied by increased activation of NF-κB, which is believed to contribute to tumor growth through inhibition of apoptosis of malignant and premalignant cells.
To make a comprehensive investigation of the expression and regulation of cytokines and other immune mediators in *Helicobacter pylori*-induced gastric cancer, we performed a cDNA microarray analysis of biopsies from tumour and tumour non-affected tissue of gastric cancer patients as well as from antrum and corpus tissues of cancer-free patients with or without *H. pylori* infection.
The analysis showed that around 10000 genes were expressed at significant levels in the stomach mucosa, and a large number of proinflammatory cytokines were upregulated in gastric cancer patients. The expression of several of these molecules was also verified by RT-PCR analysis. The upregulated genes

include both previously reported ones such as IL-1, IL-6, IL-8, IL-11, WNT5A, COX-2 and MMP3, but there were also novel findings such as increased expression of the cytokines IL-24 and LIF and the metalloproteinases ADAMTS4 and ADAMTS5 in gastric cancer tissue. Several of the upregulated cytokines were also increased in *H. pylori*-infected cancer-free subjects, but in gastric cancer patients the inflammation was uncoupled to infection since bacteria were not detectable in their stomach tissue.
In conclusion, we demonstrate an extensive upregulation of proinflammatory cytokines in the stomach mucosa of gastric cancer patients, and we believe that this cytokine imbalance may contribute to development and progression of gastric cancer.

O110

Host Osteopontin Maintains an Acute Inflammatory Response in the Tumor Microenvironment to Suppress Extrinsic Cancer Cell Progression

Yu-Hua Hsieh[1], Margaret Juliana[2], Kang-Jey Ho[3], Henri van der Heyde[4], Craig Elmets[5], **Pi-Ling Chang[1]**
[1]*Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, USA,* [2]*Department of Genetics, University of Alabama at Birmingham, Birmingham, AL, USA,* [3]*Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA,* [4]*La Jolla Infectious Disease Institute, San Diego, CA, USA,* [5]*Department of Dermatology, University of Alabama at Birmingham, Birmingham, AL, USA*

Although numerous cancer types express the matricellular protein, osteopontin (OPN), and its levels in the plasma of cancer patients are elevated implicating cancer cell-derived OPN in facilitating tumor progression, the role of OPN expressed by other cells in tumor progression is unclear, due to the lack of appropriate study model. To assess the impact of host-derived OPN on tumor progression and its contribution to levels in the serum, we established a murine cutaneous OPN-null squamous cell carcinoma (SCC) cell line (ONSC) consisting of *H-Ras* and *p53* mutations and which has the ability to develop SCC in immune-competent mice. Subcutaneous injection of ONSC cells led to the development of SCC, with a dramatic decreased incidence in wild-type compared with OPN-null mice by 8–10 wk. Histopathological, biochemical and hematological analyses of the tumor microenvironment and/or serum from tumor-bearing mice during the first few weeks indicated that 1) ONSC survival, proliferation and differentiation in a weak acute inflammatory microenvironment of OPN null mice is independent of OPN, and 2) host-derived OPN is necessary for maintaining an acute inflammatory response leading to lower incidence of SCCs in wild-type mice. Its effect is not through increasing circulating inflammatory cells or chemotaxis, instead we postulate that the response is likely accomplished by enhancing the effect of and/or extending the life of inflammatory cells in the tumor microenvironment. Further, the elevated serum levels of OPN in mice harboring ONSC tumors contributed by host cells, including activated, circulating inflammatory cells, indicates that the actual role of blood OPN in cancer patients requires further investigation.

O111

Tumour Formation Initiated by Nondividing Epidermal Cells via an Inflammatory Infiltrate

Esther N. Arwert[1], Rohit Lal[2], Fiona M. Watt[1]
[1]*Department of Epithelial cell biology, Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Cambridge, UK,* [2]*Department of Medical Oncology, Guy's and St Thomas' Foundation Trust, Guy's Hospital, London, UK*

Multi-layered epithelia, such as the epidermis, comprise a basal layer of dividing cells, including stem cells, and suprabasal layers of nondividing cells that are undergoing terminal differentiation. Since a hallmark of cancer is uncontrolled proliferation, it is widely assumed that tumours only start from dividing cells. Here I show that nondividing epidermal cells in which mitogen-activated protein kinase kinase 1 (MEK1) is constitutively active can initiate tumour formation by recruiting basal cells that lack oncogenic changes to the tumour mass. Tumour formation occurs when the skin is wounded, and is dependent on an inflammatory infiltrate including T-cells and macrophages. Tumours fail to form when the infiltrating bone marrow-derived cells lack MyD88, a scaffolding protein that acts downstream of both the IL1 receptor and Toll-like receptors. These results show that nondividing, differentiated cells can initiate tumor formation without re-acquiring the ability to divide.

O112

The Human Pro-inflammatory Antimicrobial Peptide LL-37 Supports Ovarian Tumor Progression by the Recruitment of Multipotent Mesenchymal Stromal Cells and other Immunosuppressive Cells

Seth Coffelt[2], Ruth Waterman[1], Sarah Henkle[3], Suzanne Tomchuck[3], **Aline M. Betancourt[3]**
[1]*Department of Anesthesiology, Tulane University Medical Center, New Orleans, LA, USA,* [2]*Tumor Targeting Group, University of Sheffield School of Medicine, Sheffield, UK,* [3]*Department of Microbiology and Immunology, Tulane University Medical Center, New Orleans, LA, USA*

Tumors depend on a permissive and supportive microenvironment for their growth and spread. Emerging evidence suggests that both resident and recruited bone marrow-derived cells play a critical and supportive role in creating a pro-tumorigenic host immune response. Indeed, an increased prevalence of recruited leukocytes in tumors is correlated with a poor prognosis for the affected patient. By contrast, therapies that eradicate certain immune cells from the tumor microenvironment lead to longer remission periods for the treated patient. Along with other recruited cells, multipotent mesenchymal stromal cells (MSCs) formerly known as mesenchymal stem cells are also known to proceed from the bone marrow to tumors, and once there to reside within tumor stromal microenvironments. Previous studies have shown that LL-37 (leucine, leucine-37), the C-terminal peptide of human cationic

antimicrobial protein 18, stimulates the migration of various immune cell types and is overexpressed in ovarian, breast, and lung cancers. Although there is evidence to support a pro-tumorigenic role for LL-37, the function of the peptide in tumors remains unclear. Here, we demonstrate that neutralization of LL-37 in vivo significantly reduces the engraftment of MSCs into ovarian tumor xenografts, resulting in inhibition of tumor growth as well as in the disruption of the fibrovascular network. These tumor-associated MSCs secrete pro-inflammatory and pro-angiogenic factors that further influence the immunosuppressive tumor microenvironment. The data indicate that LL-37 facilitates ovarian tumor progression through the recruitment of progenitor cell populations that further help establish a favorable ovarian tumor microenvironment.

O113

Heparanase: A Critical Determinant of Breast Cancer Metastasis to Brain

Lixin Zhang[1], Peter Calkins[1], Peggy Sullivan[3], **Dario Marchetti[1,2]**
[1]*Department of Pathology, Baylor College of Medicine, Houston, TX, USA,* [2]*Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA,* [3]*Department of Pathology, University of California-Los Angeles, Los Angeles, CA, USA*

Due to the increasing incidence of breast cancer brain metastasis (BCBM), the identification of mechanisms responsible for brain metastasis formation is imperative to develop novel therapies. Specifically, mechanistic links between Her-2 and BCBM determinants are needed to elucidate the known correlation between Her-2 overexpression and BCBM onset. Heparanase (HPSE) is the only functional mammalian endoglycosidase degrading heparan sulfate (HS), the main polysaccharide of basement membranes and tumor-surrounding extracellular matrix. HPSE relevance in cancer progression has been established: HPSE overexpression correlates with metastasis, tumor vascularity, and with shorter post-operative patient survival, making it an active target for anti-cancer therapeutics.
We hypothesized that Her-2 augments BCBM by inducing HPSE via Her-2/epidermal growth factor receptor (EGFR) signaling. We examined HPSE levels, intracellular trafficking, and activity in two human Her-2 - expressing BCBM cell systems (MDA231Br3/2/1 and MDA231Br/Her-2/neo) and BCBM clinical specimens. We demonstrate that: 1) HPSE is present and functional according to their brain metastatic propensities (231Br3 > 231Br2 > 231Br1 > 231Parental) and Her-2 content; 2) EGF induces HPSE expression and nucleolar localization in a dose/time-dependent manner; 3) DNA Topoisomerase I is a HPSE target in nucleoli of BCBM cells. Equally relevant, to determine whether microRNAs play roles in HPSE regulation, we used microRNA bioinformatic programs and identified miR-1258 as a *bona fide* microRNA targeting *hpse* 3'-UTR region. Second, to determine miR-1258 contributions modulating HPSE expression and activity in BCBM, we performed gain-/loss-of-function studies using miR-1258 mimics and inhibitors, and discovered that miR-1258 affects HPSE abilities to promote in vitro cell invasion and BCBM in xenografts.
These investigations provide first-time evidence showing that: 1) HPSE is relevant in BCBM via Her-2 – dependent modalities; 2) *hpse* is a gene target of microRNA regulation; 3) MiR-1258 is a primary *hpse* microRNA candidate; 4) MiR-1258 regulates HPSE affecting BCBM in vitro and in vivo.

O114

A Ceramide Rheostat Balances Angiogenesis and Anti-angiogenesis

Jean-Philip Truman[1], Monica Garcia-Barros[2], Matthew Kaag[3], Dolores Hambardzumyan[4], Branka Stancevic[2], Michael Chan[6], Daniel Hicklin[5], Zvi Fuks[1], Richard Kolesnick[2], **Adriana Haimovitz-Friedman[1]**
[1]*Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [2]*Laboratory of Signal Transduction, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [3]*Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [4]*Department of Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [5]*ImClone Systems, Inc., New York, NY, USA,* [6]*Wake Forest University School of Medicine, Winston-Salem, NC, USA*

Genetic data indicate an acute wave of ceramide-mediated endothelial apoptosis, initiated by acid sphingomyelinase (ASMase), regulates tumor stem cell response to single high-dose radiotherapy, obligatory for tumor cure. Here we show that bFGF or VEGF pre-treatment of cultured endothelium prevent ASMase activation, ceramide generation and endothelial apoptosis, events reversible with exogenous C_{16}-ceramide. Anti-VEGFR2 acts conversely, enhancing ceramide generation and apoptosis. *In vivo*, intravenous anti-VEGFR2 DC101 or anti-VEGF G6-31, if delivered immediately prior to radiation, synergistically increase ASMase-mediated endothelial apoptosis, and radiation cure of MCA/129 fibrosarcomas and B16 melanomas implanted in wild-type mice. However both agents fail to radiosensitize tumors in $asmase^{-/-}$ mice, which provide apoptosis-resistant vasculature, or in wild-type littermates pre-treated with anti-ceramide antibody. Hence, VEGF/bFGF fail to suppress apoptosis if ceramide levels remain elevated while anti-angiogenic therapies fail without ceramide elevation, defining a ceramide rheostat that determines outcome of single-dose radiotherapy.
Significance: Anti-angiogenic therapy is currently conceived to act by two differing mechanisms. One postulates anti-angiogenesis prevents recruitment of endothelium into nascent or damaged vasculature, effectively starving tumor, while the other proposes anti-angiogenic therapies "normalize" dysfunctional tumor vasculature thereby improving perfusion and drug delivery. The "ceramide rheostat" model provides a pharmacologically-tractable alternative paradigm for combining anti-angiogenesis with anti-cancer treatments that target tumor

stem cell clonogens directly. Understanding the ordering of biochemical events of radiation-induced, ceramide-mediated endothelial cell death and its consequence for tumor response enables design of temporal sequencing of anti-angiogenic drugs preceding irradiation that optimizes radiosensitization, enhancing tumor cure.

O115

Heparanase Role in Oral Cancer Prognosis and Cellular Differentiation

Yoav Leiser[1,4], Imad Abu-El-Naaj[1], Edmond Sabo[3], Dan Deutsch[5], Philip Lazarovici[6], Micha Peled[1,2], Israel Vlodavsky[4]
[1]*The Department of Oral and Maxillofacial Surgery, Rambam Medical Center, Haifa, Israel,* [2]*The Faculty of Medicine, Technion, Haifa, Israel,* [3]*Department of Pathology, Rambam Medical Center, Haifa, Israel,* [4]*The Bruce Rappaport Faculty of Medicine, Cancer and Vascular Biology Research Center, Haifa, Israel,* [5]*Dental Research Laboratory, Institute of Dental Sciences, The Hebrew University Faculty of Dental Medicine, Hadassah Medical Center, Jerusalem, Israel,* [6]*Department of Pharmacology and Experimental Therapeutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel*

Background:
Numerous studies have shown that metastases formation depends on the ability of tumor cells to invade basement membranes and tissue barriers in a process involving enzymes capable of degrading extracellular matrix (ECM) components. One of these enzymes is heparanase, an endoglycosidae which degrades heparan sulfate.
Purpose:
Examine the expression of heparanase in oral carcinomas and establish whether its extent, intensity and cellular localization can be of prognostic value in predicting the outcome of oral cancer patients and explore its role during cellular differentiation.
Methods:
Biopsy specimens from 50 oral carcinoma patients were immunohistochemically analyzed for the expression and cellular localization of heparanase, PC12 (pheochrocmocytoma) cultures were used as an in-vitro model of cellular differentiation induced by NGF.
Results:
Nuclear localization of heparanase was observed in all oral verrucous carcinomas, a very well differentiated tumor that rarely metastasize, as opposed to only 28% of nuclear localization detected in oral squamous cell carcinomas. Heparanase expression level also significantly correlated with the degree of tumor differentiation. Moreover, while cytoplasmic localization of heparanase was associated with high grade carcinomas, nuclear localization of the enzyme was found primarily in low grade, well differentiated tumors. Heparanase was suggested to be involved in the differentiation of PC12 cell and was up regulated 6.5 fold during NGF induced cellular differentiation. Furthermore, NGF receptor TrkA seems to be involved in heparanase up regulation in PC12.
Conclusion:
In rarely metastasizing verrucous carcinomas, heparanase was expressed in the cell nucleus, as opposed to metastasizing oral squamous cell carcinomas which exhibited mostly cytoplasmic localization of the enzyme. Expression level and cellular localization of heparanase could serve as reliable predictive indicators of oral carcinoma development, metastatic potential and patient prognosis.

O116

Perivascular Expression of CXCL9 and CXCL12 in Primary Central Nervous System Lymphoma: Chemokine Synergism Controls Cell Infiltration and Positioning

Daniel Venetz[1], Maurilio Ponzoni[2], Milena Schiraldi[1], Andres J.M. Ferreri[2], Francesco Bertoni[3], Claudio Doglioni[4], **Mariagrazia Uguccioni[1]**
[1]*Unit of Chemokines and Inflammation, Institute for Research in Biomedicine, Bellinzona, Switzerland,* [2]*Unit of Lymphoid Malignacies, Scientific Institute San Raffaele, Milan, Italy,* [3]*Laboratory of Experimental Oncology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland,* [4]*Institute of Pathology, University Vita Salute san Raffaele, Milan, Italy*

Primary central nervous system lymphomas (PCNSL) are aggressive malignancies confined to the CNS, mostly of diffuse large B cell histotype. Despite improved understanding of the malignant B cell phenotype, little is known on the tumour microenvironment and the response of the adaptive immunity against PCNSL. We investigated the phenotype of tumour infiltrating lymphocytes (TILs) and the expression of chemokines in 22 cases of PCNSL from immunocompetent patients. CD8+ T cells are selectively recruited to the tumour mass and represent the majority of TILs. They tend to accumulate in perivascular areas, are Granzyme B+, and vigorously proliferate *in situ*. Their localization and density correlates with the expression of the inflammatory chemokine CXCL9 in the perivascular microenvironment. In addition to CXCL9, CXCL12 is coexpressed on the tumour vasculature and forms heterocomplexes with CXCL9, which enhance migration of CXCR4+ malignant B cells. These findings indicate the presence of a strong chemoattractant stimulus in the perivascular microenvironment which serves as an important regulator for the recruitment of adaptive immune effectors and for the angiocentric positioning of malignant B cells in the perivascular cuff.

O117

A Molecular Signature of Melanoma Brain Metastasis: Development and Characterization of a Novel Human Melanoma Mouse Model

Sivan Izraely[1], Orit Sagi-Assif[1], Anat Klein[1], Tsipi Meshel[1], Ilana Yron [1], Galia Tsarfaty[2], Dave S.B. Hoon[3], Isaac P. Witz[1]
[1]*Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel-Aviv, Israel,* [2]*Department of Diagnostic & Imaging, Sheba Medical Center, Tel Hashomer, Israel,* [3]*Department of Mulecular Oncoloy, John Wayne Cancer Institute, Saint John's Health Center, Santa Monica, CA, USA*

Brain metastasis confers upon melanoma patients an extremely bad prognosis. The mechanisms underlying homing to and survival of metastatic melanoma cells in the brain are unknown. Our working hypothesis is that interactions of melanoma cells with microenvironmental factors of the brain regulate site specific metastasis to this organ.

Our main objective is to identify key molecules associated with melanoma brain metastasis that could serve as therapeutic targets.

We developed three systems of human melanoma variants that form either local cutaneous tumors or variants from the same human melanoma that form brain metastasis in xenografted nude mice. As these variants have an identical genetic background, any molecular differences between these variants reflect alterations associated with the ability to form brain metastasis. We are currently using these variants to establish a melanoma brain metastasis specific genetic signature.

Gelatin zymography was used to determine MMP-2 activity in the melanoma variants. Brain metastatic variants displayed a relatively higher activity level of MMP-2 than local variants, indicating a greater ability of the metastatic variants to invade through basement membrane.

To identify chemokine receptors that might be involved in melanoma homing to the brain, we analyzed the expression of chemokine receptors and the membrane-bound chemokine CX3CL1 in the local and metastatic variants. Five chemokine receptors (CCR3, CCR4, CXCR3, CXCR7 and CX3CR1) and CX3CL1 were expressed on the melanoma variants.

Other surface molecules associated with tumor progression were found to be differentially expressed on local and metastatic variants. Utilizing microarrays, we generated gene expression profiles of the melanoma variants. This analysis revealed a set of genes differentially expressed in local and metastatic variants.

Ongoing work focuses on differential interactions of local and brain metastasizing variants with brain endothelia.

This study was supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (Needham, MA, USA)

O118

Characterization of Interleukin-8 Promoted Protease Expression and Activity in Relation to Prostate Cancer Metastasis to the Bone

Ashleigh Hill[1], Johanna Pettigrew[1], Pamela Maxwell[1], David Waugh[1]
[1]*Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland, UK*

Interleukin-8 (IL-8) is a proinflammatory CXC chemokine which activates intracellular signalling downstream of two cell surface receptors CXCR1 and CXCR2. We have demonstrated increased expression of IL-8, CXCR1 and CXCR2 in malignant epithelium in human prostate cancer, with expression greatest in androgen-independent metastatic prostate cancer tissue. However, since CXCR1 and CXCR2 receptors are also expressed on endothelial cells, infiltrating neutrophils and tumour associated macrophages, the release of IL-8 from cancer cells is likely to make a significant contribution in regulating the constitution and activity of the tumour microenvironment. In addition, the detection of CXCR1 and CXCR2 expression on bone marrow stromal cells indicates that infiltrating metastatic cells with elevated IL-8 expression may have enhanced capacity to regulate the microenvironment of the bone marrow cavity. Our studies are focused on characterizing the effects of tumour-derived IL-8 signalling in modulating the function of prostate cancer and bone marrow stromal cell function in order to orchestrate the remodelling and colonisation of the bone by metastatic prostate cancer cells. Administration of IL-8 to three prostate cancer cell lines (LNCaP, PC3 and/or 22RV1 cells) and two bone marrow stromal cell lines (HS5 and HS27A) increases AP-1 and NF-κB-directed gene transcription, leading to increased expression of cathepsin K and the potent, cell-tethered collagenase, MT1-MMP, enzymes known to be implicit in promoting bone turnover. Furthermore, our studies demonstrate that IL-8 signalling promotes nuclear translocation of the transcriptional co-activator, β-catenin, underpinning increases in the expression of a downstream gene target of TCF/LEF transcripiton complex, the serine protease uPA. RNAi-mediated attenuation of β-catenin expression attenuated this IL-8 induced increase in uPA expression. Current studies are characterizing the importance of IL-8-induced protease activity within the bone microenvironment, initiating bone remodelling and promoting activation of matrix-associated growth factors to underpin the osteoclastogenic and osteoblastic phases of bone metastasis in prostate cancer.

O119

MMP-14 (MT1-MMP) Mediated Endoglin Shedding Regulates Tumour Angiogenesis

Lukas Hawinkels[1,2], Patty Kuiper[2], Hein Verspaget[2], Eliza Wiercinska[1], Roeland Hanemaaijer[4], Peter ten Dijke[1], Cornelis Sier[2,3]
[1]*Department of Molecular Cell Biology and Centre for Biomedical Genetics, Leiden University Medical Centre, Leiden, the Netherlands,* [2]*Department of Gastroenterology-Hepatology, Leiden University Medical Centre, Leiden, the Netherlands,* [3]*Department of Surgery, Leiden University Medical Centre, Leiden, the Netherlands,* [4]*TNO, Quality of Life BioSciences, Leiden, the Netherlands*

Endoglin is a TGFβ coreceptor and is highly expressed on angiogenic endothelial cells with a crucial role in angiogenesis. A soluble form of endoglin is present in the circulation, which might possess anti-angiogenic properties. Increased soluble endoglin levels are reported in pregnant women suffering from pre-eclampsia, but reports on soluble endoglin in cancer patients are contradictory. We examined soluble endoglin levels in colorectal cancer in association with the endoglin shedding mechanism.
Immunohistochemical analysis of colorectal cancer specimens revealed high endoglin expression in angiogenic endothelial cells. Interestingly, low endoglin expression on the tumour vessels was accompanied by high MMP14 expression, the most abundantly expressed membrane-type MMP. In the circulation of 23 patients soluble endoglin levels were slightly decreased compared to healthy controls. The mechanism of endoglin shedding was evaluated *in vitro* using HUVEC endothelial cells, which secrete high levels of endoglin. The release of endoglin was inhibited by addition of broad-spectrum MMP inhibitors, but not by adding specific serine- or cystein-protease inhibitors. Specific inhibitors of the gelatinase or stromelysin MMP subclasses had no effect, indicating that MT-MMPs are the primary protease candidates. Therefore, we co-transfected endoglin and MMP14 in COS cells. Co-expression of endoglin and membrane-bound MMP14 led to strongly increased soluble endoglin levels, which required direct interaction between endoglin and MMP14. Cells co-transfected with a MMP14 mutant, lacking the trans-membrane domain, did not generate soluble endoglin. Knockdown of MMP14 by shRNA in HUVECs established that endoglin shedding was decreased upon reduction of MMP14 expression. Finally, we confirmed that soluble endoglin was capable of reducing angiogenic potential of endothelial cells using endothelial sprouting assays.
In conclusion, this study shows that MMP14 mediates endoglin shedding from endothelial cells, thereby regulating the angiogenic potential of endothelial cells in the colorectal tumour-microenvironment.

O120

Neuroblastoma Macro- and Micro-Metastasis: Interactions with the Microenvironment

Shelly Maman[1], Ido Nevo[1], Liat Edry-Botzer[1], Orit Sagi-Assif[1], Ilana Yron[1], Isaac P. Witz[1]
[1]*Department of Cell Research and Immunology, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel*

Neuroblastoma (NB) is the most common extracranial solid tumor in children. Survival rates of patients with metastatic disease are poor despite extensive efforts.
We developed an orthotopic mouse model for human NB metastasis comprising local and metastatic variants originating from single tumors. The inoculation of the metastatic variants into the orthotopic site (adrenal gland) generated lung macro-metastasis within 12–16 weeks, however, the inoculation of the local variants did not. Immunohistochemical examination did not reveal NB cells in the lungs or bone marrow (BM) of the mice inoculated with the local variant. In an attempt to possibly rescue micrometastatic cells from these organs, we cultured lungs and BM from mice orthotopically inoculated with local NB variants. After 6–12 weeks an outgrowth of NB cells was observed. Immuno-phenotypying of these cells indicated that the lungs and BM of the mice contained dormant human NB cells.
We hypothesize that the lungs and BM of NB-inoculated mice contain proliferation-restraining components against which the cells that form macro-metastasis developed resistance.
We tested this hypothesis and found that:
1. BM endothelial cells contain factors that inhibit the proliferation of micro BM metastases.
2. Spent medium of normal lung tissue contains factors that inhibit the proliferation of micro and macro lung metastases.
3. Spent medium of lung tissue from tumor-bearer mice contains factors that inhibit the proliferation of micro lung metastases but enhance the proliferation of macro lung metastases.
4. Micro BM metastases contain factors that enhance the proliferation of BM endothelial cells, in an organ specific manner.
The working hypothesis for future studies is that micrometastases remain dormant for long periods of time because they are inhibited by factors in their microenvironment.
This study was supported by a grant from Bonnie and Steven Stern, New York, NY, USA

O121

Co-expression of Invasive Markers (uPA, CD44) and Multiple Drug Resistance Proteins (MDR1, MRP2) is Correlated with Epithelial Ovarian Cancer Progression

Li Wang[1,2], Hongmin Chen[1,2], Jingli Hao[2,3], **Yong Li[2,3]**
[1]*Department of Gynecologic Oncology, Henan Tumour Hospital, Zhengzhou, Henan, China,* [2]*Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia,* [3]*Cancer Care Centre, St George Hospital, Sydney, NSW, Australia*

Background: Invasion and metastases of cancer cells and the development of resistance to anticancer therapies are the main causes of morbidity and mortality from cancer. The objective of this study was to correlate the expression of urokinase plasminogen activator (uPA) and CD44 with MDR1 and MRP2 in epithelial ovarian cancer (EOC) cell lines, primary tumours and metastatic lesions during EOC progression.
Methods: The expression and co-localization of uPA, CD44, MDR1 and MRP2 were examined on primary and metastatic EOC cell lines and paraffin-embedded tissue sections from primary EOC (n=120), the matched metastatic lesions (n=40) and normal ovarian tissues (n=20) using confocal microscope by different monoclonal antibodies.
Results: The co-expression of uPA, CD44, MDR1 and MRP2 was found in primary (OVCAR-3 and A2780) and metastatic (SKOV-3 and OV-90) cell lines. The expression of uPA, CD44 and MDR1was found in 88%, 83% and 88% of primary EOC and 90%, 85% and 90% of the matched metastatic lesions respectively and but not in normal ovarian tissues. Most of tumours showed moderate to strong intensity staining. The over-expression of uPA, CD44 and MDR1 was significantly associated with various progression parameters such as tumour stage, grade, residual disease status relapse and presence of ascites (P <0.05) but not with histology type (P>0.05). Co-localization of uPA, MDR1 and CD44 in primary tumours and metastatic lesions was observed.
Conclusions: Over-expression of uPA, CD44 and MRD1 is correlated with EOC progression; both uPA and CD44 are related with drug resistance during EOC metastasis and could be useful therapeutic targets to prevent the development of incurable, recurrent and drug resistance EOC.

O122

Kinoid Vaccine, a New Immunotherapeutic Generation to Target Tumor Released Ectopic Cytokines

Daniel Zagury[1], Bernard Bizzini[1], Robert C. Gallo[2], Armand Bensussan[3], Georges Uzan[4]
[1]*Science & Research Department, Néovacs SA, Paris, France,* [2]*Department of Human Virology, Institute of Human Virology; University of Maryland, Baltimore, MD, USA,* [3]*UMR 976, Institut National de la Santé et de la Recherche Médicale (INSERM), CHU Saint-Louis, Paris, France,* [4]*U972, Institut National de la Santé et de la Recherche Médicale (INSERM), Hopital Paul Brousse, Villejuif, France*

Ectopic cytokines released by cancer or stromal cells in the microenvironment of malignant tumors contribute to the cancer pathogenesis. IL-10 and TGF-β abnormally produced by some tumors may display an immunosuppressive effect; VEGF ectopically secreted by some cancer stromal cells participates in the neoangiogenesis; TNFα overproduction contributes to the cachexia occurring at terminal stages of cancer (1).
To counteract the effects of pathogenic cytokines in various chronic diseases, anticytokine Abs have been used either passively administered or induced by an active immunization. In some cancers, anti-VEGF mAbs used in association with chemotherapy have proved to be therapeutically beneficial (2).
Our group have prepared a VEGF immunogen, constituted by a KLH-VEGF heterocomplexe, termed VEGF kinoid. Active immunization of mice with the VEGF derivative immunogen, appropriately adjuvanted, proved to be fully innocuous and mounted a high anti-VEGF neutralizing Ab titer but not a cellular response. Purified IgG from immune sera decreased by ≥50% tumor growth of human A673 rhabdomyosarcoma cells and HT29 colon carcinoma xenografted in Swiss nude and Nod/SCID mice respectively. Following active mVEGF kinoid immunization, Balb/c mice challenged with syngeneic CT26 colorectal tumor cells showed a reduced growth of metastases and a reduced tumor vascularization but had no effect on the primary tumor cell growth (3).
In cancer treatments besides VEGF kinoid other kinoids targeting pathogenic cytokines could represent future medications as TNFα kinoid (4) which is currently used in Crohn's disease clinical trials.
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O123

Comparative Uncovering of Tumors' Systems Biology by Modular Targeting of Tumor-Associated Inflammation

Albrecht Reichle[1]
[1]*Hematology/Oncology, University Hospital Regensburg, Regensburg, Germany*

As yet, it is assumed that tumors defy experimental therapeutic access from inside in a comprehensive and reconstructive way (systems view) but only comply an observation-guided, contra-intuitive knowledge about biochemical pathways. Based on this familiar assumption the rational for new therapeutic strategies is commonly derived from theme-dependent context knowledge. Comparative analyses of anti-inflammatory activities and clinical response induced by continuously administeredbiomodulatory treatment modules (module M: metronomic low-dose chemotherapy, module A: pioglitazone plus etoricoxib, module A+M, and module A+M/+: plus second transcriptional modulator (interferon-alpha or dexamethasone) in metastatic stage of different metastatic tumors (266 patients; 54% systemically pre-treated; metastatic

melanoma, sarcoma, renal clear cell carcinoma, hormone-refractory prostate cancer, gastric cancer, and Langerhans' cell histiocytosis) were performed to uncover and reconstruct tumors' systems structures mediating tumor-associated inflammation (eight phase II trials, thereof two randomized). Tumor- and stage-specific therapeutic accessibility of inflammation-related processes to induce response in all tumor types indicates a constitutive spin-off of new systems functions during the metastatic process and differential integration of inflammation into the tumor compartments' context-dependent 'living world', which is featured by tumor- and subtype-specific rationalization processes: Inflammation-related activities are communicatively promoted and differentially adapted during tumor evolution. Empirically, differences may be detected in modalities of evolutionary systems development (heterogeneity in tumor-associated inflammation-related systems), and in the acquired functional impact of inflammation-related systems (tumor-specific mechanisms of action induced by metronomic low-dose chemotherapy). Availability of markers for 'late-stage' response to systems-directed anti-inflammatory therapies supports the tumors' modular features. Biomodulatory therapies, administered as fixed modules may contribute to discover and understand novel regulatory systems in tumor biology. The study highlights the claim for validity of therapeutic inflammation control as an important prerequisite for tumor control, which is shown to be the basis for action-relevant yes/no statements generating facts on-site in the tumor via biomodulatory therapy modules.

O124

Tumor Micoenvironment Is Controled by Procathepsin L Secretion: A New Gene Therapy to Inhibit Progression of Tumors Induced by Human Melanoma Cells

Raymond Frade[1]
[1]*INSERM U.672 (former U.354), Immunochemistry of Cell Regulations and Virus Interactions, EVRY, Ile-de-France, France*

We previously demonstrated that the switch from non to highly tumorigenic phenotype of human melanoma cells is directly related to procathepsin L secretion, which modified tumor microenvironment. Indeed, we demonstrated that secreted procathepsin L cleaves human C3, the third component of complement and consequently increases cell resistance to complement-mediated cell lysis. In addition, secreted procathepsin L cleaves other extracellular components. We clearly demonstrated the involvement of procathepsin L secretion in tumor progression by developing three different assays: 1) the inhibition of secreted procathepsin L activity by preincubating human melanoma cells with polyclonal anti-cathepsin L antibodies; 2) the increase of procathepsin L secretion by transfecting non-tumorigenic cells with cathepsin L cDNA to overexpress procathepsin L and to increase its secretion; 3) the inhibition of procathepsin L secretion. This latter was triggered by intracellular expression of an anti-human cathepsin L single chain variable fragment (ScFv), prepared in our laboratory from a monoclonal anti-cathepsin L antibody. In all these previous experiments, melanoma cells were processed before their injection into nude mice. Recently, we designed a new lentiviral vector in which this anti-cathepsin L-ScFv was cloned. This anti-cathepsin L ScFv lentiviral construct was optimized to transduce human melanoma cells with the highest intracellular expression of anti-cathepsin L-ScFv. In these transduced cells, procathepsin L secretion was strongly inhibited. In addition, injection of this anti-cathepsin L-ScFv lentiviral vector into tumors already induced in nude mice, inhibits tumor progression and associated angiogenesis. This is the first report to demonstrate that targeting procathepsin L secretion with anti-cathepsin L-ScFv lentiviral construct constitutes a new gene therapy to inhibit the progression of tumors induced by human melanoma cells.

O125

Disruption of Leukemia/Stroma Cell Interactions by CXCR4 Antagonists Enhances Chemotherapy and Signal Transduction-Induced Apoptosis in Leukemias

Michael Andreeff[1], Zhihong Zeng[1], Michael Fiegl[1], Marina Konopleva[1]
[1]*Molecular Hematology & Therapy, Departments of Stem Cell Transplantation & Cellular Therapy and Leukemia, UT M. D. Anderson Cancer Center, Houston, TX, USA*

The chemokine receptor CXCR4 is critically involved in the migration of hematopoietic cells to the stroma-derived-factor-1α (SDF-1a)-producing bone marrow microenvironment. We and others have previously demonstrated that stroma/leukemia interactions mediate protection of leukemic cells from chemotherapy-induced apoptosis (*Konopleva, Leukemia 16:1713, 2002*). Inhibition of CXCR4 with a specific peptide abrogated this effect and sensitized leukemic cells to chemotherapy (Zeng et al. MCT 5, 3113, 2006). Importantly, CXCR4 is upregulated by physiological hypoxia in the bone marrow (Fiegl et al. BLOOD, 113:1504, 2009) and contributes to pro-survival signaling in hematopoietic cells, through PI3K/AKT, MAPK and STAT3 signaling. AMD3465, a second generation small-molecule CXCR4 inhibitor with greater potency than AMD3100 (Plerixafor) was used to test the hypothesis that CXCR4 inhibition disrupts stromal/leukemia cell interactions and overcomes stroma-mediated resistance. Results show that AMD3465 inhibits surface expression of CXCR4 on AML cells and SDF-1a and stroma (MS-5)-induced migration of leukemia cells. *In vitro,* stromal cells protect leukemic cell lines and primary AML cells from spontaneous, chemotherapy, and tyrosine kinase (TKI) inhibitor-induced apoptosis. CXCR4 inhibition enhanced Ara-C-, Busulfan- and Sorafenib- (FLT3-ITD inhibitor) induced apoptosis and, importantly, downregulated AKT and MAPK signaling. *In vivo* xenografts into (NOD/SCID/IL-2Rα-1-) mice and syngeneic (Ba/F3-ITD) leukemia models showed even more pronounced effects, resulting in mobilization of leukemia stem cells and much enhanced efficacy of Ara-C and Sorafenib (Zeng et al. BLOOD, e-pub Oct 2008). In patients with AML in CR, treatment with AMD3100+G-CSF mobilized up to 80% leukemic cells into circulation. Conclusion: Data suggest that SDF-1a/CXCR4 inter-

actions contribute to the resistance of leukemic cells to chemotherapy and TKI-induced apoptosis. Disruption of these interactions by CXCR4 inhibitors represents a first strategy for targeting the complex leukemia cell/microenvironment interactions. Several clinical trials to test this concept in leukemia patients are in progress.

O126

Role of Tetrahydrobiopterin in Regulation of Tumor Angiogenesis Mediated by PI3K/Akt, eNOS and Ras Pathway

Liye Chen[1], Simon Briggs[1], Eric O'Neill[2], Jiliang Li[3], Russell Leek[3], David Kerr[1], Adrian Harris[3], **Shijie Cai[1]**
[1]*Department of Clinical Pharmacology, University of Oxford, Oxford, UK,* [2]*Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, UK,* [3]*Cancer Research UK Medical Oncology Unit, University of Oxford, Oxford, UK*

Emerged evidence suggests endothelial nitric oxide synthase (eNOS)-derived NO is particularly important in tumour angiogenesis and hence a novel target for cancer treatment. eNOS activation requires tetrahydrobiopterin (BH4) as a cofactor for NO production. However, the role of BH4 in eNOS regulation, potentially involving phosphatidylinositol 3-kinase (PI-3K) signalling pathway, remains to be established. The effects of BH4 in tumour angiogenesis are not known. To investigate this pathway, we augmented BH4 levels in vascular endothelial cells by supplementing cultures with sepiaterin, a BH4 precursor for the pterin salvage pathway synthesis. We also made a genetically modified murine fibroblast cell line over-expressing GTP cyclohydrolase I (GTPCH, the rate-limiting enzyme for the de novo BH4 synthesis) under doxycycline (Dox) control and analysed the effects in a mouse xenograft. In cell cultures, sepiapterin increased Akt/eNOS phosphorylation in a dose dependent manner in COS-7 cells (no endogenous eNOS) transfected with human eNOS cDNA. This augmentation was abrogated by wortmannin or Ly294002, PI3K inhibitors. eNOS/Akt phosphorylation by sepiapterin in both HUVEC and bovine aortic endothelial cells (BAEC) was also significantly enhanced, in association with increases in NO production, cell proliferation and migration, and capillarity-like tube formation. Furthermore, sepiapterin greatly increased GTP-bound wild-type Ras protein. But this effect was diminished by L-NAME, an eNOS inhibitor. In mouse xenografts, GTPCH over-expression increased the expression of Ki67 and CD34 in tumour tissue. Conversely, switch off of GTPCH expression by Dox in drinking water or inhibition of its enzymatic activity by intraperitoneal injection of DAHP (GTPCH inhibitor) significantly decreased CD34 positive endothelial cells in mouse xenografts. This study demonstrates a critical role for BH4 in tumour angiogenesis, which is at least partially mediated by activating the pathway of PI3K/Akt/eNOS/wild-type Ras protein in vascular endothelial cells. Our findings suggest that BH4 synthesis may be a rational target for inhibiting tumour angiogenesis.

O127

Angiotensin-(1–7) Inhibits Breast Tumor Growth in an Orthotopic Murine Model by Reducing Angiogenesis and Fibrosis

Katherine Cook[1,2], E. Ann Tallant[1,2], Patricia E. Gallagher[1]
[1]*Hypertension and Vascular Research Center, Wake Forest University School of Medicine, Winston-Salem, NC, USA,* [2]*Molecular Medicine and Translational Science Program, Wake Forest University School of Medicine, Winston-Salem, NC, USA*

Angiotensin-(1–7) [A7] is an endogenous seven amino-acid peptide hormone of the renin-angiotensin system that exhibits antiproliferative properties. We showed that A7 significantly inhibited the growth of non-small cell lung adenocarcinoma in a xenograft mouse model. In this study, human BT474 HER2-amplified estrogen receptor positive breast cancer cells were injected into the mammary fat pad of athymic mice; tumors grew to approximately 200 mm^3 prior to infusion with saline or 24 μg/kg/h A7 for 18 days. A marked reduction in tumor volume (5209±419 mm^3 to 1656±124 mm^3; n=5, $p<0.05$) and weight (3.6±0.2 g to 2.2±0.1 g; n=5, $p<0.05$) was observed in mice administered A7 as compared to saline control animals. Vessel density was decreased approximately 50% by the heptapeptide, demonstating that A7 has antiangiogenic properties. Picrosirius red histochemistry showed that interstitial fibrosis (4.91±0.96 percent/field versus 1.22±0.19; n=17–20, $p<0.0005$) and perivascular fibrosis (49.32±3.20 percent/vessel versus 13.35±2.23; n=20–21, $p<0.0001$) were significantly reduced with A7 administration. This decrease in fibrosis was associated with a reduction in collagen I deposition, suggesting that A7 has an antifibrotic effect in breast cancer. Treatment with the heptapeptide significantly decreased (31% reduction, n=4, $p<0.05$) the *in vitro* growth of cancer-associated fibroblasts (CAFs) isolated from orthotopic breast tumors which could lead to a decrease in mitogenic factors and metalloproteinases produced by CAFs. A 2.3-fold increase in the mitogen-activated protein (MAP) kinase phosphatase DUSP1 was also observed, suggesting that the reduction in fibroblast proliferation may be due in part to inhibition of MAP kinase activity. Taken together, these data suggest that A7 may serve as a first-in-class chemotherapeutic agent for breast cancer targeting the tumor microenvironment through a reduction in angiogenesis and a decrease in CAF proliferation.

O128

Angiotensin-(1–7) Inhibits VEGF and PlGF to Reduce Tumor Angiogenesis in Triple Negative Breast Cancer in an Orthotopic Mouse Model

Patricia E. Gallagher[1], David R. Soto-Pantoja[1], Katherine Cook[1], E. Ann Tallant[1]
[1]*Hypertension & Vascular Research Center, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA*

Triple negative breast tumors are aggressive, highly metastatic cancers that lack estrogen and progesterone receptors and have basal expression of the human epidermal growth factor receptor HER2. Angiotensin-(1–7) [A7], an endogenous heptapeptide hormone that activates the *mas* receptor, significantly reduced the *in vivo* proliferation of human triple negative breast tumor growth in an orthotopic model. Athymic mice with tumors produced by injection of MDA-MB-231 cells into the mammary fat pad were treated for 28 days with saline or 1000 μg/kg A7, delivered by daily subcutaneous injection. A7 markedly reduced tumor volume (170.8±21.4 mm^3 versus 546.7±87.9 mm^3; n=5, $p<0.05$) and tumor wet weight (0.5±0.1 g versus 1.0±0.2 g; n=5, $p<0.05$) compared to the tumors from control animals. A7 decreased ERK1/ERK2 MAP kinase activities and increased MAP kinase phosphatase DUSP1 in both parent cells and orthotopic tumors, while an siRNA to DUSP1 prevented the attenuation of ERK activities by the heptapeptide. These results suggest that A7 upregulates a MAP kinase phosphatase to reduce MAP kinase activities and decrease tumor growth. The inhibition in tumor growth by A7 was associated with a reduction in vessel density (32.0±7.0 vessels/field to 87.8±6.4, $p<0.05$), a 59% decrease in placental growth factor (PlGF) and a 72% reduction in vascular endothelial growth factor (VEGF), indicating an inhibition of angiogenesis. Incubation of the parent cells with A7 reduced PlGF and VEGF by more than 60% in a receptor-mediated process. Transfection of siRNAs to DUSP1 into breast cancer cells reversed the decrease in PlGF and VEGF with A7 treatment, suggesting that reduction of angiogenic cytokines was mediated by an increase in DUSP1. Based on the antiproliferative and antiangiogenic properties of the heptapeptide, A7 may serve as an effective, first-in-class compound for the treatment of triple negative breast tumors targeting the specific receptor *mas*.

O129

Tamoxifen and the Lignan Enterolactone Increase *in vivo* Levels of IL-1Ra and Decrease Tumor Angiogenesis in Estrogen Dependent Breast Cancer Explants

Gabriel Lindahl[1], Niina Saarinen[2], Annelie Abrahamsson[1], **Charlotta Dabrosin[1]**
[1]*Division of Oncology, Linköping University, Linköping, Sweden,* [2]*Functional Foods Forum, University of Turku, Turku, Finland*

Phytoestrogens have been shown to be potential compounds in breast cancer prevention and treatment by poorly understood mechanisms. One of the most abundant phytoestrogen in Western diet is the mammalian lignan enterolactone (ENL). We have previously reported a pro-angiogenic action of estradiol counteracted by the anti-estrogen tamoxifen in breast cancer. The proinflammatory cytokines IL-1alpha and IL-1beta have been shown to be major pro-angiogenic whereas the IL-1 receptor antagonist (IL-1Ra) seems to inhibit tumor angiogenesis. Here we show that estradiol decreased secreted IL-Ra of breast cancer cell *in vitro* and that the addition of tamoxifen or ENL to the cells reversed this decrease. Moreover, tamoxifen exposure alone increased IL-1Ra levels significantly compared to control cells. Mice bearing estrogen dependent breast cancers were treated with subcutaneous injections of tamoxifen, fed with ENL, or continued exposure of estradiol only. In these solid breast cancer tumors extracellular IL-1Ra was sampled *in situ* using microdialysis after 2–3 weeks of treatment. Tumors of mice treated with tamoxifen or fed with ENL had significantly increased extracellular IL-Ra levels compared with control tumors exposed to estradiol only in a similar fashion as shown *in vitro* and these tumors also exhibited decreased vessel area. Moreover, treatment with subcutaneous injections of recombinant IL-Ra protein resulted in tumor regression *in vivo* despite continued estradiol exposure. We conclude that estradiol down-regulate IL-1Ra in breast cancer cells. In addition, we show that an anti-estrogenic effect of ENL in breast cancer include restoration of IL-1Ra levels and that one of the anti-tumorigenic effects of tamoxifen may be mediated via potent increase of IL-1Ra levels in estrogen dependent breast cancer. Taken together our results suggest that increasing IL-1Ra may be a possible anti-estrogen therapeutic option for breast cancer treatment and prevention.

O130

Non Invasive Molecular Monitoring of Tumor Angiogenesis

Laura Ciarloni[1,2], Francesca Botta[1], Curzio Rüegg[1,3], **Francesca Botta[1]**
[1]*Division of Experimental Oncology, Centre Pluridisciplinaire d'Oncologie (CePO), Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland,* [2]*Diagnoplex SA, Epalinges, Switzerland,* [3]*National Center for Competence in Research, Molecular Oncology, ISREC-EPFL, Lausanne, Switzerland*

Tumor angiogenesis is a critical event in tumor growth and progression. Anti-angiogenic drug such as Avastin, Sutent, Nexavar and Torisel, have been approved for the treatment of advanced human cancers, opening the way to anti-angiogenic therapy in clinical oncology. However, the improved use of current approved drugs, or the development of novel ones, is limited by the lack of reliable surrogate markers that may allow a non-invasive and cost-effective monitoring of angiogenesis and identification of responding patients.Several studies performed using mouse models showed that bone marrow-derived (BMD) myeloid cells and several cell subpopulations, are important modulators of tumor angiogenesis. Once those cells are attracted at the tumor site by tumor-released factors, they promote angiogenesis, tumor growth, invasion and metastasis. Moreover, it appears that the tumor could educate these cells before they enter the tumor microenvironment. Previous studies suggested the possibility that circulating BMD myeloid cells may be imprinted by tumor-derived signals even before they reached the tumor. If induced changes can be detected by non-invasive procedures, these cells, and associated molecular events, could be used to identify surrogate markers of tumor angiogenesis. Following the screening of different human and murine tumor models, we observed a myeloid cell population mobilized in mice bearing 4 T1-breast cancer cells-derived tumors. Particularly, we detected

a striking accumulation of a subpopulation of F4/80+ cells (monocytic lineage) and neutrophils in the peripheral blood of orthotopically-injected 4 T1 mice. Microarray-based gene expression analysis of F4/80+ cells isolated from the peripheral blood of control, 4 T1-bearing and anti-angiogenic drug treated 4 T1-bearing mice is ongoing with the purpose to identify relevant genes associated with tumor growth or angiogenesis. These results will be validated in human peripheral blood cells collected from healthy volunteers, and cancer patients before, during and after anti-angiogenic therapies.

O131

Intravital Imaging of Human Prostate Cancer Using Bombesin-Targeted Viral Nanoparticles

Amber Ablack[1], Nicole Steinmetz[3], Jennifer L. Hickey[2], Jailal Ablack[1], Leonard Luyt[2], Marianne Manchester[3], **John D. Lewis[1]**
[1]*Department of Oncology, University of Western Ontario, London, ON, Canada,* [2]*Department of Chemistry, University of Western Ontario, London, ON, Canada,* [3]*Department of Cell Biology, Center for Integrative Biosciences, The Scripps Research Institute, La Jolla, CA, USA*

Viral nanoparticles offer an attractive multivalent platform for diagnostic *in vivo* imaging of prostate and other cancers. We have developed a nanoparticle platform based on the c*owpea mosaic virus* (CPMV) that offers discrete control over the conjugation of detection moieties, solubilization polymers and targeting ligands to the viral capsid. We report here the specific targeting and imaging of human PC-3 prostate cancer cells *in vitro* and *in vivo* with PEGylated fluorescent viral nanoparticles conjugated to a pan-bombesin peptide. The amphibian tetradecapeptide, bombesin, selectively interacts with the gastrin-releasing peptide (GRP) receptor family that is over-expressed on human prostate cancer cells. Bombesin peptide was conjugated to CPMV particles functionalized with a near-infrared (NIR) dye (Alexa Fluor 647) and polyethylene glycol (PEG) using the copper(I)-catalyzed azide-alkyne cycloaddition reaction. Absorbance measurements indicated that each nanoparticle contained 90 NIR dyes and 80–95 PEG or bombesin-PEG units. The integrity of CPMV particles was verified by FPLC, SDS PAGE and transmission electron microscopy. The bombesin-targeted CPMV particles showed a marked increase in uptake by PC-3 cells compared to a non-targeted control as measured by flow cytometry, and specificity was confirmed by successful blocking with an excess of soluble bombesin peptide. Targeting of PC-3 cells *in vitro* was confirmed by confocal microscopy. Bombesin conjugated CPMV showed impressive targeting and uptake in human prostate tumors *in vivo*, using a shell-less avian embryo tumor model. Taken together, we have shown here that bombesin-targeted viral nanoparticles offer a highly selective imaging tool for human prostate tumors, using a platform with future potential for clinical non-invasive imaging strategies and drug delivery.

O132

Novel Multiple Myeloma Biomarker Candidates Identified in the Secretome of Bone Marrow Fibroblasts and Endothelial Cells

Christopher Gerner[1], Thomas Mohr[1], Astrid Slany[1], Verena Haudek[1], Christoph Zielinski[1], Johannes Drach[1]
[1]*Department of Medicine I, Medical University of Vienna, Vienna, Austria*

The microenvironment of tumor cells in the bone marrow may be actively involved in the progression of malignant diseases. In multiple myeloma (MM), interactions of bone marrow stromal cells with the malignant plasma cells have gained significant importance as targets for novel therapeutic agents. Based upon these observations, we aimed at analyzing in detail the secretory capacity of bone marrow fibroblasts obtained from patients with MM in order to better understand their contribution to disease progression. We therefore analyzed the secretome of primary bone marrow fibroblasts of MM patients by proteome profiling based on highly sensitive mass spectrometry. Normal skin and bone marrow fibroblasts were found to secrete various extracellular matrix (ECM) proteins including fibronectin, collagens and laminins, in addition to some chemokines and cytokines including CXCL12, follistatin-like 1, insulin-like growth factor binding proteins 4, 5 and 7; and SPARC. In contrast, bone-marrow-derived fibroblasts from MM patients secreted increased amounts of ECM proteins and alpha-fetoprotein in addition to insulin-like growth factor II, stem cell growth factor and matrix metalloproteinase-2. Co-culture of primary MM cells with these fibroblasts further stimulated the secretion of ECM proteins, of cytokines such as inhibin beta A chain and growth factors such as connective tissue growth factor, which might be relevant to support the malignant clone. Analyses of the secretion capacity of bone marrow fibroblasts from patients with MGUS show that their secretome profile is also different compared to that of normal bone marrow fibroblasts. Proteome profiling of secreted proteins may thus help to identify relevant tumor-associated proteins, to increase our understanding of cell cooperativity and thereby increase our understanding of progression events in monoclonal gammopathies.

O133

How do Endothelial Cells Shape the Tissue Microenvironment? A Proteomic Approach

Thomas Mohr[1], Stefan Stättner[2], Nina Gundacker[1], Verena Haudek[1], Astrid Slany[1], Christine Brostjan[3], Reinhard Horvat[4], Josef Karner[2], Michael Micksche[1], Christopher Gerner[1]
[1]*Department of Medicine I, Institute of Cancer Research, Medical University of Vienna, Vienna, Austria,* [2]*Department of Surgery, Social Medical Center South, Vienna, Austria,* [3]*Department of Surgery Research Laboratories, Medical University of Vienna, Vienna, Austria,* [4]*Institute of Clinical Pathology, Medical University of Vienna, Vienna, Austria*

Endothelial cells (EC) substantially shape the tissue microenvironment which plays a critical role in tumor progression. We established protein maps of the secretome of human umbilical vein endothelial cells (HUVEC), human liver endothelial cells (HLEC) and human tumor derived endothelial cells (HTEC) from ovarian carcinoma. HLEC and HTEC were isolated using magnetic beads (anti CD31). Phenotype and function was confirmed by FACS analysis (positive for CD31, CD34, CD54 and CD62e after stimulation with TNF-α, podoplanin expression <10%, CD91 negative). Cells from passages 3–5 were cultured in proteinfree medium. After 24 hrs, supernatants were subjected to 1D gel electrophoresis followed by nanoflow liquid chromatography and MS/MS fragmentation analysis. Data were organized by the CPL/MUW proteomics database. We identified more than 250 proteins encompassing extracellular matrix proteins (collagens, fibrillin-1, fibulin-3, endothelial cell-selective adhesion molecule, dystroglycan, laminins, multimerin-1, proteoglycan-I, perlecan), proteases (MMPs, ADAMs, legumain, serine proteases 23 and HTRA1), peptidases (aminopeptidases, angiotensinase C, carboxypeptidase C and E, dipeptidyl-peptidase 2 and gamma-glu-X carboxypeptidase), protease inhibitors (TIMPs, PAI-1, serpin I2), growth factors (CTGF, PDGFs, SDF) and cytokines (interleukin-6, -8). By comparison with various other cell types (fibroblasts, VEGF and Il-1β activated HUVEC) we could establish protein profiles typical for various functional states. HLEC generated a proinflammatory microenvironment (secretion of IL-6, IL-8, several other inflammation associated proteins). The microenvironment generated by HTEC was characterized by growth factors (PDGF-A, CTGF) and other proteins associated with angiogenic activation, promotion of cell survival and cell growth. These results provide the up to now most comprehensive protein maps of the secretome of endothelial cells and demonstrate the value of proteomics to investigate the tissue microenvironment.

O134

Changes in Proteomic Expression Patterns of Tumour Associated Fibroblasts (TAF) by Interaction with Urinary Bladder Carcinoma Cells

Astrid Enkelmann[1], Niko Escher[2], Martina Walter[1], Michaela Weidig[3], Heiko Wunderlich[1], Kerstin Junker[1]
[1]*Department of Urology, University Hospitals Jena, Jena, Germany,* [2]*Core Unit Chip Application, University Hospitals Jena, Jena, Germany,* [3]*Department of Pathology, University Hospitals Jena, Jena, Germany*

Background: Tumour development and progression are strongly affected by interaction of tumour cells and tumour stroma. For different tumour models (e.g. breast cancer) a supportive effect of TAF on the tumour genesis was demonstrated. Aims of the present work are the isolation and proteomic characterisation of TAF from primary urinary bladder tumour specimen. A further part of this study will deal with the influence of urinary bladder carcinoma cell lines on protein expression of TAF.
Material and Methods: TAF were isolated from cultured urinary bladder tumour specimen. Therefore, primary tumour material was treated with EDTA followed by differential trypsinisation. Non-tumour fibroblasts were isolated from foreskin and normal urinary bladder tissue. Analyses of protein patterns were carried out on cultivated fibroblasts by SELDI-TOF-MS. TAF and foreskin fibroblasts were co-cultivated with several urinary bladder cancer cell lines in separated cell culture compartments followed by SELDI-TOF-MS analyses.
Results: By optimizing cell culture routines it was possible to isolate and subsequently cultivate TAF from primary tumour material of the urinary bladder. SELDI-TOF-MS measurements reveal differences in the proteomic patterns of TAF and non-tumour fibroblasts. Co-cultivation of urinary bladder carcinoma cells and TAF or non-tumour fibroblasts induces modified protein patterns in the different cell types.
Conclusion: TAF can be isolated and cultivated separately from primary tumour material. They are characterised by the expression of a specific protein pattern in comparison to non-tumour fibroblasts. Co-cultivation with tumour cells revealed the induction of a modified expression profile in fibroblasts and vice versa. The present results will provide a more detailed knowledge of the role of TAF in tumour development of urinary bladder carcinoma.

O135

The Serum Soluble HLA Class I Peptidome as a Source for Cancer Biomarkers and a Possible Modulator of the Tumor Microenvironment

Michal Bassani-Sternberg[1], Eilon Barnea[1], Ilan Beer[2], Irit Avivi[3], Tami Katz [3], **Arie Admon[1]**
[1]*Department of Biology, Technion - Israel Institute of Technology, Haifa, Israel,* [2]*IBM Haifa Research Laboratory, IBM, Haifa, Israel,* [3]*Department of Hematology & Bone Marrow Transplantation, Rambam Health Care Campus, Haifa, Israel*

One of the possible main route by which tumor cells modulate the response of the immune system within the tumor microenvironment is by secretion of soluble human leukocytes antigens (sHLA) carrying their peptide cargo. The HLA molecules are normally considered only to be transporters that carry peptides from the cytoplasm to the cell surface for surveillance by circulating T lymphocytes. However, many types of cancer cells are known to release into the serum large amounts of soluble HLA molecules still bound with their authentic peptides repertoires (the sHLA-peptidomes). Since the sHLA peptidomes are largely derived from the diseased cells, these monomeric sHLA-peptide complexes bind to circulating T cells and can modulate their anti-cancer cytotoxic activities. Furthermore, the identified serum sHLA peptidome provide a rich source of information about the tumor cells and the analysis of these peptidomes can be used as a sensitive serum-based cancer diagnostic. In this study we show that a few milliliters of fresh human plasma are sufficient for detailed analyses of sHLA-peptidomes, composed of thousands of peptides. The methodology comprises of a single-step immunoaffinity purification of the sHLA molecules from fresh human plasma, followed by analysis of the bound peptides by capillary chromatography and tandem mass spectrometry. This sHLA-

peptidomics approach was validated with plasma of a few patients with multiple-myeloma and leukemia, resulting in identification of numerous cancer related sHLA peptides, potentially useful as disease biomarkers, which were absent from the sHLA-peptidomes of the healthy controls. The repertoires of sHLA peptides recovered from the plasma of the cancer patients or from the bone marrow plasma were mostly identical. Therefore, the analysis of the blood sHLA peptidome provides an exciting glimpse onto the tumor microenvironment and may facilitate intervening in its immune suppressive properties.

O136

Hypoxia and PMA-Induced Maturation Inhibit TIMP-2 Secretion from Human Monocytes and Enhance Angiogenesis

Nitza Lahat[1], Miri Engelmayer-Goren[1], Haim Bitterman[2], Doron Rozsenzweig[1], Lea Weiss-Cerem[2], Michal A. Rahat[1]
[1]*Immunology Research Unit, Carmel Medical Center and The Ruth and Bruce Rapapport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel,* [2]*Ischemia-Shock Laboratory, Carmel Medical Center, Haifa, Israel*

Hypoxia, characteristic of fast growing solid tumors, recruits and immobilizes macrophages and enhances angiogenesis. Monocytes extravasation from the circulation across the basement membrane and extracellular matrix, which is mediated by matrix metalloproteinases (MMPs), is accompanied by their maturation into macrophages. However, the mechanisms evoked by hypoxia that regulate monocyte/macrophage behavior are largely unknown. We show that hypoxia reduces TIMP-2 secretion from primary monocytes or from U937 and THP-1 monocytic cell lines by 3–4 folds ($p<0.01$), by inhibiting its transcription. PMA-induced maturation of these cells, irrespective of hypoxia, also causes a 2–3- fold reduction of TIMP-2 ($p<0.05$), not by enhancing its intracellular or extracellular degradation, but by inhibiting its translation. We demonstrate involvement of SP-1 in transcriptional inhibition of TIMP-2 in monocytes, and suggest that hypoxia-induced enhancement of SP-1 phosphorylation dissociates it from TIMP-2 promoter, and disrupts coordinative recruitment of other transcription factors, such as NFY. Hypoxia reduces TIMP-2 secretion from endothelial cells by 2-folds ($p< 0.05$), and increases endothelial cell migration/proliferation in a TIMP-2-dependent manner, whereas the reduced TIMP-2 secretion from monocytes and macrophages do not affect their migration. Thus, we suggest that various mechanisms control TIMPs synthesis and expression in different cell types and processes, and that overall reduced TIMP-2 secretion in the hypoxic tumoral microenvironment contributes to enhance angiogenesis.

O137

The Unfolded Protein Response Protects Cells during Hypoxia through Preservation of Autophagic Capacity

Kasper Rouschop[1], Twan van den Beucken[1,2], Ludwig Dubois[1], Hanneke Niessen[3], Johan Bussink[5], Kim Savelkouls[1], Hilda Mujcic[1], Willy Landuyt[4], Philippe Lambin[1], Albert van der Kogel[5], Marianne Koritzinsky[1,2], Bradly Wouters[1,2]
[1]*Department of Radiation Oncology (Maastro Lab), GROW Research Institute, Maastricht University, Maastricht, The Netherlands,* [2]*Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, Toronto, ON, Canada,* [3]*Department of Molecular Genetics, GROW Research Institute, Maastricht University, Maastricht, The Netherlands,* [4]*Laboratory of Experimental Oncology/Radiobiology, K.U. Leuven, Leuven, Belgium,* [5]*Department of Radiation Oncology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*

Hypoxia is a common feature of tumors that contributes to malignancy and treatment resistance. The basis for these effects derives in part from a transcriptional response mediated by the HIF family of transcription factors. Hypoxia also has been shown to activate the unfolded protein response (UPR) which induces a protective response against hypoxia induced cell death both in vitro and in xenografts in vivo. Here we show that the protective effect of the UPR during hypoxia is mediated through regulation of autophagy. We discovered that the UPR induces the transcription of the essential autophagy genes LC3B and ATG5 during hypoxia through its ability to regulate the transcription factors ATF4 and CHOP respectively. LC3B and ATG5 are not required for the initiation of autophagy, but instead mediate phagophore expansion and formation of the autophagosome. Transcriptional induction of LC3B during hypoxia functions to replenish LC3B protein levels which are normally turned over during the process of autophagy, and thus allow autophagy to continue during extended hypoxic exposures. We show that cells engineered with various defects in PERK/UPR signalling fail to transcriptionally induce LC3B and thus become rapidly depleted of LC3B protein during hypoxia. Activation of autophagy and induction of LC3B was also observed in hypoxic areas of tumor xenografts derived from cell lines and in a series of 12 human head and neck xenografts established directly from tumors. Importantly, pharmacological inhibition of autophagy sensitized cells to hypoxic exposure, reduced the viable fraction of hypoxia in xenografts, and sensitized tumors to irradiation. These data suggest that regulation of autophagy via the UPR facilitates cell survival during hypoxia and that this pathway is an interesting therapeutic target in combination with radiotherapy.

O138

Molecular and Cellular Characterization of The Brain Tumor Microenvironment with Focus on Peritumoral Brain Swelling

Nic Savaskan[1], Ilker Y. Eyüpoglu[2]
[1]*Institute of Cell Biology & Neurobiology, Charité-Universitätsmedizin Berlin, Berlin, Berlin, Germany,* [2]*Department of Neurosurgery, University of Erlangen-Nurenberg, Erlangen, Bavaria, Germany*

Brain edema is a hallmark of human malignant brain tumors and contributes to the clinical course and outcome of brain tumor patients. The so-called peritumoral edema or brain swelling imposes in T2-weighted MR scans as high intensity areas surrounding the bulk tumor mass. The mechanisms of this increased fluid attraction and the cellular composition of the microenvironment are only partially understood. Here, we focus on the molecular and cellular characterization of this particular zone. We identified that areas of perifocal edema not only include the tumor invasion zone but also are associated with the occurance of neuronal cell death and increased astrocytic distribution surrounding the bulk tumor mass. Moreover, a high number of activated microglial cells accumulate at the tumor border. Thus, the area of perifocal edema is mainly dominated by reactive changes of vital brain tissue. We further analyzed the peritumoral zone by biochemical means and identified augmented levels of the neurotransmitter glutamate. RNA interference or pharmacological approaches towards glutamate modulations attenuated neuronal cell death and brain swelling. We will present further data which corroborate the concept that brain swelling may in part be a consequence of the neurotoxic tumor microenvironment.

O139

Importance of Differential Stress-Induced CXC-chemokine Expression and Signaling in Regulating Cancer and Stromal Cell Function in PTEN-deficient Prostate Tumours

David Waugh[1], Pamela Maxwell[1]
[1]*Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland, UK*

We have shown that expression of the proinflammatory CXC chemokine, interleukin-8 (IL-8) and its receptors CXCR1 and CXCR2 is elevated in malignant prostate cancer (CaP) epithelium. Published studies confirm that hypoxia and/or chemotherapy-induced stresses underpin AP-1, HIF-1 and NFκB-mediated transcription-driven increases in IL-8, CXCR1 and CXCR2 expression in CaP cells. The current study determines the relevance of PTEN, a commonly mutated or deleted tumour suppressor gene in CaP, in regulating the induction of CXC-chemokine signaling and the cellular response of stressed CaP cells. Time-dependent increases in CXCL8, CXCR1 and CXCR2 mRNA were observed in PTEN-deficient LNCaP and PC3 cells. ELISA confirmed increased IL-8 secretion following hypoxia, while immunoblotting confirmed elevated CXCR1 and CXCR2 expression in both cells. In contrast, CXCL8, CXCR1 and CXCR2 expression was only marginally up-regulated in PTEN wild-type DU145 and 22Rv1 cells under hypoxia. Subsequently, PTEN status was shown to regulate the magnitude and duration of CXC-chemokine-promoted signaling and altered gene expression profiles. For example, CXCL8 administration increased expression of HIF-1α and increased the activity of this transcription factor in PTEN-deficient LNCaP and PC3 cells but not in PTEN wild-type cells. Furthermore, expression of HIF-1 target genes (VEGF, TGFα) was also induced following CXCL8 stimulation in PTEN deficient but not PTEN wild-type cells. Attenuation of PTEN in the DU145 and 22Rv1 cells using siRNA revealed the CXCL8-induced responses including the increase in HIF-1 expression and activation. Functionally, the transcription-mediated elevation in IL-8 signalling underpins an increased survival of hypoxic prostate cancer cells to DNA-damage-based chemotherapy. In summary, our studies suggest that the magnitude of CXC-chemokine signaling and it subsequent effects is functionally important in PTEN-deficient prostate tumours, promoting proliferation, survival, chemoresistance and an androgen-independent transition in the cancer cells, while simultaneous effects on the tumour stroma will potentiate angiogenesis and recruitment of a disease-progressing immune cell infiltrate.

O140

Cancer-Related Inflammation: The Seventh Hallmark of Cancer

Alberto Mantovani[1]
[1]*Istituto Clinico Humanitas IRCCS, Milan, Italy*

Inflammatory conditions in selected organs increase the risk of cancer. An inflammatory component is present also in the microenvironment of tumours that are not epidemiologically related to inflammation. Recent studies have begun to unravel molecular pathways linking inflammation and cancer. Schematically, an intrinsic (driven by genetic events that cause neoplasia) and an extrinsic (driven by inflammatory conditions which predispose to cancer) pathway link inflammation and cancer. Smouldering inflammation in the tumour microenvironment contributes to proliferation and survival of malignant cells, angiogenesis, metastasis, subversion of adaptive immunity, response to hormones and chemotherapeutic agents. As such, cancer-related inflammation (CRI) represents a target for innovative diagnostic and therapeutic strategies. We surmise that CRI represents the seventh hallmark of cancer.

O141

How Anticancer Therapies Switch on the Immune System?

Laurence Zitvogel[1]
[1]*CICBT507, Institut Gustave Roussy, Villejuif Cedex, France*

Conventional therapies of cancer rely upon radiotherapy and chemotherapy. Such treatments supposedly mediate their effects via the direct elimination of tumor cells. However, anticancer such therapies can also modulate the host immune system in

several ways. Drugs can inhibit immunosuppressive pathways, or activate distinct immune effectors, or sensitize tumor target cells to CTL attack or generate an immunogenic cell death modality, all culminating in eliciting or enhancing anticancer immune responses contributing to the tumoricidal activity of the drug. Indeed, we reported that anthracycline-mediated cell death is immunogenic in tumor bearing hosts through a molecular pathway involving membrane exposure of calreticuline (CRT) by tumor cells[1,2,3]. CRT is mandatory for the uptake by dendritic cells of dying tumor cells. More generally, anthracyclines, X-Rays and platinum based-therapies mediate a tumoricidal activity relying on CD8+ T cells, CD11c+DC, IFNg/IFNgR signalling pathway but not IL-12. We addressed which biochemical or metabolic components expressed or released by dying tumor cells could trigger the immune system and participate to the immunogenicity of cell death. While HMGB1/TLR4 are mandatory for the processing of dying bodies by DC and the activity of chemotherapy, other components recently unravelled will be presented at the meeting. These results delineate a clinically relevant immunoadjuvant pathway triggered by tumor cells.

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O142

Inflammation and Cancer: Insights into Organ-specific Immune Regulation of Cancer Development

Lisa M. Coussens[1]
[1]*Department of Pathology and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA*

The concept that leukocytes are components of malignant tumors is not new; however, their functional involvement as promoting forces for tumor progression has only recently been appreciated. We are interested in understanding the molecular mechanisms that regulate leukocyte recruitment into neoplastic tissue and subsequent regulation those leukocytes exert on evolving cancer cells. By studying transgenic mouse models of skin, lung and breast cancer development, we have recently appreciated that adaptive leukocytes differentially regulate myeloid cell recruitment, activation, and behavior, by organ-dependent mechanisms. Thus, whereas chronic inflammation of premalignant skin neoplasms is B cell–dependent, during mammary carcinogenesis, T cells appear to play more of a dominant role in regulating pro-tumor and pro-metastatic properties of myeloid cells. To be presented will be recent insights into organ and tissue-specific regulation of epithelial cancer development by adaptive and innate immune cells, and thoughts on how these properties can be harnessed for effective anticancer therapeutics.

Funding from the National Institutes of Health and a Department of Defense Era of Hope Scholar Award.

O143

Intratumoral Immune Reaction: A Novel Paradigm for Cancer

Jerome Galon[1]
[1]*Integrative Cancer Immunology, INSERM U872, Paris, France*

To date the anatomic extent of tumor (TNM classifications) has been by far the most important factors to predict the prognosis of colorectal cancer patients. However, the impact of immune responses and tumor escape on patient prognosis in human cancer is poorly understood.

We showed that tumors from human colorectal cancer with a high density of infiltrating memory and effector memory T-cells (TEM) are less likely to disseminate to lymphovascular and perineural structures and to regional lymph-nodes. We showed that the combination of immune parameters associating the nature, the density, the functional orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host immune reaction on patients prognosis. We proposed to define these immune criteria as “immune contexture”. Investigation of the CRC primary tumor microenvironment allowed us to uncover the association of favorable outcomes with efficient coordination of the intratumoral immune response. We described four major immune coordination profiles within CRC primary tumors depending on the balance between tumor escape and immune coordination.

In conclusion, the density and the immune-cell location within the tumor have a prognostic value that are superior of those of the TNM classifications. Tumor invasion is statistically dependent on the host immune reaction.

O144

Regulation of Macrophage Function by the Tumor Microenvironment : Role of Hypoxia and Angiopoietin-2

Claire Lewis[1], Seth Coffelt[1], Craig Murdoch[2]
[1]*Department of Infection & Immunity, University of Sheffield Medical School, Sheffield, UK,* [2]*Department of Oral Pathology, University of Sheffield Dental School, Sheffield, UK*

Tumor-associated macrophages (TAMs) are abundant in virtually all types of malignant tumour. These highly versatile cells respond to the presence of stimuli in different tumour regions with the release of a distinct repertoire of growth factors, cytokines, chemokines, and enzymes that regulate tumor progression. The

distinct tumour microenvironments where TAMs are found include areas of invasion where TAMs promote tumour cell motility; stromal and peri-vascular areas where TAMs may promote metastasis; and avascular and peri-necrotic areas where they are thought to stimulate angiogenesis. In fact, TAMs accumulate in hypoxic areas of tumours in large numbers and our most recent data show that hypoxia, necrotic debris and/or hypoxia-induced cytokines like angiopoietin-2 stimulate expression of important tumour-promoting genes like VEGF, EGF and IL-6 by TAMs. This may explain why high TAM density in these areas correlates with increased tumour angiogenesis and metastasis. Large areas of hypoxia and necrosis form in tumors after administration of chemotherapeutic agents, radiotherapy or drugs that disrupt the tumor vasculature. This is often accompanied by a marked influx of macrophages into the tumor residue where they are activated to stimulate its revascularisation and re-growth. In this way, macrophages act as a powerful ally in tumor resistance and recovery. We are currently exploiting the natural ability of macrophages to migrate into to such poorly vascularised tumor areas to deliver therapeutic virus. To do this, we have developed a novel technology to genetically manipulate macrophages to synthesise and release therapeutic virus under the control of hypoxia-responsive promoter elements. This restricts viral production (and thus therapeutic gene expression in the virus) to cells in hypoxic/necrotic tumor areas. In this way, the responses of macrophages to tumor hypoxia can be exploited to deliver gene therapy to tumors.

O145

Regulation of In Situ to Invasive Breast Carcinoma Transition

Min Hu[1], Guillermo Peluffo[1], Jun Yao[1], Rebecca Gelman[1], Stuart schnitt[2], Craig Allred[3], **Kornelia Polyak[1]**
[1]*Dana-Farber Cancer Institute, Boston, MA, USA,* [2]*Beth-Israel Deaconess Medical Center, Boston, MA, USA,* [3]*Washington University, St. Louis, MO, USA*

The progression of ductal carcinoma in situ (DCIS) to invasive ductal carcinoma is a key yet poorly understood event in breast tumor progression. Comparative molecular analyses of tumor epithelial cells from in situ and invasive tumors have failed to identify consistent tumor stage-specific differences. However, the myoepithelial cell layer and basement membrane, present only in DCIS, are key distinguishing and diagnostic features. To determine the contribution of non-epithelial cells to tumor progression, we analyzed the role of myoepithelial cells and fibroblasts in the progression of DCIS using a xenograft model of human DCIS. Progression to invasion was promoted by fibroblasts, but was inhibited by normal myoepithelial cells. The progression-promoting effects of fibroblasts could be eliminated by COX-2 inhibitors. Invasive tumor epithelial cells from these progressed lesions formed DCIS rather than invasive cancers when re-injected into naïve mice. Molecular profiles of myoepithelial and luminal epithelial cells isolated from primary normal and cancerous human breast tissue samples corroborated findings obtained in the xenograft model. These results provide the proof of principle that breast tumor progression could occur in the absence of additional genetic alterations in tumor epithelial cells. Furthermore, our data suggest that a key event of tumor progression is the disappearance of the normal myoepithelial cell layer and basement membrane due to defective myoepithelial cell differentiation provoked by microenvironmental signals. Thus, myoepithelial cells could be considered gatekeepers of the in situ to invasive breast carcinoma transition and understanding the pathways that regulate their differentiation may open new venues for breast cancer therapy and prevention.

O146

Role of the Tumour Microenvironment in Angiogenesis and in Prediction of Breast Cancer Metastasis

Adriana Albini[1], Ulrich Pfeffer[2], Giuseppina Pennesi[1], Douglas Noonan[3]
[1]*Oncology Research, MultiMedica group, Milano, Italy,* [2]*Functional Genomics, National Institute for Cancer Research, Genova, Italy,* [3]*Clinical and Biological Sciences, University of Insubria, Varese, Italy*

Breast cancer a common malignancy and a leading cause of cancer-related mortality. Currently, it is clear that a significant percentage of patients respond well to first line therapy and will not relapse or evolve to metastatic disease. However, discrimination of these patients from those that will progress is poor. To avoid over-treatment and to administer a tailored therapies we still need to further improve diagnostic and prognostic tools. We must look beyond the tumor cells themselves, and into the tumor microenvironment, to have additional clues to predict probability of progression and metastatic dissemination. Gene expression profiling could be a useful approach to characterize not only tumor cells but also the surrounding microenvironment. We used a gene expression dataset of 159 breast cancer cases with follow-up information of at least 8 years to discriminate the tumors that will eventually give rise to recurrence or metastases from those that will progress. We performed a hierarchical clustering by considering genes involved in cell-cell and cell-matrix interactions and signaling that were or were not associated with tumor relapse. We found two main clusters, one is enriched in cases with metastases and the other containing only a few metastatic cancer samples. We then compiled a list of genes that are significantly differently expressed between correctly classified cases with metastases and the most frequently misclassified cases using a permutation test. The tumor-microenvironment signature set used here gave prediction of progression rates that were essentially superimposable on larger previously published gene signature sets. Interestingly, we found that there was a cluster of frequently misclassified cancers using the diverse gene signature sets. Gene expression profiles of the tumor microenvironment may permit additional levels of selection that could identify the outlying samples that cluster with non-progression profiles but are malignant.

O147

Molecular Basis of Growth Factor-Induced Mammary Cell Migration: Implications to HER2-positive Breast Cancer

Yosef Yarden[1]
[1]*Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel*

Growth factors and their transmembrane receptors contribute to all steps of tumor progression, from the initial phase of clonal expansion, through angiogenesis to metastasis. An important example comprises the epidermal growth factor (EGF) and the respective receptor tyrosine kinase, namely ErbB-1/EGFR, which belongs to a prototype signaling module that implicated in carcinoma development. The extended module includes two autonomous receptors, EGFR and ErbB-4, and two non-autonomous receptors, namely: a ligand-less oncogenic receptor, HER2/ErbB-2, and a kinase-dead receptor (ErbB-3). This signaling module is richly involved in human cancer and already serves as a target for several cancer drugs. Along with regulation of cell proliferation, EGFR family members control cellular motility through a process requiring newly synthsized RNA molecules. Using DNA arrays and immortalized mammary cells we study mechanisms underlying enhanced cell motility upon EGFR activation. These studies will be described and their relations to clinical observations will be discussed.

O148

The Metastatic Niche: Adapting the Foreign Soil

David Lyden[1], Rosandra Kaplan[1], Jared Wels[1], Till-Martin Theilen[1]
[1]*Pediatrics, Weill Medical College of Cornell University, New York, NY, USA*

Steven Paget's 'seed and soil' hypothesis introduced the concept that a receptive microenvironment in distant organs is required for the colonization, survival and outgrowth of metastatic tumor cells. Meanwhile, the early molecular and cellular events taking place within distant tissues that "prime the soil" for tumor cell colonization are only beginning to be discovered. Previously, we showed that tumor-specific growth factors promote the mobilization of vascular endothelial growth factor 1 $(VEGFR1)^+$ hematopoietic progenitor cells (HPCs) and $VEGFR2^+$ endothelial progenitor cells (EPCs) to the developing tumor vasculature. However, the role of BM-derived cells in tumor metastasis was largely unidentified. We have demonstrated that BM-derived HPCs promote a conducive microenvironment for tumor growth termed the "pre-metastatic niche". Here, secretory factors of the primary tumor induce modifications within pre-metastatic tissues prior to the arrival of tumor cells and stromal cells. Blocking VEGFR1 function was seen to abrogate HPC recruitment and consequent metastasis, whereas inhibiting VEGFR2 function prevented micrometastatic to macrometastatic transitioning. The HPCs at the pre-metastatic sites maintained their progenitor cell status, expressing markers such as CD34, CXCR4, CD11b, c-Kit and Sca-1. Prior to the arrival of HPCs at the pre-metastatic niche, focal upregulation of fibronectin isoforms occurred. BM-derived cells expressing VLA-4 integrin preferentially bound to regions with enriched fibronectin expression, contributing to site-specificity for tumor metastasis. Despite these findings, the precise function of VEGFR1 expression within these hematopoietic cell types is not understood. By lentiviral gene transfer targeting the haematopoietic compartment, we found that downregulation of VEGFR1 expression in the BM drastically reduced the occurrence of metastatic tumor burden, whereas overexpression of VEGFR1 enhanced progression of macrometastasis in the lung. Studies to determine the functional role of VEGFR1 expression within BM-derived cells in promoting metastatic progression are ongoing and will likely enhance our understanding of the factors that enable metastatic progression.

O149

Heparanase: One Molecule with Multiple Functions in Cancer Progression

Israel Vlodavsky[1], Liat Fux[1], Gil Arvatz[1], Eyal Zcharia[2], Immanuel Lerner[2], Michael Elkin[2], Neta Ilan[1]
[1]*Cancer and Vascular Biology Research Center, The Rappaport Faculty of Medicine, Technion, Haifa, Israel,* [2]*Department of Oncology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel, Jerusalem, Israel*

Heparanase activity is strongly implicated in cell invasion associated with tumor metastasis, angiogenesis and inflammation, a consequence of structural remodeling of the extracellular matrix (ECM). Heparanase upregulation correlates with increased tumor vascularity and poor postoperative survival of cancer patients. Moreover, heparanase levels in the urine and plasma of cancer patients often correlate with the severity of the disease and response to anti-cancer treatments, indicating that the enzyme is a valid target for anti-cancer drug development and a promising tumor marker. Given the potential tissue damage that could result from inappropriate cleavage of heparan sulfate (HS), tight regulation of heparanase expression and function are essential. Apart of stimulatory elements along the heparanase promoter, we identified AU-rich element in the 3' untranslated region that suppresses heparanase gene expression. Regulation at the protein level includes modulation of its cell surface expression, cathepsin L-mediated processing, cellular uptake, secretion, and cytoplasmic vs. nuclear localization. Heparanase also augments cell adhesion and signaling cascades leading to enhanced phosphorylation of selected protein kinases and increased transcription of genes associated with aggressive tumor progression. This function of heparanase appears independent of its enzymatic activity and HS

substrate and is mediated by a protein domain localized at the C-terminus (C-domain) of the protein. The C-domain is critical for heparanase secretion and signaling functions and for maintaining the 3D structure of the active enzyme. The functional repertoire of heparanase is further expanded by its regulation of syndecan clustering and shedding. Studies applying heparanase over-expressing and knock-out mice emphasize its role in tissue morphogenesis and as a master regulator of other ECM degrading enzymes. Heparanase is causally involved in inflammation and accelerates colon tumorigenesis associated with inflammatory bowel disease. Inhibitors directed against the C-domain, combined with inhibitors of heparanase enzymatic activity are being developed to halt tumor growth, metastasis, angiogenesis and inflammation. A lead compound (non-anticoagulant glycol-split heparin), highly effective against myeloma tumors, was selected toward a clinical trial in cancer patients.

O150

Microenvironment-Dependent Support of Self Renewing Ovarian Cancer Stem Cells

Karl Skorecki[1], **Maty Tzukerman[1]**
[1]*Department of Molecular Medicine, Rapport Faculty of Medicine, Rambam Medical Center and Technion, Israel Institute of Technology, Haifa, Israel*

One of the main stumbling blocks in establishing personalized cancer therapy has been the paucity of pre-clinical experimental models in which the actual cancer cells from a patient can be successfully grown in a manner which mimics growth in the human body for testing of anti-cancer treatments tailored to the individual patient. We have demonstrated that human embryonic stem cells (hESC) - derived microenvironment provide a niche which enables the growth of important subsets of ovarian cancer stem cells, which evade growth in conventional systems. Six different subpopulations of ovarian cancer cells from one patient have been generated and characterized. Remarkably – in the embryonic stem cell based model – the ovarian cancer cells from a single patient – recapitulated the broad repertoire of properties that can be seen in tumors observed across the entire spectrum of many different patients. Moreover, the embryonic stem cell platform, exposed the key subpopulations of ovarian cancer stem cells – which are believed to be the most important target for a sustained response with anti-cancer therapy. These subpopulations show the capacity for both self-renewal and tumorigenic differentiation in a niche-dependent manner, and are characterized by the expression of specific markers for cancer stem cells. This study underscore the potential experimental utility of the hESC-derived cellular microenvironment to expose certain cancer cell sub-populations that do not grow into a tumor in the conventional direct tumor xenograft platform and therefore are most probably not readily accessible to characterization and testing of anticancer therapies.

O151

Hepatomimetic Properties of Colon Cancer Cells: Microenvironmental Regulation and Clinical Implications

Fernando Vidal-Vanaclocha[1], Javier Beaskoetxea[2], Naiara Telleria[2], Amaia Del Villar[2], Andrés Valdivieso[3], Jorge Ortiz de Urbina[3]
[1]*Department of Cell Biology and Histology, Basque Country University School of Medicine, Leioa, Bizkaia, Spain,* [2]*Pharmakine SL, Derio, Bizkaia, Spain,* [3]*Hepatobiliar Tumor Surgery Sevice, Cruces Hospital, Cruces-Baracaldo, Bizkaia, Spain*

Organ-specific colonization of cancer cells is an important feature of metastasis and it has been reported that distinct alterations in gene expression underlie metastasis to defined organs. However, the regulation and clinical projection of this tropism are unknown. DNA microarrays and RT-PCR were used to determine the gene expression profile of hepatic colorectal carcinoma metastases and tumor-unaffected liver tissue from same patients. HT-29 human colon carcinoma and primary cultured human hepatocytes and liver myofibroblasts were used to determine if both tumor and liver cells are mutually influencing their expression of metastasis-associated genes. Three microenvironment-related gene expression categories were detected: 1) Hepatic metastases genes not expressed by tumor-unaffected liver tissue. Some of them were already expressed at primary tumors of patients having hepatic colon carcinoma metastases in less than five years, and were expressed by both HT-29 cells given cultured liver cell-conditioned media (CM) and liver cells given HT-29 cell-CM. 2) Genes co-expressed by hepatic metastases and tumor-unaffected liver tissue. These were not expressed by primary tumors. This category also included both liver-specific genes expressed by HT-29 cells given liver cell-CM, and colon cancer-specific genes expressed by liver cells receiving HT-29-CM. 3) Genes of tumor-unaffected liver tissue not expressed at hepatic metastases. These were expressed by liver cells, but not by colon cancer cells, and represented the genetic background of the hepatic metastasis microenvironment. In addition, gene expression profiles of hepatic metastases demonstrated a resemblance to tumor-unaffected hepatic tissue, suggesting hepatomimetic properties of metastatic colon cancer. Hepatic tissue also adopted colon cancer-specific genes induced by tumor-derived factors. Mutual gene expression mimic phenomena stem from exposure of metastatic cells to the hepatic microenvironment, and of liver cells to tumor factors. The distinct clinical features of microenvironment-related hepatic metastasis gene categories suggest their implications in the hepatotropism and metastatic development of colon carcinoma.

O152

Disabled-2 a Potential Integrator of TGF-β Signaling and Trafficking in Epithelial to Mesenchymal Transition and Dedifferentiated Tumor Cell Lines

David Chetrit[1], Galit Horn[1], Keren Shapira[1], Tal Hirshhorn[1], Lior Barzilay[1], Nechama Smorodinsky[1], Yoav Henis[1], **Marcelo Ehrlich[1]**
[1]*Departments of Cell Research and Immunology and Neurobiology, Tel Aviv Unviersity, Tel Aviv, Israel*

Dedifferentiation of epithelial carcinomas and epithelial to mesenchymal transition (EMT) involve complex and coordinated changes to the trafficking and signaling apparatuses of the cell. Two of the main signaling pathways which induce and react to these phenotypic-morphological changes are the TGF-β and Ras signaling pathways. Thus, proteins which interact with components of both pathways have the potential to integrate the different signaling stimuli. Furthermore, alterations to signal compartmentalization, by modifications to the intracellular localization of signaling molecules through trafficking, are a potential mode of regulation of their signaling output. In this context, Disabled-2 (Dab2), a multidomain endocytic adaptor that interacts with the TGF-β receptors, SMAD proteins, Dab2IP (a Ras-GAP), Grb2, Src and integrins is a candidate regulator of dedifferentiation and EMT. Here, we report that in contrast to epithelial-like tumor cells, Dab2 is expressed in undifferentiated carcinomas and in mouse mammary tumor cells which undergo EMT. These cells also present enhanced activation of Ras and its downstream effectors and differences in the expression of proteins related to TGF-β signaling. Furthermore, Dab2 enhances the internalization of TGF-β receptors and alters their signaling output. In addition, elevation of the expression levels of Dab2 leads to an enhancement of cell spreading on fibronectin, a characteristic of the EMT-like cells. Moreover, manipulations to the levels of activation of Ras or ERK entail an abrogation of this enhanced spreading capacity. We propose that TGF-β and Ras signaling regulate EMT and that Dab2 is involved in the determination of the phenotype-specific signaling output.

O153

Integrins in EMT and Tumor Microenvironment

Andrei Bakin[1], Anna Bianchi[1], Andrea Varga[1], Alfiya Safina[1]
[1]*Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY, USA*

Cancer progression and metastasis are linked to epithelial-mesenchymal transition (EMT) and the invasive potential of tumor cells. In tumor microenvironment, transforming growth factor β (TGF-β) cytokines are prominent inducers of EMT and tumor invasion. The EMT process is implicated in the acquisition of the metastatic potential, the generation of cancer-initiating stem cells and resistance to chemotherapy. The development of anti-TGF-β therapy is a challenging task because TGF-β is a potent tumor-suppressor in early-stage cancers, inhibiting cell growth and promoting cell death. For the past several years, our research has been focused on the identification of key molecules responsible for oncogenic activities of TGF-β.

Our study of TGF-β-induced EMT in the context of carcinoma and normal epithelial cells has uncovered major elements of the Ras and TGF-β pathways controlling cell invasion and the EMT process. The study revealed that oncogenic Ras does not induce EMT but alters the EMT response to TGF-β. In normal cells, TGF-β up-regulates TPM1 expression thereby inducing actin fibers and stable cell-matrix adhesions that reduce cell motility and invasion. In malignant cells, oncogenic Ras and epigenetic pathways silence TPM1 expression, enhancing cell-invasive capacity. This discovery explains the switch in the TGF-β function in cancer as well as reveals risk factors of metastasis and molecular targets for anti-cancer therapy.

To further dissect the role of matrix-adhesion components we used siRNA approach. The functional studies assessed EMT markers, integrins, cell adhesion, migration and invasion *in vitro*, as well as the tumorigenic potential in an orthotopic xenograft model *in vivo*. Our data indicate changes in the expression of specific integrins in advanced-stage cancers. These molecules may represent novel biomarkers and targets for anti-cancer drug discovery research.

O154

Vascular Co-option in Brain Metastasis

Ruth J. Muschel[1], W. Shawn Carbonell[1], Lukxmi Balathasan[1], Sebastien Serres[1], Thomas Weissensteiner[1], Martina L. McAteer[1], Daniel C. Anthony[1], Robin P. Choudhury[1], Nicola R. Sibson[1]
[1]*Gray Institute of Radiation Oncology and Biology, University of Oxford, Oxford, UK*

One source of a tumour blood supply is of course the native host vessels also termed vascular co-option. We have examined brain metastases for the use of host vessels in both experimental brain metastasis models and in clinical specimens. Indeed, over 95% of early micrometastases examined demonstrated vascular cooption with little evidence for isolated neurotropic growth. This vessel interaction was adhesive in nature implicating the vascular basement membrane (VBM) as the active substrate for tumor cell growth in the brain. Accordingly, VBM promoted adhesion and invasion of malignant cells and was sufficient for tumor growth prior to any evidence of angiogenesis. Blockade or loss of the b1 integrin subunit in tumor cells prevented adhesion to VBM and attenuated metastasis establishment and growth *in vivo*.

The engagement of the tumour cells with the host vasculature also had the effect of inducing expression of the endothelial activation protein VCAM-1. VCAM-1 can be detected by magnetic resonance imaging using a new targeted contrast agent, in which VCAM-1 antibodies have been coupled to microparticles of iron oxide (MPIOs). Using this imaging method we have been able to detect microscopic brain metastases in experimental models.

Our data establishes a new understanding of CNS metastasis formation and identifies the neurovasculature as the primary functional compartment for such growth. It also provides a detection strategy for microscopic brain metastases.

O155

The Aging Host Microenvironment May Reduce Tumor Progression by Reducing Genomic Instability

Judith Leibovici[1], Orit Itzhaki[1], Tatiana Kaptzan[1], Ehud Skutelsky[1], Judith Sinai[1], Moshe Michowitz[1], Monica Huszar[1]
[1]*Department of Pathology, Sackler Faculty of Medicine, Tel- Aviv University, Tel- Aviv, Israel*

Numerous cancers display a lower aggressiveness in aged as compared to young patients. The mechanisms underlying this phenomenon are not yet elucidated. Several mechanisms have nevertheless been demonstrated: reduced tumor cell proliferation in the old, increased apoptosis, decreased angiogenesis and immune response modification. We have found another mechanism of the age- dependent reduced tumor progression: a decreased DNA ploidy in B16 melanoma grown in old (near diploidy) as compared to those developing in young mice (near tetraploidy) (Exp. Gerontol., 43: 164, 2008).
Morphologically, tumor cells from aged mice were of smaller cell and nuclear size than those of young animals. Flow cytometry forward scatter data also showed a smaller cell size of melanoma cells from old mice. According to DNA flow cytometry profile, while B16 melanoma cells from young animals contained a high tetraploid cell percentage, those derived from old animals were mostly near diploid. Tetraploidy is considered to precede aneuploidy which, in turn, is at the origin of neoplasia genetic instability. The tetraploidy to near euploidy transit in melanoma cells of aged mice might therefore constitute a mechanism by which the genetic instability inherent to tumor progression is attenuated.
Our findings indicate that the aging microenvironment can actually affect the tumor cell genome. In tissues of aged organisms, tumor progression might possibly be prevented via normalization of a tetraploid checkpoint. We propose that the previously described mechanisms of the reduced tumor progression in the aged might lead to a reduced genetic instability. The aging microenvironment, with its reduced availability of growth factors and hormones which reduces tumor cell proliferation, with its higher content of apoptosis-inducing agents (cortisone, TNF) and with its reduced angiogenesis – which in turn reduces tumor cell proliferation - , this aging microenvironment constitutes a non-permissive surrounding for genomic instability, a prerequisite for tumor progression.

O156

FoxF1 Regulates Tumor-promoting Properties of Cancer-associated Fibroblasts in Lung Cancer

Roy-Akira Saito[1,2], Karin Jirström[3], Kohei Miyazono[2], Arne Östman[1]
[1]*Department of Oncology-Pathology, Cancer Center Karolinska, Karolinska Institutet, Stockholm, Sweden,* [2]*Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan,* [3]*Center for Molecular Pathology, Department of Laboratory Medicine, Lund University, Malmö University Hospital, Malmö, Sweden*

Cancer-associated fibroblasts (CAFs) promote tumor growth and progression. Significant progress has been made in the last years concerning identification of novel CAF-derived factors. However, the mechanisms underlying the conversion of fibroblast to CAF phenotype remain largely unknown. Epigenetic and genetic mechanisms have been suggested, although the latter remains controversial. In this study we hypothesized that CAF phenotype can be induced by activation of developmentally important mesenchymal transcription factors. To test this hypothesis, we focused on the role of FoxF1 in lung cancer since this factor is critically involved in mesenchymal-epithelial interactions during lung development. Ectopic FoxF1 expression in murine fibroblasts induced upregulation of ASMA, HGF, FGF-2 and integrin beta3. Moreover, ectopic FoxF1 enhanced fibroblast collagen gel contraction. Consistent with these findings, knockdown of endogenous FoxF1 in lung fibroblasts resulted in downregulation of HGF, FGF-2 and integrin beta3. Upregulation of FoxF1 in fibroblasts increased their ability to stimulate migration of A549 lung cancer cells and, conversely, downregulation of FoxF1 in lung fibroblasts reduced this ability. Most interestingly, co-injection experiments demonstrated that fibroblasts with high FoxF1 expression were more potent than control fibroblasts in supporting subcutaneous tumor growth following co-injection of A549 cells and either of the two types of fibroblasts. Tumors with FoxF1 expressing fibroblasts also displayed higher vessel density. Clinical relevance of these findings was supported by the demonstration of significant association between short survival and high FoxF1 CAF expression in lung cancer. Ongoing experiments include analyses of clinical samples to identify upstream regulators of CAF FoxF1 expression.
Taken together, our observations suggest that FoxF1 confers tumor-promoting properties on lung fibroblasts, and thus provide experimental support for the concept that CAF phenotypes can be induced by activation of developmentally important transcription factors.

O157

Effect and Regulation of Gr-1+CD11b+ Immature Myeloid Cells in Tumor Microenvironment and Beyond

Li Yang[1]
[1]*Laboratory of Cancer Biology and Genetics, National Cancer Institute, Bethesda, MD, USA*

Significantly increased immature myeloid cells are found in peripheral blood and tumor tissues of cancer patients. The number of these cells correlates with staging of tumor progression. However, their impacts on tumor progression and underlying mechanisms remain to be elucidated. In mouse models, these cells are identified as Gr-1+CD11b+ cells. They are well known for their immune suppression function thus are called myeloid immune suppressor cells or myeloid derived suppressor cells in tumor immunology field. Recent years, we found these cells infiltrate into tumor microenvironment, produce high levels of multiple matrix metalloproteinases (MMPs) such as MMP13,

MMP14, MMP2 and MMP9, as well as TGFß1. They significantly contribute to vasculature remodeling and tumor cell invasion. In addition, these cells are recruited to breast carcinomas lack of TGFß signaling through SDF-1/CXCR4 and CXCL5/CXCR2 chemokine axes. They mediate the switch of TGFß signaling from a tumor suppressor to a tumor promoter. Furthermore, Gr-1+CD11b+ cells are also significantly increased in lungs of mice bearing mammary adenocarcinomas prior to tumor cell arrival. These immature myeloid cells decrease INF-γ production and increase pro-inflammatory cytokines in the premetastatic lung. Interestingly, MMP9 produced by these cells disrupt VE-cadherin junction of endothelial cells. Deletion of MMP9 normalizes aberrant vasculature in the premetastatic lung, and diminishes lung metastasis. The production and activity of MMP9 is selectively restricted to lungs and organs with large number of Gr-1+CD11b+ cells. Our data suggest that Gr-1+CD11b+ cells alter premetastatic lung into an inflammatory and proliferative environment, diminish immune protection and promote metastasis. Our studies demonstrate that Gr-1+CD11b+ cells exert pro-tumor activities in tumor microenvironment and distant premetastatic lung. Thus inhibition of Gr-1+CD11b+ cells could normalize host environment, improve host immunosurveillance and inhibit tumor metastasis.

O158

Ets2 in Lung Fibroblasts Promotes the Growth of Metastatic Breast Cancer Cells

Jillian L. Werbeck[1], Fu Li[2], Martina Gutik[2], Thomas J. Rosol[1], Michael C. Ostrowski[2]
[1]*Veterinary Biosciences, The Ohio State University, Columbus, OH, USA,* [2]*Molecular and Cellular Biochemistry, The Ohio State University, Columbus, OH, USA*

The Ets family of transcription factors have been shown to play a key role in promoting the growth of breast cancer cells. Work from our laboratory has shown that Ets2 is involved in regulating growth and metastasis through a tumor-independent mechanism in the *MMTV-PyMT* model. Therefore, the goal of this work is to understand the role of Ets2 signaling in the tumor microenvironment at both the primary and metastatic site.
Our hypothesis is that Ets2 activation in the lung stroma promotes the growth of breast cancer lung metastases. In order to test this hypothesis *in vivo,* we used a genetic approach to conditionally delete Ets2 from only fibroblasts. A fibroblast-specific (Fsp) promoter was used to drive expression of cre recombinase to functionally delete a floxed Ets2 allele. We then injected highly metastatic murine breast cancer cells derived from *MMTV-PyMT* tumors (Met-1) into the lateral tail vein of both wild-type and *Fsp-cre;* $Ets2^{-/-}$ mice in order to assess metastatic incidence and tumor burden in the lung (N=15). Metastases were tracked using *in vivo* bioluminescence imaging (BLI) and final tumor burden was assessed by quantitative histomorphometry.
In conclusion, we determined that the deletion of Ets2 in lung fibroblasts delayed the incidence of breast cancer lung metastases ~4 weeks. Furthermore, metastatic tumor burden was significantly reduced in the lung ($p<0.02$). We further demonstrated that this decrease in tumor burden was not related to a decrease in endothelial cell recruitment (angiogenesis) or local macrophage infiltration (inflammation). This therefore suggests that Ets2 action in the tumor microenvironment may have a novel role in promoting lung metastases and we are currently investigating other potential mechanisms. Our overall understanding of the genetic contributions of the tumor microenvironment at the metastatic site will be essential to delay or inhibit metastasis.

O159

C-reactive Protein Protects Myeloma Cells from Apoptosis via Activating ITAM-containing FcgRII

Qing Yi[1], Jing Yang[1]
[1]*Department of Lymphoma and Myeloma, MD Anderson Cancer Center, Houston, TX, USA*

It is well recognized that multiple myeloma (MM), a hematologic cancer that is still incurable, is protected by the bone marrow microenvironment consisting of stromal cells, matrix, and cytokines such as IL-6 and IGF-1. However, our studies have also suggested that myeloma cells induce systemic changes in patients that promote myeloma cell growth and protect myeloma cell apoptosis. One of the changes is the presence of high levels of circulating C-reactive protein (CRP) in myeloma patients. Elevated levels of CRP are present in patients with infections, inflammatory diseases, necrosis, or malignancies including MM. Recently we made a striking discovery that CRP enhances myeloma cell proliferation under stressed conditions and protects myeloma cells in vitro from apoptosis induced by chemotherapy drugs, IL-6 withdrawal, or serum deprivation. In vivo injections of human CRP around subcutaneous tumors protected tumor cells and significantly undermined the therapeutic effects of dexamethasone or melphalan in xenografted myeloma-SCID and SCID-hu mouse models. CRP protected tumor cells from apoptosis via binding Fcg receptors (FcgRs), preferentially the activating FcgRIIA/C, but not the inhibitory FcgRIIB, leading to PI3K/Akt, ERK, and NF-kB pathway signaling and inhibited activation of caspase cascades induced by chemotherapy drugs. CRP also enhanced myeloma cell secretion of IL-6 and synergized with IL-6 to protect myeloma cells from chemotherapy drug-induced apoptosis. These findings are clinically relevant, since we found CRP accumulating on myeloma cells from all myeloma-patient bone marrow biopsies examined; no CRP was found on marrow cells from healthy individuals (Yang et al., Cancer Cell, 2007; 12:252–265). To confirm the results, we recently generated two stable human myeloma cell lines, ARP-1 and MM1-144, which secrete CRP after infection with lentiviral vectors containing the human *crp* gene. These two cell lines became significantly less

sensitive to dexamethasone-induced apoptosis, which could be reversed by CRP-neutralizing antibodies. Thus, our results provide strong evidence for a novel effect of CRP on myeloma cells.

O160

Bone Marrow-Derived Hematopoietic Progenitor Cells as Mediators of Metastasis

Rosandra Kaplan[1,2], Daniel Rutigliano[1,3], Selena Granitto[1], Lauren Rotman[1], Daniel Rafii[1], Elan Bomsztyk[1], Kendra Kadas[1], John Lawrence[1], Emma Sidebotham [3], Elisa Port[5], Allyson Ocean[4], Linda Vahdat[4], David Lyden[1,2]
[1]*Department of Pediatric Hematology/Oncology, Weill Cornell Medical Center, New York, NY, USA,* [2]*Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [3]*Department of Pediatric Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [4]*Department of Medical Oncology, Weill Cornell Medical Center, New York, NY, USA,* [5]*Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

The role of host cells in tumor progression and metastasis is now well recognized. We show that bone marrow-derived hematopoietic progenitor cells (HPCs) help to initiate the metastatic cascade by creating a supportive microenvironment in distant tissue sites. In addition to detection of these cells in pre-metastatic and metastatic tissues, we can now monitor HPCs in the circulation in mouse models as well as for patients in the clinical setting. Patients with advanced carcinoma show elevated levels of circulating HPCs by flow cytometry compared to low levels in healthy controls. We identify a defined circulating cell population that correlates with the presence of tissue-specific HPCs at the pre-metastatic niche. These circulating cells express CD34 and VEGFR1 as well as cKit, CD133, and CXCR4, with a subset expressing CD11b. Moreover, the degree of elevation of these cells correlates with clinical stage with significant increase in mobilized HPCs in patients with metastatic disease as compared to localized disease at presentation and in ongoing studies is being correlated with metastatic progression. We also show that patients with high circulating HPCs have greater colony forming assay capacity than healthy controls, suggesting these cells functionally maintain their progenitor status. Beyond the HPC elevation observed in newly diagnosed patients, these cells appear to be mobilized in the setting of tumor surgical resection and may explain the finding shown previously of enhanced metastasis observed after surgical removal of the primary tumor in mouse models. This process can potentially be inhibited and thereby derail the early systemic changes occurring even in those patients with so-called localized cancers. Targeting these cells at different clinical time points may significantly impact the outcome of metastatic spread, and monitoring patients for HPC mobilization may help define a population of cancer patients at higher risk for metastatic disease, enabling more tailored therapies.

O161

The Microenvironment of Hepatic Nodules is Necessary for Tumor Progression

Silvia Doratiotto[1], Fabio Marongiu[1], Maria Paola Serra[1], **Ezio Laconi[1]**
[1]*Department of Biomedical Sciences and Technologies, University of Cagliary, Cagliari, Italy*

Preneoplastic hepatocytes isolated from liver nodules are unable to grow or progress to cancer when orthotopically transplanted into normal syngenic recipients. However, we have reported that these cells can selectively expand upon transplantation into the liver of animals pre-exposed to retrorsine (RS), a compound that blocks endogenous hepatocyte cell cycle. Furthermore, such expanding clusters form new hepatic nodules that rapidly progress to hepatocellular carcinoma. Thus, it would appear that if the original nodular architecture is disrupted, the resulting isolated cells display no evidence of growth autonomy when seeded in a normal orthotopic environment and can only progress to cancer via formation of new nodular lesions in the host liver.
To further extend these observations, in present study we re-isolated nodular hepatocytes from the first RS-treated and transplanted host and performed a second serial orthotopic transplantation in the liver of either normal or RS-treated recipients. Animals were treated according to our original protocol and 100 thousands nodular hepatocytes were infused via a mesenteric vein. Results were striking: while transplanted cells grew very rapidly in the liver of animals pre-treated with RS (several macroscopically visible nodules, up to 2 mm in diameter, were already apparent at 2 weeks after cell infusion), no evident growth was seen in the corresponding untreated recipients. However, the growth rate of second-passage nodular cells was higher compared to that observed following the first transplant in the RS-treated host.
We interpret these results to suggest that (i) isolated nodular hepatocytes do not display any significant degree of growth autonomy after multiple in-vivo passages; (ii) an appropriate tissue microenvironment is essential for their selective expansion; (iii) once a nodular lesion is re-formed in the host, this sets the stage for tumor progression to occur within such a unique microenvironment.
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O162

The Differential Role of Microenvironmental IL-1α and IL-1β In Tumor Angiogenesis

Elena Voronov[1], Yaron Carmi[1], Shahar Dotan[1], Ron N. Apte[1]
[1]*The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences and the Cancer Research Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel*

Previously, we have shown the importance of IL-1, mainly IL-1b in tumor-mediated angiogenesis. Here, we describe some of the

mechanisms by which host-derived IL-1 participates in angiogenesis. We assessed invasiveness, inflammatory and angiogenesis patterns of B16 melanoma cells in Matrigel plugs in mice deficient in IL-1 expression (IL-1β and IL-1α KO mice) and in mice deficient in IL-1Ra, as compared to control mice. Reduced tumor invasiveness and angiogenesis was observed in Matrigel plugs in mice deficient in IL-1 expression, as compared to control mice. In contrast, mice deficient in IL-1Ra, where there is overexpression of IL-1, show the most intensive angiogenic response. CD34-positive hemopoietic stem cells were the earliest and most abundant infiltrating population; in control mice, their levels in Matrigel plugs were higher than in mice deficient in IL-1 expression. CD34-positive cells are probably key players in tumor-mediated angiogenesis in this model. Reconstitution of the bone marrow of IL-1 deficient mice by cells from control mice leads to an increased number of CD34-positive cells, as well as increased tumor invasiveness and angiogenesis, comparable to control mice. We found that several populations of CD34-positive cells invaded the Matrigel after injection of melanoma cells to different KO mice. Both IL-1α and IL-1β are probably involved in the induction of $CD11b^+$, $CD34^+$ and $VEGFR1^+$ cells, designated as hematopoietic precursor cells, whereas IL-1β is mostly involved in $CD34^+$, $VEGFR2^+$, $CD31^-$ cells, known as endothelial precursor cells. It was found that both cell types can produce VEGF and thus promote tumor induced angiogenesis. At the same time, only inhibition of IL-1β reduces the angiogenic response induced by injection of B16 melanoma cells in control mice. Thus, inhibition of IL-1β at early stages of tumor development may prove to be effective for use in anti-tumor therapy.

O163

VEGF-A165A and IL-6 in Human Colon Cancer: A Microenvironment Cooperation Leading to Cell Death Escape through microRNAS Dysregulation

Sabina Pucci[1], Paola Mazzarelli[1], Maria J. Zonetti[1], Luigi G. Spagnoli[1]
[1]*Department of Biopathology, University of Rome Tor Vergata, Rome, Italy*

Cooperation through the sharing of diffusible factors of tumor microenvinoment and the redirection of some specific guardian pathways raises new questions about tumorigenesis and has implication on designing new therapeutic approaches.Tissue microenvironment strongly influences tumorigenesis and neovascularization, redirecting some pathways versus a persisting pro-survival state. Recent studies suggest a potential role of IL-6-sIL6R in the pathogenesis of colon cancer, although data on the possible relationship between IL-6 production and tumour progression are still conflicting. Increased formation of IL-6-sIL-6R complexes that interact with gp130 on the cell membrane leads to increased expression and nuclear translocation of STAT3, which can cause the induction of anti-apoptotic genes, such Bcl-x_L. Moreover, as it has been observed in critical conditions (hypoxia, oxidative stress), STAT 3 activation influences the preferential expression of VEGF-A165a, leading to the inhibition of programmed cell death inducing Bcl-2.In colon cancer progression we observed that the cooperative production of IL-6 released as by the tumour itself as by tumour associated macrophages and the IL-6 induced VEGF-A, could influence tumour cell proliferation, favour apoptotic escaping and cell migration.The IL-6 and VEGFA165 treatment of a colon cancer cell line, *Caco-2*, modulated the expression of genes involved in tumor invasion and apoptosis, as observed by microarrays. In particular, IL-6 downmodulated Bax expression at mRNA level. Concomitantly, IL-6 exposure influenced Bax also at protein level acting on the Bax-Ku70-sCLU physical interactions in the cytoplasm, by affecting the Ku70 acetylation and phosphorylation state. Moreover, we demonstrate that IL-6 together with VEGF are able to inhibit Bax-dependent cell death also by increasing the production of the pro-survival form of Clusterin, shifting death into survival. Strikingly we observed that the cooperation between IL-6 and VEGFA165 influenced the expression of tumor suppressing miRNAs affecting the epigenetic HDAC-1 activity and the epithelial to mesenchymal transition, turning the neoplastic cell from epithelial to mesenchimal, strongly correlated to the malignization of many types of cancers. These still obscure molecular interactions, underlie the relevant role of these microenvironmental factors in the complicated cross talk among molecules that could effectively turn the cell fate.

O164

Receptor "Hijacking" by Malignant Glioma Cells: A Tactic for Tumor Progression

Ji Ming Wang[1], Keqiang Chen[1], Wanghua Gong[1], Jian Huang[1]
[1]*Cancer and Inflammation Program, National Cancer Institute at Frederick, Frederick, MD, USA*

Gliomas are the most common and deadly tumors in the central nervous system (CNS). In the course of studying the role of chemoattractant receptors in tumor growth and metastasis, we discovered that highly malignant human glioblastoma and anaplastic astrocytoma specimens were stained positively for the formylpeptide receptor (FPR), which is normally expressed in myeloid cells and accounts for their chemotaxis and activation induced by bacterial peptides. Screening of human glioma cell lines revealed that FPR was expressed selectively in glioma cell lines with a more highly malignant phenotype. FPR expressed in glioblastoma cell lines mediates cell chemotaxis, proliferation and production of angiogenic factors, vascular endothelial growth factor (VEGF) and CXCL8 (IL-8), in response to agonists released by necrotic tumor cells.Furthermore, FPR in glioblastoma cells activates the receptor for epidermal growth factor (EGFR) by increasing the phosphorylation of a selected tyrosine residue in the

intracellular tail of EGFR. Thus, FPR hijacked by human glioblastoma cells senses agonists in the tumor microenvironment and exploits the function of EGFR to promote rapid tumor progression.

O165

Recruitment of Mast Cells to the Tumor Microenvironment Via a High Affinity Leukotriene B_4 Recepter Signaling Enhances Tumor-Associated Angiogenesis and Tumor Growth

Masataka Majima[1], Yasuharu Kosaka[1], Tatsunori Suzuki[1], Kanako Hosono[1], Hirotsugu Okamoto[1], Takehiko Yokomizo[2]
[1]*Department of Pharmacology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan,* [2]*Department of Medical Biochemistry, Kyushu University, Fukuoka, Fukuoka, Japan*

Recent results suggest that bone marrow derived hematopoietic cells regulate angiogenesis in tumor microenvironment. Leukotriene B_4 (LTB_4), a 5-lipoxygenase (5-LOX) metabolite of arachidonic acid has been well-documented to be a potent chemotactic factor for granulocytes. LTB_4 exerts its biological activities through two distinct LTB receptors: BLT1, a high affinity receptor, and BLT2, a low affinity receptor. Although other 5-LOX metabolites, LTC_4 and LTD_4 were reported to be proangiogenic in chick chorioallantoic membrane system, roles of LTB_4 in enhancement of tumor-associated angiogenesis have not been clarified. We developed BLT1 knockout mice (BLT1-KO), and tested whether or not LTB_4-BLT1 signaling enhanced the recruitment of hematopoietic cells to the tumor microenvironment and tumor-associated angiogenesis. When Lewis lung carcinoma (LLC) cells were implanted to the subcutaneous tissues of mice, tumor growth in BLT1-KO mice was significantly less than that in wild type counter parts (WT). This reduction was accompanied with the reduced angiogenesis estimated by CD31 expression and mean vascular density in the stoma tissues. LLC growth and tumor-associated angiogenesis in this model were dependent on the vascular endothelial growth factor (VEGF). The expression of VEGF in the stromal tissues in BLT1-KO mice was reduced in the stromal tissues compared with that in WT mice. Myeroperoxidase mRNA levels in the stromal tissues in BLT1-KO mice were not reduced compared with those in WT, however, the accumulation of mast cell in the stromal tissues were significantly less in BLT1-KO than in WT. The same was true in WT treated with a 5-LOX inhibitor, AA861. Mast cells from WT mice expressed BLT1, and LTB_4 enhanced the chemotaxis of mast cells. Disodiumcromoglycate sodium that suppresses the mast cell function blunted the growth rate of LLC tumors together with reduction in angiogenesis. These results suggested that recruitment of mast cells to the tumor microenvironment via BLT1 signaling enhances tumor-associated angiogenesis, and that blockade of BLT1 signaling may be promising to treat solid tumors.

O166

Invasion of Human Breast Cancer Cells In Vivo Requires both Paracrine and Autocrine Loops Involving the Colony Stimulating Factor-1 Receptor

Antonia Patsialou[1], Jeffrey Wyckoff[1,2], Yarong Wang[1], Sumanta Goswami[1,4], E. Richard Stanley[3], John Condeelis[1,2]
[1]*Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, USA,* [2]*Gruss Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY, USA,* [3]*Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY, USA,* [4]*Department of Biology, Yeshiva University, New York, NY, USA*

Metastasis of adenocarcinomas involves the dissemination of tumor cells from the primary tumor, and their transport to, arrest and growth in the target organ. We have used intravital imaging to observe tumor cell invasion and intravasation directly in living mouse and rat primary mammary tumors and have shown that dissemination of tumor cells involves active motility and transendothelial migration into blood vessels. Infiltrating macrophages promote these behaviors of carcinoma cells via a colony-stimulating factor-1/epidermal growth factor (CSF-1/EGF) paracrine loop. In this macrophage-dependent invasion, tumor cells secrete CSF-1 and sense EGF, while the macrophages secrete EGF and sense CSF-1.

In patients, CSF-1 and its receptor (CSF-1R) have been implicated in the progression of breast cancer. This is based on high levels of circulating CSF-1 in patient sera with aggressive disease and increased CSF-1R staining in tumor tissues. However, there have been no direct *in vivo* studies to determine whether a CSF-1 autocrine signaling loop functions in human breast cancer cells *in vivo* and whether it contributes to invasion in a mechanism similar to the rodent models.

We have tested this hypothesis directly *in vivo* using MDA-MB-231 cell-derived mammary tumors in SCID mice. We show for the first time that *in vivo* invasion in a human mammary tumor model is dependent on both the EGF/CSF-1 paracrine signaling with host macrophages, as well as autocrine signaling in the tumor cells that express both CSF-1 and CSF-1R. In particular, we show that the autocrine-mediated invasion is a tumor microenvironment specific event, as it is evident only in the mouse xenograft *in vivo* and not in the cultured cell line. Furthermore, we show that this amplification of the autocrine invasion in the xenograft is due to an upregulation of the CSF-1R inside the primary tumor that is dependent on transforming growth factor-beta1 signaling *in vivo*.

O167

Regulation of Tumorigenesis, Angiogenesis and Metastasis by the Proprotein Convertases (PCs)

Nathalie Scamuffa[1], Fabien Calvo[1], **Abdel-Majid Khatib[1]**
[1]*Equipe Avenir, Inserm, Paris, France*

To attain their biological active forms, a variety of protein precursors are processed by proteases named proprotein convertases (PCs). These include PC1, PC2, Furin, PC4, PACE4, PC5 and PC7. Our previous studies were the first to demonstrate the importance of the maturation of protein precursors such as matrix metalloproteases, adhesion molecules, growth factors, and growth factors receptors by these enzymes in carcinogenesis and angiogenesis. We found that inhibition of the PCs in various tumor cells inhibited their malignant phenotypes and their ability to mediate tumor growth and angiogenesis. We also identified PDGF-A, PDGF-B, VEGF-C as new PCs substrates. Inhibition of PDGFA and VEGF-C processing by directed mutagenesis inhibited tumor growth and the formation of tumor vascular and lymphatic vessels, respectively. Similarly, over-expression of the general PC inhibitor alpha1-PDX and knockdown of the convertases expression in tumor cells using siRNA strategy inhibited processing of IGF-1 receptor and its subsequent activation by IGF-1 to induce IRS-1 and Akt phosphorylation. These tumor cells when injected into the liver circulation of mice prevented tumor cells interaction with liver endothelial cells and adhesion and showed a significantly reduced ability to form liver metastases. Based on these and other findings we postulate that PCs play a key role in the growth, survival and metastatic potential of tumor cells by regulating the activity of their cognate substrates and downstream effectors. Regulation of PCs activities may provide a powerful adjunct approach in cancer therapy.

O168

Characterization of the Immunological Microenvironment in Follicular Lymphoma

Camille Laurent[1], Sabina Muller[1], Pierre Brousset[1], Talal Al Saati[1], Salvatore Valitutti[1]
[1]*INSERM U563, Institut Claude Preval, Toulouse, France*

We applied confocal microscopy to the study of thick section of follicular lymphoma (FL) biopsies. We investigated the expression of different phenotypic markers characterizing the immunological microenvironment (CD3, CD8, CD20, CD4, CD56), together with activation markers such as granzyme B, perforin, g-interferon and phosphotyrosines. We observed, in most cases, a rich infiltrate of lytic granules-bearing cytotoxic cells, representing about 25% to 40% of the immunological FL microenvironment, that was not observed in control reactive lymph nodes. Cytotoxic cells were not localized in follicular areas but rather in the peri-follicular areas. Only a part of lytic granules-bearing cytotoxic cells were $CD8^+$, indicating that the immunological infiltrate in FL contains CTL and other not yet identified subsets of killer cells. The enrichment of cytotoxic cells in the peri-follicular areas of FL affected lymph nodes could have an impact in the control of the disease progression. As an initial approach to test this hypothesis we investigated whether FL derived B cells might be susceptible to lysis mediated by CTL cells *in vitro*. Our results show that primary polyclonal $CD8^+$ T cells from healthy donors or from FL patients efficiently annihilate super FL derived cells (KARPAS 422) loaded with a cocktail of bacterial super-antigens.
Taken together our results indicate that CTL and other killer cells are selectively recruited in FL affected lymph nodes and might be involved in the immune surveillance against malignant B cells.

O169

The Proteolytic Cascade in Metastasis

Steven Mason[1], Nick Gauthier[1], Robbie Samstein[1], Paula Bos[1], Don Nguyen[1], Jemila Kester[1], Xiang Zhang[1], Nicholas Socci[2], Joan Massagué[1], Johanna Joyce[1]
[1]*Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [2]*Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

Metastasis is a late event in cancer development, and is responsible for 90% of deaths from solid tumors. The proteolytic cascade can play an important role in metastasis as proteolytic activity can be channeled down specific pathways, and several proteases have been implicated in various stages in metastasis. In order to better understand the role of the proteolytic cascade in metastasis, we have utilized a novel microarray that has the ability to distinguish human and mouse protease and protease inhibitor expression in the tumor microenvironment. With this microarray, we have profiled the protease and inhibitor expression patterns of a xenograft model system in which metastatic breast cancer cells that home specifically to the bone, brain, or lung are used to generate tumors of shared parental origin in distinct locations. Several different proteases and their endogenous inhibitors, including multiple cysteine cathepsins, exhibit temporal, cell type-, and location-specific patterns of expression. In vitro invasion and co-culture experiments reveal that monocytes and astrocytes, two significant stromal components of the metastatic tumor microenvironment, are able to modulate the invasiveness of bone- and brain-homing metastatic derivatives, respectively. Additionally, tumor cells in turn can regulate the expression of proteases and endogenous inhibitors in stromal cells. Finally, shRNA knockdown of cathepsin B in tumor cells significantly impairs the invasion of brain-homing metastatic cells in culture, and knockdown of cathepsins B or L has contrasting effects on the development of metastatic brain tumors in vivo. These results indicate that many different proteases and their endogenous inhibitors play a significant role in the development of metastatic tumors, and that their selective, and likely combinatorial, inhibition may have significant therapeutic benefit.

O170

EGFL7 Protein Expression Effects Tumor Progression by Influencing the Rate of Angiogenesis

Laura Fung[1], Amber Ablack[2], Desmond Pink[3], Wendy Schulte[3], John D. Lewis[2,3,4]
[1]*Department of Medical Biophysics, The University of Western Ontario, London, ON, Canada,* [2]*London Regional Cancer Program, London Health Sciences Centre, London, ON, Canada,* [3]*Innovascreen Inc., Halifax, NS, Canada,* [4]*Department of Oncology, London Health Sciences Center, London, ON, Canada*

Tumor growth depends on establishment of new blood vessels through *de novo* angiogenesis, which in turn provide a route for metastasis. It has been shown that EGFL7 is highly up-regulated in endothelial cells during angiogenesis, and that it accumulates on the basal side of endothelial cells in nascent sprouts. While a number of reports have suggested a role in the remodeling of the extracellular matrix, the precise function of EGFL7 in angiogenesis is yet to be elucidated. We have recently discovered that some metastatic human tumor cell lines, including the human fibrosarcoma HT1080, express elevated levels of EGFL7 protein. We hypothesized that EGFL7 influences the metastatic progression of HT1080 by modulating tumor angiogenesis. For this reason, we investigated the role of EGFL7 expression in the metastatic progression of the HT1080 cell line *in vitro* and *in vivo*. We found that over-expression of EGFL7 in HT1080 cells does not affect their proliferation *in vitro*. In an *in vivo* chorioallantoic membrane angiogenesis assay, over-expression of EGFL7 significantly reduced angiogenesis compared to controls. When tumors were grown in an avian xenograft model, those expressing high levels of EGFL7 grew more slowly and showed significantly delayed vascularization. Analysis of the vascular ultrastructure suggested that the vasculature in EGFL7 over-expressing tumors was less tortuous and leaky compared to controls. Metastasis of HT1080 cells to the brain and liver was reduced by more than 80% in EGFL7 over-expressing tumors. Taken together, these results indicate that expression of EGFL7 by tumors influences the stability of the neovasculature and therefore, it may be a novel therapeutic target for anti-cancer strategies.

O171

A Novel Role for Megakaryocytes in the Bone Marrow Microenvironment of Prostate Cancer Metastasis

Xin Li[1], Serk In Park[1], Amy Koh[1], Ken Pienta[2,4], **Laurie McCauley**[1,3]
[1]*Periodontics & Oral Medicine, University of Michigan, Ann Arbor, MI, USA,* [2]*Urology, University of Michigan, Ann Arbor, MI, USA,* [3]*Pathology, University of Michigan, Ann Arbor, MI, USA,* [4]*Internal Medicine, University of Michigan, Ann Arbor, MI, USA*

Bone marrow is an accommodating microenvironment for prostate cancer cell localization and growth; however, host countermeasures likely exist to constrain tumor occupation of the skeleton. Megakaryocytes develop adjacent to bone and migrate to the vascular sinusoids before releasing platelets to the circulation. Hence, they have the potential to encounter tumor cells early in their progression into the bone. The purpose of this study was to determine the impact of megakaryocytes (MKs) on prostate cancer (PCa) cells using *in vitro* and *in vivo* approaches. K562 (human megakaryocyte precursors) and primary MKs induced from mouse bone marrow hematopoietic precursor cells were used in co-culture experiments with PCa cells (PC-3, VCaP, C4-2B). K562 potently suppressed PC-3, VCaP, and C4-2B cell numbers in co-culture; whereas they increased osteoblastic SaOS2 cells. The MK/PCa restrictive effect was more potent when cells were cultured in direct contact, and also when less differentiated MKs were used. The inhibitory effect of MKs was pro-apoptotic as determined by propidium iodide (PI) and annexin V flow cytometric analysis in addition to a restrictive proliferative effect seen via reduced levels of cyclin D1 mRNA. A PCR pathway analysis followed by quantitative RT-PCR revealed increased PC-3 mRNA levels for the pro-apoptotic genes apoptosis-associated speck-like protein containing a CARD (aka ASC/TMS1/PYCARD) and death-associated protein kinase 1 (DAPK1) after co-culture with K562 cells. *In vivo*, athymic mice were administered thrombopoietin (TPO) to expand their megakaryocyte populations prior to intracardiac PC-3 luciferase tagged (PC-3^{luc}) cell inoculation. TPO significantly increased MKs in the bone marrow and reduced numbers of luciferase positive prostate tumors in the long bones. These data show a novel role for megakaryocytes as potential gatekeepers in the bone marrow microenvironment of the prostate skeletal metastatic lesion.

O172

Culture of Human Laryngeal Carcinoma Cell Line Hep-2 in Presence of Fibronectin Increases MMP-9 Expression with the Involvement of Multiple Signaling Pathways

Triparna Sen[1], Anindita Dutta[1], Gargi Maity[1], **Amitava Chatterjee**[1]
[1]*Department of Receptor Biology and Tumor Metastasis, Chittaranjan National Cancer Institute, Kolkata, India*

The microenvironment is being increasingly recognized as critical component in tumor progression and invasion. During cell migration, there is a continuous interaction between cell surface receptors and ECM proteins. In the present communication we studied the effect of Fibronectin-integrin interaction in human laryngeal carcinoma cell line, Hep-2 and the downstream effectors. The study indicates that culture of Hep-2 cells in SFCM in presence of FN enhances MMP-9 expression. FN induces the activity and expression of MMP-9 by binding to its receptor a5b1 in Hep-2 cells. This induction occurs through the possible involvement of multiple signaling pathways. We propose that there is a "cross-talk" between the signaling pathways. The silencing of FAK with FAK siRNA and its subsequent effect on FN-induced MMP-9 expression has confirmed the involvement of

FAK as an important modulator in the pathway. When FN binds to its receptor, it causes the phosphorylation of FAK, which in turn causes activation and nuclear translocation of PI-3 K and subsequent activation of ERK finally leading to MMP-9 transactivation and stimulation. PI-3 K on the other hand, upon integrin ligand interaction, could also independently activate ILK. These signaling pathways work in concert with each other and disruption of one could affect the function of another. The signals from the signaling pathways finally leads to the increased DNA binding activity of important transacting factor on MMP-9 promoter and thus transcription of MMP-9 in turned on. Our study provides scopes for future clinical interventions by targeting these signaling pathways in FN-induced MMP-9 upregulation and invasion of laryngeal cancer cells.

O173

Transforming Growth Factor Induced Protein TGFβI Promotes Ovarian Cancer Cell Motility and Adhesion to Peritoneal Cells

Miranda Ween[1], Hoffmann Peter[2], Raymond Rodgers[1], Carmela Ricciardelli[1], Martin Oehler[1,3]
[1]*Discipline of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia, Australia,* [2]*Adelaide Proteomics Centre, University of Adelaide, Adelaide, South Australia, Australia,* [3]*Department of Gynaecological Oncology, Royal Adelaide Hospital, Adelaide, South Australia, Australia*

Ovarian cancer is characterized by metastasis to the peritoneal surface lining the abdominal cavity. It remains unclear which factors promote this process. We have investigated the interaction between ovarian cancer (OVCAR-5, OVCAR-3, and SKOV-3) and peritoneal cells (LP-9) by co-culture and proteomic screening of conditioned media. One of the molecules found to be differentially expressed was the extracellular matrix adhesion protein, transforming growth factor-beta-induced protein (TGFβI, also known as big-H3 or keratoepithelin). Non-malignant ovarian surface epithelial cells and peritoneal mesothelial cells expressed high TGFBI levels. In contrast primary serous and matching metastatic tumour cells had very low levels of TGFBI. In functional experiments recombinant TGFβI significantly increased adhesion of the ovarian cancer cell lines to LP-9 peritoneal cells by up to 25% ($P<0.01$) and increased motility of OVCAR-5 cells by 62% ($P<0.001$). Furthermore, addition of neutralising TGFβI antibody reduced OVCAR-5 adhesion to LP-9 by 21% ($P<0.001$). TGFβI was found to be predominantly produced by the peritoneal cells and to be processed to smaller forms in the ovarian cancer-peritoneal cell co-culture. MALDI-TOF/TOF mass spectrometry identified TGFβI processing at both the N and C terminal domains. The addition of broad spectrum protease inhibitors blocked the TGFβI processing and reduced OVCAR-5 adhesion to LP-9 cells by 40% ($P<0.001$). We conclude that TGFβI produced by peritoneal cells can promote ovarian cancer cell adhesion and motility.

O174

Membrane Hsp72 from Tumor-Derived Exosomes Mediates p-Stat3 Dependent Function of Myeloid Suppressor Cells through the TLR2-MyD88 Pathway

Grégoire Mignot[1], Chalmin Fanny[1,2], Ladoire Sylvain[1,2,3], Vincent Julie[1,2], Apetoh Lionel[4], Rébé Cédric[1,3], Ghiringhelli François[1,2,3]
[1]*INSERM U866, Dijon, France,* [2]*Faculty of Medecine and Pharmacy, Dijon, France,* [3]*Anti-cancer center Georges François Leclerc, Dijon, France,* [4]*Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*

Myeloid suppressor cells (MDSCs) have been identified in humans and mice as a population of immature myeloid cells with ability to suppress T cell activation. MDSCs, which accumulate in tumor bearing hosts, have been shown to contribute to cancer development in mice and humans. Recent evidence suggests that the transcriptional factor Stat3 is constitutively activated in many mouse and human cancer cells. Indeed, tumors that constitutively express phosphorylated-Stat3 (p-Stat3) released some tumor derived factors that induced Stat3 activation in myeloid cells, a phenomenon which leads to MDSCs accumulation and immune suppressive activity. However, the exact nature of the tumor-derived factors accounting for this immunosuppression has not been investigated.
Here, we studied the factors released by 3 tumor cell lines that constitutively expressed p-Stat3 and induced MDSCs suppressor activity in a Stat3 dependent manner. Surprisingly, we showed that tumor derived exosome (TDE) -and not a tumor derived soluble factor- determines MDSCs Stat3-dependent suppressive activity. Moreover, we could demonstrate that, in both mice and humans, membrane Hsp72 from TDE triggers Stat3 activation in MDSCs in a TLR2/MyD88 dependent manner through an autocrine production of IL-6. Accordingly, targeting exosome production *in vivo* using dimethylamiloride blunts the suppressive activity of MDSC and enhanced the efficacy of cyclophosphamide treatment in three different mouse tumor models. Finally, we also demonstrated that this mechanism supporting suppressive MDSCs activity is relevant in cancer patients. Collectively, our findings show for the first time in both mice and human settings that membrane TDE associated Hsp72 restrained tumor immune surveillance by supporting MDSCs suppressive functions.

O175

Immune Cell Homing in Preinvasive HPV Disease

Cornelia Trimble[1], Christopher Thoburn[1], Shiwen Peng[1], Nicole Hanson[3], Jodie Tassello[3], Denise Frosina[3], Ferdynand Kos[1], Rachael Clark[2], Achim Jungbluth[3]
[1]*Johns Hopkins University School of Medicine, Baltimore, MD, USA,* [2]*Dermatology, Brigham and Women's Hospital, Boston, MA, USA,* [3]*Ludwig Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

Globally, human papillomavirus (HPV) causes more human malignancies than any other virus. High grade cervical intraepithelial neoplasia (CIN2/3) occurs only in the setting of persistent mucosal infection with an oncogenic strain of HPV, and presents a compelling opportunity to test immunotherapies because expression of two viral proteins, E6 and E7, are functionally required to initiate and maintain disease. We have a large prospective cohort of subjects with CIN2/3 who are followed for a brief, 15-week window prior to definitive excision of the cervical squamocolumnar junction (cervical conization or LEEP procedure). Not all dysplastic lesions progress to cancer; 25% of HPV16+ CIN2/3 undergo complete regression in this timefrqme. However, systemic HPV16 E6 and E7 T cell responses are marginally detectable, and do not correlate with lesion regression. However, CIN2/3 does recruit inflammatory infiltrates. Memory T cells accumulate in dysplastic mucosa, and spectratyping provides strong evidence that these often contain clonally expanded populations. In our cohort, intraepithelial CD8+ infiltration at t_0 was predictive of regression by t_{wk15}. In contrast, in lesions that failed to regress in the study window, inflammatory infiltrates were restricted to the cervical stroma, whilst intraepithelial CD8+ infiltrates were minimal. Detectable IFNγ immune responses to E6 and E7 measured in patient-matched peripheral blood obtained at the same visits did not correlate with lesional CD8+ infiltrates. These observations suggest that in the setting of natural infection, cellular immune responses to HPV antigens measured in the peripheral blood do not accurately reflect the composition or function of immune infiltrates in the cervical mucosa. Our work is focused on determining the immunologic signature of lesions which allow intraepithelial effector cell access. The identification of homing mechanisms for genital immune surveillance could suggest optimal routes of vaccination, and inform monitoring of immune responses likely to traffic to the genital mucosa.

O176

Tumor Conditioning: Modulation of the Tumour Microenvironment by Signalling Inhibition as a Strategy for Improving Cancer Therapy

W. Gillies McKenna[1], Naseer Qayum[1], Eric J. Bernhard[2], Ruth J. Muschel[1]
[1]*Gray Institute for Radiation Oncology & Biology, Oxford University, Oxford, UK,* [2]*Radiotherapy Development Branch, National Cancer Institute, Rockville, MD, USA*

Tumour hypoxia is an important determinant of the efficacy of cancer therapy since well-oxygenated cells are more sensitive to drugs and radiation and less likely to be metastatic than hypoxic cells. Reducing tumour hypoxia is thus a potential strategy for improving cancer treatment. We previously showed that targeting Ras activity improves oxygenation in tumours expressing oncogenic RAS and contributes to the radiation response. Upstream inhibition of Ras at EGFR, and downstream inhibition at PI3K and Akt also improve tumour oxygenation.

We have used multi-modality imaging studies of tumour microenvironmental changes induced by inhibitors of signalling proteins. Two cell lines were studied one driven by overexpression of EGFR and the other by mutation of N-ras. We have also made studies in a spontaneous MMTV neu breast cancer mouse tumour model. The EGFR kinase inhibitor Iressa, the prenyltransferase inhibitor L-778,123, the PI3K inhibitor PI-103 and the HIV protease inhibitor Nelfinavir were used to block signalling at EGFR, at Ras, PI-3 K and at Akt respectively.

Bioluminescence imaging in vivo demonstrated that HIF-1 promoter activity is reduced with inhibition of downstream signalling. Confirmation of tumour oxygenation was obtained immunohistochemically using nitroimidazole (EF5) binding and evaluating Carbonic Anhydrase-9 levels. Tumour vascular function was improved as measured by contrast-enhanced ultrasound power doppler. Confocal/multiphoton imaging revealed increased tumour vascularity and an increase in extravascular perfusion.

These data suggest that it is possible by targeting signalling intrinsic to the tumor cells themselves to manipulate the tumor microenvironment in a manner that renders the tumor more susceptible to cytotoxic therapy with drugs or radiation. We will present data supportive of this hypothesis both from Radiation growth delay assays and cytotoxic drug uptake and metabolism.

O177

M-CSF Inhibition Selectively Targets Pathological Angiogenesis and Lymphangiogenesis

Yoshiaki Kubota[1], Toshio Suda[1]
[1]*Department of Cell Differentiation, Keio University, Tokyo, Japan*

Anti-angiogenic therapy for the treatment of cancer and other neovascular diseases is desired to be selective for pathological angiogenesis and lymphangiogenesis. Macrophage-colony stimulating factor (M-CSF), a cytokine required for the differentiation of monocyte-lineage cells, promotes the formation of high-density vessel networks in tumors (Lin et al. 2001; 2006) and therefore possesses therapeutic potential as a M-CSF inhibitor (Aharinejad et al. 2004; Paulus et al. 2006). However, the physiological role of M-CSF in vascular and lymphatic development, as well as the precise mechanisms underlying the anti-angiogenic effects of M-CSF inhibition, remains unclear. Moreover, therapeutic potential of M-CSF inhibition in other neovascular diseases has not yet been evaluated. In this study, we used osteopetrotic (*op/op*) mice to demonstrate that M-CSF deficiency reduces the abundance of LYVE-1^+ and LYVE1^- macrophages, resulting in defects in vascular and lymphatic development. In ischemic retinopathy, M-CSF was required for pathological neovascularization, but was not required for the recovery of normal vasculature. In mouse osteosarcoma (established from c-Myc–overexpressing, *Ink4a/ARF*$^{-/-}$, bone marrow-derived stromal cells), M-CSF inhibition effectively suppressed tumor angiogenesis and lymphangiogenesis, and disorganized extracellular matrices. In contrast to VEGF blockade, interruption of M-CSF inhibition did not promote rapid

vascular regrowth. Continuous M-CSF inhibition did not affect healthy vascular and lymphatic systems outside tumors. These results suggest M-CSF-targeted therapy is an ideal strategy for treating ocular neovascular diseases and cancer (Kubota et al. *J. Exp. Med.* 2009).

O178

Pre-Clinical Evaluation of a Potent and Selective CXCR4 Peptide Antagonist Currently in Phase 1 Trials for Cancer

Sheng-Bin Peng[1], Liang Zeng Yan[1], Wayne Kohn[1], Qinyuan Lou[1], Lisa Russell[1], Datian Lin[1], Xiaoyi Zhang[1], William Roell[1], John Wijsman[1], Kelly Credille[1], Yu-Hua Hui[1], Maciej Zamek-Gliszczynski[1], Jacqueline Akunda[1], John Stille[1], Donald Thornton[1], Jonathan Yingling[1]
[1]*Eli Lilly and Company, Indianapolis, IN, USA*

Emerging evidence demonstrates that SDF-1 (or CXCL12) and CXCR4, a chemokine and chemokine receptor pair, play important roles in multiple stages of tumorigenesis. We have recently developed a series of potent and selective CXCR4 peptide antagonists, and one of which is currently in Phase 1 clinical trials for cancer. This peptide antagonist specifically blocks SDF-1 binding to human and monkey CXCR4 with IC50 values of 0.079 and 0.097 nM, respectively. It inhibits SDF-1-induced GTP binding with Kb value of 0.38 nM. In human lymphoma U937 cells expressing endogenous CXCR4, the peptide inhibits SDF-1-induced cell migration with IC50 value of 0.26 nM. It also inhibits SDF-1/CXCR4-mediated intracellular signaling, exhibiting a dose-dependent inhibition of SDF-1-stimulated pERK and pAkt in multiple tumor cell lines. Biochemical and cellular analysis revealed that this peptide has no apparent agonist activity. Pharmacokinetic analysis demonstrated that the terminal elimination half life of this peptide is 1.5, 3.3, and 3.3 hr, and the subcutaneous bioavailability is 100, 68 and 100% in rat, dog and monkey, respectively. In a mouse pharmacodynamic model, this peptide induces a dose and time-dependent increase of circulating white blood cells/neutrophils and hematopoietic progenitor cells with an ED50 value of 0.74–0.85 mg/kg, and this PD effects last 6–24 hr depending on dose. Similar pharmacodynamic effects were observed in monkey based on an increased level of circulating CD34+ cells, white blood cells and neutrophils. Analysis of pharmacokinetic and pharmacodynamic data from multiple species supports a once daily subcutaneous injection dosing regimen in the clinic. Additionally, the peptide has shown dose-dependent inhibition of tumor growth in multiple human xenograft models utilizing cell lines that express high levels of CXCR4, such as non-Hodgkin's lymphoma and lung tumor models. It also inhibits tumor cell metastasis in an experimental breast tumor metastasis model.

O179

Inhibition of Cathepsin Proteases Synergizes with Maximum-Dose and Low-Dose Chemotherapy to Block Malignant Progression in a Mouse Model of Metastatic Breast Cancer

Tanaya Shree[1,2], Benelita T. Elie[1], Alfred Garfall[1], Katherine Bell-McGuinn[1], Kenishana Simpson[1], Violetta Barbashina[1,3], Johanna A. Joyce[1]
[1]*Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY, USA,* [2]*Tri-Institutional MD-PhD Program, Well Cornell Medical College/Rockefeller University/Memorial Sloan Kettering Cancer Center, New York, NY, USA,* [3]*Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Cysteine cathepsin proteases are deregulated in many human tumors, and have been implicated in promoting angiogenesis, invasion, and metastasis. Their genetic ablation or pharmacological inhibition significantly impairs tumor progression in several mouse models. Oncologists rely heavily on maximum tolerated dose (MTD) chemotherapy to treat cancer, but this frequently leads to chemoresistance and has limited efficacy against metastasis, the primary cause of cancer deaths. Continuous low dose (CLD) chemotherapy delivers lower doses at greater frequency, and has been shown to be anti-angiogenic. We hypothesized that combining cathepsin inhibition with agents targeting cancer cells and vasculature could dramatically improve anti-tumor efficacy and prevent metastatic progression.
Using a mouse model of breast cancer (MMTV-PyMT), we treated mice with MTD paclitaxel (Tax^{MTD}), CLD cyclophosphamide (Cyc^{CLD}), and a cathepsin inhibitor (JPM), alone and in combinations. While JPM alone had no effect on mammary tumor burden, it significantly impaired tumor growth when combined with Tax^{MTD} (52% reduction vs. 37% for Tax^{MTD} alone). Adding JPM to the Tax^{MTD}+Cyc^{CLD} combination accomplished a significant 83% reduction compared to 62% without JPM. Interestingly, tumor lysates from Tax^{MTD}–treated mice contained higher levels of cathepsin activity and mRNA. As infiltrating immune cells are the primary source of cathepsins in these tumors, we reasoned that tumors may mobilize cathepsin-positive cells from the bone marrow after Tax^{MTD} treatment to promote recovery from the cytotoxic assault, potentially explaining why cathepsin inhibition in the context of Tax^{MTD} treatment is more effective than treating with either drug alone. Indeed, increased cathepsin activity-positive cells were found in the blood 48 hours after Tax^{MTD} treatment. Our current data also suggests that cathepsin inhibition specifically impairs the development of lung metastases. These analyses clearly support a therapeutic benefit from adding cathepsin inhibition to chemotherapeutics in the treatment of breast cancer and the prevention of metastases.

O180

The Effect of the PAX2 Oncogene on the Tumor Microinvironment, Tumor Progression and its Potential as a Therapeutic Target for Prostate Cancer

Carlton Donald[1]
[1]*Phigenix, Inc., Atlanta, GA, USA*

Inhibition of cell death is a critical pathophysiological factor that contributes to the initiation and progression of cancer. Recently, much attention has focused on developing therapeutic agents aimed at cancer cell survival pathways involving factors such as MEK kinase and AKT. Unattenuated, tumour-associated expression of PAX2, a transcriptional regulator implicated in oncogenesis and cancer development, has been observed to play a direct role in these pathways. PAX2 expression is aberrantly turned on in a number of cancers such as Wilm's Tumor, breast, ovarian, bladder and prostate. We have discovered a novel mechanism by which PAX2 promotes cancer cell survival through the suppression of the host defense peptide and putative tumor suppressor Human Beta Defensin-1 (hBD-1). Our current findings provide the first indication of the cellular factors responsible for deregulated PAX2 expression in prostate cancer and how targeting these factors promote cancer cell death. Collectively, these data offers substantial evidence of the therapeutic potential of inhibiting PAX2 for the treating prostate cancer.

O181

Targeting the Tumor Stroma - a Novel Therapeutic Strategy Based on Separate Analysis of the Malignant and Stromal Cell Compartments in Brain Tumors

Jian Wang[1], Anne M. Øyan[2], Oleg Tsinkalovsky[2], Hege Jacobsen[1], Peng Zhao[3], Linda Sleire[1], Lars Prestegarden[1], Rene Brons[5], Emmet Cormark[4], Xingang Li[3], Simone Niclou[5], Bjorn Tore Gjertsen[4], Rolf Bjerkivg[1,5], Karl-Henning Kalland[2], Per Øyvind Enger[1,6]
[1]*Department of Biomedicine, Univeristy of Bergen, Bergen, Norway,* [2]*The Gade Institute, University of Bergen, Bergen, Norway,* [3]*Neurosurgical Department, Qilu Hospital, Shandong University, Jinan, China,* [4]*Department of Internal medicine, Hematology Section, Haukeland University Hospital, Bergen, Norway,* [5]*NorLux Neuro-Oncology, Centre Recherche de Public Sante, Luxembourg, Luxembourg,* [6]*Department of Neurosurgery, Haukeland University Hospital, Bergen, Norway*

The recruitment of host vasculature and the infiltrative behaviour of gliomas underscore the significance of tumor-stroma interactions in brain tumor pathogenesis. The aim of this project is to identify cancer-related changes in the stroma during brain tumor progression that can be targeted therapeutically. However, targeting tumor-activated stromal cells require further insight into the mechanisms that regulate the tumor-stroma interplay. Since, any tumor biopsy contains a mixture of cancer cells and stromal cells, we are unable to determine whether a given gene expression profile or protein signature is derived from stromal or cancer cells. For the same reason, we are also unable to specify the directions of cross-talk between compartments; whether an influence is excerted upon the tumor by the surrounding stroma, or vice versa. In this project, we have generated a green fluorescent protein (GFP) -expressing on the nude rat by crossing nude rat with a transgenic GFP-expressing line. We implant human glioma biopsies in green-fluorescent (GFP) immunodeficient rats. The resulting xenograft tumors are dissociated into a cell suspension and FACS-sorted into GFP-positive stromal cells and GFP-negative tumor cells. We also obtained cell suspensions of stromal cells from normal brain. Human specific nuclei antibody staining has confirmed that sufficient purity of the sorted cells. Using this tool, we intend to delineate the gene expression profiles and protein signatures unique to the tumor-activated stromal cells. This information will subsequently be used to tailor drug regimens that target tumor-activated stroma and tumor-stroma interactions.

O182

Does Hypoxia Play a Role in the Failure of Androgen Ablation Therapy for Prostate Cancer?

Jenny Worthington[1], Louise Ming[1], Maxwell Omabe[1], Christopher Mitchell[1], Stephanie McKeown[1]
[1]*Biomedical Sciences Research Institute, University of Ulster, Coleraine, UK*

Introduction: Androgen-dependent prostate cancer is frequently treated with androgen ablation therapy (AAT), however tumours often recur in 1 – 3 years with an aggressive, androgen-independent phenotype. It is proposed that treatment-induced stress factors in the tumour microenvironment, may contribute to this failure.
Method: LNCaP tumours were grown on the backs of male SCID mice. Tumour oxygenation was measured before and (a) 24 hours after treatment with a panel of anti androgens (b) during 28 days of daily dosing with bicalutamide (2 mg/kg). LNCaP tumour fragments were implanted into a dorsal skin flap (DSF) onto the backs of SCID mice. The animals were treated with bicalutamide (2 mg/kg) daily and tumour vasculature was imaged weekly for 21 days.
Results: Flutamide (25 mg/kg) and bicalutamide(10 mg/kg) significantly reduced tumour oxygenation after 24 hours. Bicalutamide administered daily at a clinically relevant dose (2 mg/kg) over 28 days resulted in profound decrease in tumour oxygenation within 1 – 3 days which persisted for a further 10 days until levels gradually began to increase, reaching almost pre-treatment levels by day 28. In tandem, tumour vasculature began to decrease until day 14 when only large feeder vessels were present however by day 21 the re-emergence of connecting vessels was apparent (imaged in DSF). Tumours excised 0 – 28 days) show altered genetic profiles and by day 28 excised tumour cells were more invasive. This was confirmed *in vivo* when metastatic deposits in the lungs were quantified in bicalutamide-treated animals and compared to vehicle-treated animals.
Conclusion: This study shows that AAT alters tumour oxygenation as early as 24 hours after treatment initiation causing profound hypoxia for 10 – 14 days. Within this time we propose that a selection pressure is created, which favours a more aggressive androgen-independent phenotype. This could explain why this treatment ultimately fails and suggests that new therapeutic strategies should be developed.

O183

Inhibition of Fibroblast-to-myofibroblast Transition as a Modality for Cancer Treatment: Effect of Halofuginone

Mark Pines[1]
[1]*Department of Animal Sciences, Volcani Center, Bet-Dagan, Israel*

Most solid tumors consist of a mixture of neoplastic and non-neoplastic cells together with ECM components. This cellular microenvironment directly modulates tissue architecture, cell morphology and cell fate and the ECM–stromal cell interaction contribute to the neoplastic phenotype. The conversion of fibroblasts into myofibroblasts, as mediated by TGFb is the most prominent stromal reaction in many epithelial lesions thus emerges as a viable target for pharmacological intervention.
Halofuginone is an inhibitor of Smad3 phosphorylation downstream of the TGFb signaling. Halofuginone inhibited myofibroblasts activation and their ability to synthesize ECM resulted in inhibition of tumor progression in various cancer xenografts such as Wilm's tumor, pancreas and renal carcinoma. In prostate cancer xenografts, halofuginone inhibition of tumor progression was correlated with reduction of plasma PSA. The myofibroblasts are essential for tumor establishment and progression. Pancreatic tumor cells when implanted alone in mice produce few tumors that progress at a low rate. Addition of myofibroblasts resulted in more tumors that developed at much higher rate. Inhibition of myofibroblasts activation by halofuginone prior to implantation reduced tumor number. Moreover, in an orthotopic model, more tumors were developed in the fibrotic pancreas compare to the normal pancreas. Halofuginone treatment inhibited pancreas fibrosis and reduced tumor number. Halofuginone is an ideal candidate for combination therapy, because of its unique mode of action and the dissimilarity of its targets from chemotherapy. In various xenografts halofuginone synergizes with low dose of chemotherapy resulting in a major reduction in tumor progression comparable to that observed by high dose of chemotherapy.
We suggest that targeting fibroblast-to-myofibroblast transition with halofuginone significantly slow tumor progression and when combined with low doses of chemotherapy a major anti-tumoral effect is achieved, avoiding the need of high dose of chemotherapy without impairing treatment efficacy.

O184

Stromal Caveolin-1 Predicts Recurrence and Clinical Outcome in DCIS and Human Breast Cancers

Agnes K. Witkiewicz[1], Abhijit Dasgupta[1], Isabelle Mercier[1], Gordon Schwartz[3], Celina Kleer[2], Richard G. Pestell[1], Federica Sotgia[1], **Michael P. Lisanti**[1]
[1]*Cancer Biology; Medical Oncology; and Pathology, Kimmel Cancer Center; Thomas Jefferson University, Philadelphia, PA, USA,* [2]*Pathology, University of Michigan, Ann Arbor, MI, USA,* [3]*Surgery, Thomas Jefferson University, Philadelphia, PA, USA*

Previously, we showed that caveolin-1 (Cav-1) expression is down-regulated in human breast cancer-associated fibroblasts. Here, we discuss recent evidence that an absence of stromal Cav-1 expression in human breast cancers is a powerful single independent predictor of early disease recurrence, metastasis and poor clinical outcome. These findings have now been validated in two independent patient populations. Importantly, the predictive value of stromal Cav-1 is independent of epithelial marker status, making stromal Cav-1 a new "universal" or "widely-applicable" breast cancer prognostic marker. We propose based on the expression of stromal Cav-1, that breast cancer patients could be stratified into high-risk and low-risk groups. High-risk patients showing an absence of stromal Cav-1 should be offered more aggressive therapies, such as anti-angiogenic approaches, in addition to the standard therapy regimens. Mechanistically, loss of stromal Cav-1 is a surrogate biomarker for increased cell cycle progression, growth factor secretion, "stemness", and angiogenic potential in the tumor microenvironment. Since almost all cancers develop within the context of a stromal microenvironment, this new stromal classification system may be broadly applicable to other epithelial and non-epithelial cancer subtypes, as well as "pre-malignant" lesions (carcinoma in situ). We conclude that Cav-1 functions as a tumor suppressor in the stromal microenvironment.
An absence of stromal caveolin-1 expression predicts early tumor recurrence and poor clinical outcome in human breast cancers.
Witkiewicz AK, Dasgupta A, Sotgia F, Mercier I, Pestell RG, Sabel M, Kleer CG, Brody JR, Lisanti MP.Am J Pathol. 2009 Jun;174(6):2023–34.

O185

Antimetastasic Action of Parp Inhibition in Melanoma trough Counteracting Angiogenesis and emt Transition

F. Javier Oliver[1], Andreína Peralta-Leal[1], Maribel Rodríguez-Lara[1], Francisco O'Valle Ravassa[2], Antonio Garcia de Herreros[3], Françoise Dantzer[4]
[1]*Department of Cell Biology and Immunology, IPBLN, CSIC, Granada, Consejo Superior de Investigaciones Científicas, Armilla, Granada, Spain,* [2]*Department of Pathology, Universidad de Granada, Granada, Spain,* [3]*IMIM, Universitat Pompeu Fabra, IMIM, Barcelona, Spain,* [4]*INTEGRITÉ DU GENOME UMR 7175, CNRS, Illkirch, Strasbourg, France*

Tumor angiogenesis and Epithelial-mesenchymal transition (EMT) are key step toward cancer metastasis. Inhibition of PARP has been reported to have anti-neoplasic effect as monotherapy or in combination with chemo or radiotherapy in different tumor settings. In this study we present results that PARP inhibition, as monotherapy, is able to countertact metastasis of melanoma cells to lung and other organs by interfering with tumor angiogenesis through alterations in vimentin and v-cadherin expression levels and EMT, resulting in down regulation and inactivation of Snail1. We also show that PARP-1 is a potent regulator of SNAIL-1 activation through modification of SNAIL-1 by poly(ADP-ribosylation) and direct protein-protein

interaction. These results suggest that inhibition of PARP through its ability to interfere with key metastasis-promoting processes, could suppress invasion and colonization of distant organs by aggressive metastatic cells.

O186

Targeting Cancer-Associated Fibroblasts (CAFs) with Small Molecule Inhibitors to Enhance Sensitivity of Tumors to Conventional Chemotherapy

Silke Haubeiss[1], Maike Sonnenberg[1], Godehard Friedel[2], Heiko van der Kuip[1], Walter E. Aulitzky[3]
[1]*Dr. Margarete-Fischer-Bosch-Institute for Clinical Pharmacology, Stuttgart, Germany,* [2]*Hospital Schillerhöhe, Stuttgart, Germany,* [3]*Department of Hematology and Oncology, Robert-Bosch Hospital, Stuttgart, Germany*

Cancer-associated fibroblasts (CAFs) are important modulators of tumor growth, invasion, and metastasis. Recently, we demonstrated that the response to chemotherapy of an individual tumor also depends on CAFs. Therefore, targeting CAFs with small molecule inhibitors may be an attractive strategy to enhance sensitivity of solid tumors to conventional chemotherapy.
We isolated CAFs from 62 lung tumors. A subset was analyzed for their sensitivity to a panel of 162 kinase inhibitors and to Cisplatin. Sensitivity of CAFs from individual tumors to Cisplatin was highly variable (GI50 2.8–29.0 μM). CAF strains responding to Cisplatin in isolated culture turned out to be significantly less sensitive when cocultivated with the tumor cell line H1299 indicating a protective effect of the tumor cells on CAFs. All CAF strains investigated were sensitive to PDGFR inhibitors such as Dasatinib. In addition, the Mdm2 antagonist Nutlin-3 turned out to be a promising compound for targeting CAFs. Both PDGFR inhibitors and Nutlin-3 blocked CAF proliferation without inducing cell death. Nutlin-3 also protected CAFs from Cisplatin-induced cell death.
Microarray analysis of CAFs cultivated in presence or absence of Dasatinib identified 368 genes whose expression was changed significantly at least twofold. 87 of these encoded cell cycle related proteins with only 3 of them being upregulated by Dasatinib. 265 genes were downregulated in the presence of Dasatinib. Among these we found genes encoding repair proteins like FANC family members and BRCA1. 103 genes were upregulated such as genes encoding PDGFRB, ECM components and adhesion proteins. Further analysis will reveal whether this signature may have prognostic value and if CAFs can be modulated by Dasatinib to be less supportive to tumor cells.
In conclusion, we identified several small molecule inhibitors with significant effects on CAFs. Our study may guide the development of novel treatment strategies combining these inhibitors with conventional chemotherapy.

O187

Monitoring Tumour Response to the Anti-angiogenic Therapy Sunitinib with an F18-labeled Angiogenesis Imaging Agent

Lucy Allen[1], Mark Battle[1], Luisa Contreras[1], Joanne Cooper[1], Rochelle Lear[1], Julian Goggi[1], Clare Durrant[1]
[1]*Medical Diagnostics, GE Healthcare, Amersham, Buckinghamshire, UK*

Introduction*:* The RGD-binding integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ play key roles in tumour angiogenesis. We examined an [^{18}F] labeled small peptide (AH111585) containing an RGD (Arg-Gly-Asp) sequence. AH111585 binds with high affinity (nM) to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, which are highly expressed on tumour neovasculature. In this study, [^{18}F]AH111585 was used to examine the response of human glioblastoma (U87) xenografts to treatment with the anti-angiogenic therapy Sunitinib.
Materials & methods: U87 tumour uptake of [^{18}F]AH111585 was determined by microPET imaging (% id/g) following administration of the anti-angiogenic therapy, such as Sunitinib. Tumour microvessel density (MVD) was also analysed post-therapy.
Results*:* Dymanic mircoPET imaging of [^{18}F]AH111585 uptake demonstrated that tumour uptake peaked ~30 mins post-injection of the tracer (5% id/g). Whole body biodistribution studies confirmed rapid clearance of [^{18}F]AH111585 from the blood with predominantly urinary excretion. Following administration of the clinically relevant anti-angiogenic therapy Sunitinib, a reduction in [^{18}F]AH111585 tumour uptake was demonstrated compared to vehicle controls. Skeletal muscle, used as a reference tissue, demonstrated equivalent [^{18}F]AH111585 uptake pre- and post-therapy. A reduction in MVD was also seen in anti-angiogenic therapy treated tumours.
Conclusions*:* The data demonstrate that [^{18}F]AH111585 can detect changes in tumour uptake following acute anti-angiogenic therapy. The results suggest this imaging agent may provide clinically important information to guide patient management and monitor response to anti-angiogenic therapies.

POSTERS

Poster No. 1

Mesenchymal Stromal Cells (MSC) in AML Bone Marrows Carry Clonal Genomic Abnormalities

Michael Andreeff[1], Teresa McQueen[1], Marina Konopleva[1], Christopher Williams[2], Vicki Hopwood[3], Taylor Appleberry[2], Corinn Rich[2], Steven Kornblau[1], Rui-Yu Wang[1]
[1]*Molecular Hematology & Therapy, Department of Stem Cell Transplantation and Cellular Therapy, UT M. D. Anderson Cancer Center, Houston, TX, USA,* [2]*PerkinElmer, Inc., Waltham, MA, USA,* [3]*School of Health Sciences, UT M. D. Anderson Cancer Center, Houston, TX, USA*

Bone marrow-derived mesenchymal stromal cells (BM-MSC) have the capacity to differentiate into various cell types to support normal and malignant hematopoiesis. However, little is known about the molecular genetics of these cells. We therefore isolated MSC from normal donors and from patients with acute myeloid leukemias (AML). Purity of MSC preparations was >95%. Ten samples from AML patients with normal (n=7) and abnormal leukemia karyotypes (n=3) were analyzed by conventional cytogenetics, array-CGH and FISH. Genomic DNA from MSC was extracted and array comparative genomic hybridization (aCGH) was performed using the PerkinElmer Constitutional Chip 4.0 that contains 5,200 BAC clones with human inserts that detects and maps changes in DNA copy number variations. DNA from AML MSC and a normal reference genome were differentially labeled with fluorescent dyes and hybridized to the array. Abnormalities detected by aCGH require the presence of at least 20% of cells carrying identical aberrations. For confirmation, individual BAC DNAs were labeled using the Invitrogen DNA labeling kit for FISH. Results: Conventional cytogenetics (G-banding analysis) showed normal diploid chromosomes in 9/10 cases, except in one sample (47, XX, +5). The corresponding AML karyotype was apparently unrelated 46, XX, der(16)t(1;16) (q21; q12.1). This finding was confirmed by FISH and aCGH. At variance to BM-MSC derived from normal donors (n=4), AML-derived MSC showed abnormalities (gains and losses) in different chromosomal regions in all cases. The most frequently involved chromosomes were No. 3, 4, 6, 7, 8, 10, 15, 16, 19, and 22. All abnormalities were confirmed by FISH using the identical BAC clones employed on the array. Conclusion: Results suggest that stromal cells from newly diagnosed leukemias carry clonal genomic abnormalities at high frequency. Hence, AML bone marrows contain two populations of clonally abnormal cells (AML and MSC).

Poster No. 2

Differential Expression of Epithelial-Mesenchymal Transition-Related Gene Markers between Primary Colorectal Carcinomas and Liver Metastases

Richard H. Argent[1], Philip Clarke[1], Elisabeth Whelband[1], Dileep N. Lobo[2], Kate Shepherd[2], Peter King[3], Martin Page[3], Rajendra Kumari[1], Anna M. Grabowska[1], Sue A. Watson[1]
[1]*Division of Pre-Clinical Oncology, University of Nottingham, Nottingham, UK,* [2]*Division of Gastrointestinal Surgery, University of Nottingham, Nottingham, UK,* [3]*Division of Janssen Pharmaceutica N.V., OrthoBiotech Oncology Research and Development, Beerse, Belgium*

Background:
Epithelial-mesenchymal transition (EMT) is frequently activated during carcinogenesis resulting in metastatic spread. EMT activation downregulates E-cadherin expression leading to increased motility and gain of a more mesenchymal phenotype. The c-Met receptor and MACC1 (metastasis-associated in colon cancer-1) are upregulated in CRC metastases and can be considered to be markers of metastatic potential. Here we assessed the expression of genes associated with EMT in CRCs and liver metastases (LMs).
Methods:
Human primary CRC (n=11) and LM (n=21) samples were obtained under full ethical approval from Queen's Medical Centre, Nottingham, UK. Samples were stored in RNA*later* prior to RNA extraction, cDNA synthesis, and real-time quantitative PCR to determine expression levels of EMT markers (Snail, Slug, Zeb1, E-cadherin), mesenchymal markers (vimentin, s100a4), as well as the c-Met receptor, MACC1, hepatocyte growth factor (HGF), and TGFβ1 relative to the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase. A student's t-test was used for statistical analysis.
Results:
Snail ($p<0.005$), vimentin ($p<0.0001$), s100a4 ($p<0.005$), and TGFβ1 ($p<0.005$) were significantly upregulated in LMs compared to normal liver. MACC1 was significantly uregulated in CRCs and LMs ($p<0.01$), and only weakly expressed in normal liver. In CRCs, c-Met ($p<0.005$) expression was significantly increased compared to normal colonic mucosa, whereas HGF ($p<0.05$), Slug ($p<0.01$), Zeb1 ($p=0.005$), s100a4 ($p<0.05$), and vimentin ($p<0.001$) expression were significantly downregulated. E-cadherin expression was significantly decreased in CRCs ($p<0.01$), and liver metastases ($p<0.005$) compared to normal colon. Comparison of expression of EMT markers between CRCs and LMs showed that HGF ($p=0.001$), Snail ($p<0.001$), Slug ($p=0.026$), Zeb1 ($p<0.001$), vimentin ($p<0.005$), and TGFβ1 ($p<0.005$) were all significantly upregulated in LM tissue.

Conclusion:
EMT markers were significantly increased in LMs compared to CRCs. MACC1 was significantly increased in CRCs, and for the first time shown to be significantly increased in LMs. Snail, TGFβ1, and vimentin, provide the best markers for LM.

Poster No. 3

Post Transcriptional Regulation of Human Heparanase by AU-Rich Element

Gil Arvatz[1], Ofer Nativ[2], Neta Ilan[1], Israel Vlodavsky[1]
[1]*Cancer and Vascular Biology Reasearch Center, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel,* [2]*Department of Urology, Bnai-Zion Medical Center, Haifa, Israel*

Heparanase is an endo-β-D-glucuronidase, the predominant enzyme that degrades heparan sulfate side chains of heparan sulfate proteoglycans. Traditionally, heparanase activity was correlated with the metastatic potential of tumor-derived cells, attributed to enhanced cell dissemination as a consequence of heparan sulfate cleavage and remodeling of the extracellular matrix barrier. More recently, heparanase up-regulation was documented in an increasing number of human carcinomas and hematological malignancies. In many cases, heparanase induction correlated with increased tumor metastasis, vascular density, and shorter post operative survival of cancer patients, thus providing a strong clinical support for the pro-metastatic and pro-angiogenic functions of the enzyme and encouraging the development of heparanase inhibitors as anti-cancer drugs. Mechanisms responsible for heparanase induction are largely unknown. We hypothesized that heparanase may be regulated post-transcriptionally by regulatory sequences located at the 3′-untranslated region (3′-UTRs) of the gene. We provide evidence that the 3′-UTR of heparanase contains an adenosine/uridine (AU)-rich element [5′-$(AUUU)_n$-3′] present within the 3′-UTRs of many proto-oncogene and cytokine mRNAs. This element confers post-transcriptional gene regulation by decreasing mRNA stability and/or by inhibition of mRNA translation. PCR amplification of heparanase 3′ UTR revealed the existence of two products in all human cell lines examined, in a similar ratio. Sequencing of the lower molecular weight PCR product identified a deletion of 185 nucleotides, resulting in loss of the highly conserved AU-rich element. Loss of this element was associated with increased heparanase enzymatic activity and cell invasion. Moreover, heparanase transcript lacking the AU-rich element was elevated in renal carcinoma biopsies compared with the adjacent normal looking tissue, indicating that this regulatory mechanism is clinically relevant.

Poster No. 4

Characterisation of the Effects of the Metastasis-Inducing Calcium-Binding Protein S100P on Cell Activity

Valery Attignon[1], Philip Rudland[1], Roger Barraclough[1]
[1]*School of Biological Sciences, University of Liverpool, Liverpool, Merseyside, UK*

S100P is a member of the S100 family of small regulatory calcium-binding proteins[1], which has been shown to play a role in the metastatic phase of cancer. Intracellular overexpression of S100P under physiological conditions has been correlated to metastasis and poor overall survival in breast cancer patients[2]. The mechanism of the Metastasis-Inducing Calcium-binding protein, S100P in metastasis has not yet been fully elucidated.
To investigate the role of metastatic S100P on cell activity, several analyses such as motility assays, gene expression using microarrays and Real-Time PCR, changes in intracellular signalling induced by addition of extracellular S100P have been performed. These experiments were carried out using an expression system developed in our laboratory consisting of a human S100P cDNA inserted in a tetracycline inducible vector transfected into non-metastatic benign rat mammary tumour-derived cells (Rama 37), and human cervical carcinoma cells (HeLa).
Results observed after microarray hybridisation showed 8 upregulated genes and 3 downregulated genes, after intracellular overexpression of S100P in rat mammary tumour cells. Extracellular addition of S100P has been shown to increase cell motility suggesting alternative cell motility stimulation via a cell surface receptor. Extracellular S100P has been shown to activate intracellular phosphorylation of a member of Serine/Threonine-specific protein kinase family: Akt and Extracellular signal-Regulated Kinases: Erk 1/2 through the Receptor for Advanced Glycosylation End-Product (RAGE).
Results enabled the Metastasis-Inducing Calcium-binding protein mechanisms to become clearer as S100P that could represent a potential target for novel diagnostic and therapeutic applications.
[1] Becker, T., et al., Eur. J. Biochem. 207, 541–547.
[2] Wang G., et al., Cancer Res. 60,1199–1207.

Poster No. 5

Differential Expression of Exonuclease Activity in Cytoplasm by Activated p53 Protein

Sanaz Derech-Haim [1], Shai Grinberg[1], Racheli Kadosh[1], Galia Rahav[1], Benjamin Sredni[2], **Mary Bakhanashvili[1]**
[1]*Department of Infectious Diseases, Sheba Medical Center, Tel-Hashomer, Israel,* [2]*Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel*

The p53 protein is responsible for control of the cell cycle, apoptosis and DNA repair. The abundance of p53, sub-cellular localization, and the interaction with cofactors play a central role in the regulation of its different biochemical functions. p53 in cytoplasm is functional and exhibits a spectrum of different biological effective pathways. p53 in cytoplasm exerts intrinsic 3¢®5¢ exonuclease activity with various RNA and DNA substrates. p53 may act as an external proofreader for errors introduced by exonuclease-deficient DNA polymerases. p53 can remove 3′-terminal nucleotides from RNA substrates containing an ARE element (localized to the 3′ un-translated region of many proto-oncogene and cytokine mRNAs). The sub-cellular localization of p53 and its functions are influenced by various external stimuli. Hence, the exonuclease activity in cytoplasm with activated p53 induced by drug treatment or following g-irradiation was elucidated. The treatment of HCT116(p53+/+) cells with Doxorubicin (Doxo) or DL-a-difluoromethyl-ornithine (DFMO) enhanced the cytoplasmic levels of p53. Interestingly, the exonuclease activity with various ARE-RNA substrates in cytoplasmic extracts of Doxo- or DFMO-treated cells was lower than in controls. Conversely, there was no decrease in exonuclease activity with DNA substrates. Apparently, the observed reduction in exonuclease activity with RNA substrates after Doxo- or DFMO-treatment is not a general phenomenon. The cytoplasmic extracts of HCT116(p53+/+) cells were further examined for exonuclease activity following g-irradiation (IR) or treatment by low-molecular weight immunoenhancer ammonium trichloro(dioxyethylene-O,O'-) tellurate (AS101). The increase in the level of p53 is concomitant with an increase in constitutive excision capacity in IR-exposed or AS101-treated cytoplasmic extracts with ARE-RNA and DNA substrates. Altogether, the data demonstrate the difference in expression of exonuclease activity in cytoplasmic fractions when p53 is stabilized under various stress scenarios.

Poster No. 6

High Stromal Expression of CAIX is Predictive of Poorer Overall Survival in Patients with HPV-negative Head & Neck Cancer Treated with Concurrent Chemoradiotherapy

Nigel Brockton[1,2], Joseph Dort[2,4], Harold Lau[2], Desiree Hau[2], Sony Brar[1], Roman Diaz[3], Corinne Doll[2], Alexander Klimowicz[3], Alan Box[3], Anthony Magliocco[2,3]

[1]*Population Health Research, Alberta Health Services, Calgary, AB, Canada,* [2]*Department of Oncology, University of Calgary, Calgary, AB, Canada,* [3]*Department of Pathology, University of Calgary, Calgary, AB, Canada,* [4]*Department of Surgery, University of Calgary, Calgary, AB, Canada*

Background:
Human Papilloma Virus (HPV) is an important etiologic factor in the development of head and neck squamous cell carcinoma (HNSCC). Although HPV + tumours typically present at a more advanced stage, they are associated with a more favourable prognosis. Tumour hypoxia has been associated with radioresistance but direct measurement of tumour oxygenation has practical limitations. Consequently, candidate endogenous markers of hypoxia (EMH) (e.g. Glucose Transporter 1 (GLUT1) and Carbonic Anhydrase IX (CAIX)) have been evaluated. No previous studies have stratified EMH analysis by HPV status. Moreover, there have been no previous studies quantifying EMH expression within the stromal compartment of these tumours.
Methods:
Ninety-two patients diagnosed with locally advanced HNSCC and treated with concurrent cisplatin and radiotherapy between 2000 and 2005 were identified. Fifty-five patients had pre-treatment FFPE tumours available for analysis. Triplicate 0.6 mm cores were assembled into TMAs. Semi-quantitative p16 immunohistochemistry (IHC) staining was used as a surrogate for HPV status. Automated, quantitative IHC (AQUA HistoRx™) was used to quantify staining for CAIX and GLUT1, as candidate EMH. We analysed the tumour and stromal expression of each candidate EMH, stratified by tumour p16 status. Overall survival was estimated from Kaplan-Meier method and curves compared using a log rank test.
Results:
53% of tumours were p16+ and 47% were p16-. For patients with p16- tumours and high stromal CAIX expression, 2-year overall survival was 33%, compared to 91% with low stromal CAIX expression ($p<0.05$). At 5 years, this overall survival difference remained significant (42% vs 22%, respectively, $p<0.05$). Epithelial CAIX expression was not a statistically significant predictive factor.
Conclusion:
High stromal CAIX expression is a significant negative predictive factor for survival in locally advanced HNSCC patients with p16- tumours. This finding may impact therapeutic targeting for this patient group, including use of hypoxic radiosensitizers.

Poster No. 7

Avastin Has a Direct Deleterious Effect on Multiple Myeloma Cell Lines

Oshrat Attar[1,2], Michael Lishner[1,2,3], Shelly Tartakover Matalon[1,2], **Liat Drucker[1,2]**

[1]*Oncogenetic Laboratory, Meir Medical Center, Kfar Saba, Israel,* [2]*Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel,* [3]*Internal Medicine Department, Meir Medical Center, Kfar Saba, Israel*

Introduction: Multiple myeloma (MM) is an incurable malignancy of plasma cells. Intensive interaction of the neoplastic cells with their microenvironment undermines effective disease treatment. MM cells secrete VEGF that promotes cytokine production and proliferation of the tumor cells. The angiogenic effect of VEGF in the bone marrow is established yet less is known about VEGF signaling in MM cells. Here we evaluated the anti-myeloma effect of VEGF inhibition by Avastin (humanized anti-VEGF monoclonal antibody). Moreover, we aimed to identify VEGF dependent signaling cascades in MM cell lines with specific emphasis on pathways that regulate protein translation initiation.
Methods: MM cell lines (8226, U266, ARK, ARP1) were cultured 5 days with Avastin (0.01 μg/ml - 4 mg/ml) and tested for: viability (WST1), proliferation (cell count), cell death (Annexin/7AAD, LC3II), cell cycle (flow cytometry), and VEGF targets (mTOR, ERK, eIF4E, etc; immunoblot). Autophagy inhibitor used: 3-methyladenine (3MA).
Results: Dose dependent reduced viability was demonstrated in all Avastin treated MM cell lines. RPMI 8226 and ARK demonstrated a G1 cell cycle arrest and decreased total cell number whereas U266 and ARP1 showed elevated autophagy (LC3II). Co-administration of 3MA and Avastin to U266 and ARP1 yielded a synergistic decrease in viability and elevated apoptotic cell death suggesting that autophagy rescued the VEGF- inhibited cells from death. Changes in VEGF targets included decreased pmTOR, pERK and peIF4E. Reduced eIF4E dependent translation was evidenced by decreased Cyclin D1 in G1 arrested RPMI 8226 and ARK. Additional VEGF signaling pathways will be assessed.
Significance: Our findings so far, establish that VEGF is critical to MM cell lines' viability and that Avastin has a significant deleterious effect on MM cell lines that is independent of its anti-angiogenic mechanism. Identification of VEGF dependent targets in MM cell lines will promote the design of effective drug combinations.

Poster No. 8

Rac-1 GTPase Controls the Capacity of Human Malignant pre-B Lymphoblasts to Migrate on Fibronectin in Response to SDF-1 alpha (CXCL12)

Manuel Freret[1], Flore Gouel[1], Jean-Pierre Vannier[1], Marc Vasse[1,2], **Isabelle Dubus[1]**
[1]*Laboratoire MERCI - EA 3829, IUHRBM & Faculte de Médecine et Pharmacie, Universite de Rouen, Rouen, France,* [2]*Departement of hematology, IUHRBM & CHU de Rouen, Rouen, France*

Childhood acute lymphoblastic leukaemia (ALL) relapse is characterized by malignant cell infiltration of medullary and extramedullary tissues. Thus it is important to better understand the mechanisms governing migration and dissemination of leukemic cells. We investigated the role of the small GTPase Rac1 in the control of CXCL12-induced migration of leukemic cells on fibronectin, which plays a key role in leukemic cell invasion. Nalm-6 cells (a human B-ALL cell line), transformed to overexpress either wild-type or a constitutively inactive form (N17 mutant) of Rac1, were seeded on fibronectin-coated wells. Adherent cells were kept in an incubation chamber under a phase-contrast microscope. Time-lapse video was numerized before and after addition of 100 ng/ml CXCL12, and images were used to quantitate cell migration speed. Spontaneous migration was not significantly different between control and transformed cells. After addition of CXCL12, the migration speed of control, non-transformed cells increased to reach a maximum within 2 hours, and returned to baseline values after 4 hours. In cells tranformed with the N17 mutant, the stimulation of cell migration by CXCL12 was more intense than in control cells ($p < 0.001$) and was still observable after 5 hours. Flow cytometry analysis showed that modifications in Rac1 expression or activity did not significantly affect cell surface expression of the integrins VLA-4 and VLA-5, which are involved in Nalm-6 cells migratory process on fibronectin. However, the SDF-1 receptor CXCR4 was up-regulated (+93%) at the surface of cells overexpressing Rac1, an effect that was prevented by a 24-hour treatment with the Rac inhibitor NSC23766. Taken together, these results suggest that Rac1 plays an important regulatory role in the response of B-ALL cells to the chemoattractant cytokine CXCL12, and thus may control mechanisms involved in leukemic cell dissemination.

Poster No. 9

Down-Expression of RB18A/MED1, a Co-Factor of Transcription, Regulates Modifications of the Tumor Microenvironment to Trigger Strong Tumorigenic Phenotype of Human Melanoma Cells

Raymond Frade[1]
[1]*INSERM U.672 (former U.354), Immunochemistry of Cell Regulations and Virus Interactions, Evry, Ile-de-France, France*

The human gene RB18A/MED1, also named TRAP220 or DRIP205, encodes for a single 205 kDa co-factor of transcription that interacts with nuclear receptors and transcription factors essential for cell growth. We originally identified this human gene and demonstrated that RB18A/MED1 is antigenically and functionally related to p53. In addition, RB18A/MED1 chromosome localization on locus 17q12-q21.1 suggested its involvement in human cancers. Since, others described over expression of RB18A/MED1 in breast, colon and prostate cancers.
We herein analyzed RB18A/MED1 expression in human melanoma cells. We found that RB18A/MED1 is either highly or weakly expressed in melanoma cells, depending on their respectively non or highly-tumorigenic phenotype. Therefore, we analyzed whether a relationship could exist between RB18A/MED1 expression and melanoma cell phenotype. For this purpose, we down-regulated RB18A/MED1 expression by transfecting melanoma cells with a RB18A/MED1 siRNA specific for the 3′-untranslated region of native RB18A/MED1 RNA, already demonstrated to inhibit specifically RB18A/MED1 protein expression. A non-specific (scramble) siRNA was used as control. The specificity of this RB18A/MED1 siRNA was also supported as, in transfected cells, lamin A/C expression or cathepsin L and MMP2 expression and secretion were not modified. The use of microarray membrane carrying 113 cancer-related genes, western blot and specific

activities demonstrated that RB18A/MED1 knockdown significantly inhibits TIMP-3 expression and increases uPAR expression, two genes well known to be involved in melanoma cell invasion by modifying tumor microenvironment. Indeed, RB18A/MED1 knockdown in melanoma cells *in vitro* increased their invasive properties, without modification of cell proliferation.
Furthermore, RB18A/MED1 knockdown *in vivo* switched melanoma phenotype from non- to strongly-tumorigenic in nude mice. Thus, our data demonstrated for the first time that down-expression of RB18A/MED1 in human melanoma cells strongly increases tumor progression by modifications of the tumor microenvironment.

Poster No. 10

SNAI1 Expression in Colon Cancer Related with *CDH1* and *VDR* Downregulation in Normal Adjacent Tissue

Jose Miguel Garcia[1], Cristina Peña[1], Maria Jesus Larriba[2], Vanesa Garcia[1], Javier Silva[1], Gemma Dominguez[1], Rufo Rodriguez[3], Antonio Garcia de Herreros[4], Jose Ignacio Casal[5], Alberto Muñoz[2], Felix Bonilla[1]
[1]*Deparment of Medical Oncology, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain,* [2]*Instituto de Investigaciones Biomedicas "Alberto Sols", Consejo Superior de Investigaciones Cientificas-Unoversidad Autonoma de Madrid, Madrid, Spain,* [3]*Deparment of pathology, Hospital Virgende la Salud, Toledo, Spain,* [4]*Unitat de Biologia Cellular i Molecular, Institut Municipal D'investigacio Medica, Universitat Pompeu Fabra, Barcelona, Spain,* [5]*Biotechnology Program, Centro Nacional de Investigaciones Oncologicas, Madrid, Spain*

SNAIL1 *(SNAI1*), *ZEB1*, E-cadherin (*CDH1)* and vitamin D receptor (*VDR*) genes regulate the epithelial-mesenchymal transition (EMT) that initiates the invasion process of many tumor cells. We hypothesized that this process could also affect the behavior of normal cells adjacent to the tumor. To verify this hypothesis, the expression level of these genes was determined by quantitative RT-PCR in tumor, normal adjacent and normal distant tissues from 32 colorectal cancer patients. In addition, we extended the study to human SW480-ADH colon cancer cells co-cultured with derivative cells over-expressing the mouse *Snai1* gene. Of 18 CC cases with *SNAI1* expression in tumor tissue, 5 also had *SNAI1* in normal adjacent tissue. Expression of *SNAI1*, but not of *ZEB1*, in tumor tissue correlated with downregulation of *CDH1* and *VDR* genes in both tumor (p=0.047 and p=0.014, respectively) and normal adjacent tissue lacking *SNAI1* expression (p = 0.054 and p=0.003). *ZEB1* expression was directly related to *VDR* expression in tumor tissue (r=0.39; p=0.027) and inversely to *CDH1* in normal adjacent tissue (r=−0.46; p=0.010). *CDH1* was also downregulated in SW480-ADH cells co-cultured with *Snai1*-expressing cells. Furthermore, proteomic analysis showed differences in the conditioned media obtained from the two cell types. These results indicate that histologically normal tissue adjacent to tumor tissue expressing the EMT-inducing gene *SNAI1* shows alterations in the expression of epithelial differentiation genes such as *CDH1* and *VDR*.

Poster No. 11

Pigment Epithelium Derived Factor (PEDF) and Adipose Tissue Triglyceride Lipase (ATGL) are Down-Regulated by the Microenvironment and TNFalpha in Rat Prostate Tumors

Sofia Halin[1], Stina Rudolfsson[2], Pernilla Wikström[1], Anders Bergh[1]
[1]*Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden,* [2]*Department of Surgical and Perioperative sciences, Urology and Andrology, Umeå University, Umeå, Sweden*

PEDF is a potent angiogenesis inhibitor (Dawson et al., 1999). We have earlier shown decreased PEDF levels in metastatic prostate tumors in rats and humans, compared with non-metastatic disease implying that the loss of PEDF contributes to the progression to a metastatic phenotype (Halin et al., 2004).
To study the effects of PEDF over-expression on prostate tumor growth and metastasis, MatLyLu rat prostate tumor cells were transfected with a plasmid expressing human PEDF. PEDF over-expression slowed orthotopic rat prostate tumor growth and decreased the number and size of lymph node metastasis. Vascular growth was affected both in the tumor and in the surrounding normal tissue.
Recently, ATGL was described as a receptor/binding protein for PEDF (Notari et al., 2006). In addition, we therefore examined if PEDF and ATGL expressions were regulated by the prostate tumor microenvironment. Both PEDF and ATGL mRNA and protein levels were markedly down-regulated in AT-1 tumors growing in the prostate compared to the tumor cells in vitro suggesting that some factor in the prostate microenvironment suppresses the intratumoral PEDF system. ATGL mRNA levels were also significantly suppressed in the normal prostate tissue surrounding the tumor compared to normal prostate tissue from naive rats. In previous studies we have shown that orthotopic AT-1 tumors accumulate macrophages in the tumor and in the surrounding normal tissue (Halin et al., 2009). Here we show that these macrophages express TNFα and that TNFα down-regulate the expression of both PEDF and ATGL in vitro. This suggests that tumor associated macrophages could downregulate the PEDF system in prostate tumors by secreting TNFα and thereby facilitate tumor angiogenesis.

Poster No. 12

Gli3 siRNA Suppresses Cell Growth in Association with p53

Han Na Kang[2], Myoung Hee Kang[2], Jung Lim Kim[2], Sang Cheul Oh[3],
Jun Suk Kim[3], Young A. Yoo[1]
[1]*Brain Korea 21 Program for Biomedical Science, Korea University College of Medicine, Korea University, Seoul, Korea Republic,* [2]*Graduate School of Medicine, Korea University, Seoul, Korea Republic,* [3]*Division of Oncology/Hematology, Department of Internal Medicine, Korea University, Seoul, Korea Republic*

Sonic Hedgehog (Shh) signaling pathway regulates the epithelial stem cell proliferation and development of organs, and activation of this pathway is observed in a variety of cancers. However, the precise role of Shh signaling pathway in the development of colon cancer cells is poorly understood. Herein, we investigated the role of Shh signaling pathway in tumorigenesis of colon cancer cells and the molecular mechanisms underlying these effects. We showed that colon cancer cell lines express all the components of Shh signaling, albeit to different extents. Moreover, blockade of the Shh pathway by KAAD-Cyclopamine (a Shh signaling inhibitor) or Gli3 siRNA led to decreased proliferation of various colon cancer cells. Importantly, inhibition of Gli3 by treatment with its siRNA resulted in the enhanced expression of p53 proteins compared to treatment with control siRNA. On the contrary, treatment of colon cancer cells with KAAD-Cyclopamine, Gli1 siRNA, or Gli2 siRNA, did not show the increase in the levels of p53 expression, but not transcription. Treatment with cyclohexamide showed that the stability of the p53 protein in the colon cancer cells transfected with Gli3 siRNA was higher than in the cells transfected with control siRNA. Furthermore, treatment with MG132, a specific inhibitor of proteasomes, led to accumulation of p53 in Gli3 siRNA-overexpressing cells. To identify the mechanism by which Gli3 siRNA induces p53 stabilization, co-immunoprecipitationan and in vivo ubiquitination assay was performed. Importantly, we found that Gli3 siRNA results in the stabilization and activation of p53, via the prevention of MDM2-mediated p53 ubiquitination and degradation. These results, taken together, suggest that Gli3 regulates the proliferation of colon cancer cells by inducing turnover of p53.

Poster No. 13

FGF2 Expression Change as an Acute Radiotherapy Responsive Marker in Sequential Biopsy Samples from Cervical Cancer Patients during Fractionated Radiotherapy

Mayumi Iwakawa[1], Miyako Nakawatari[1], Kaori Imadome[1], Tatsuya Ohno[2], Shingo Kato[2], Etsuko Nakamura[1], Minako Sakai[1], Yu Ohkubo[2], Tomoaki Tamaki[2], Takashi Imai[1]
[1]*RadGenomics Research Group, National Institute of Radiological Sciences, Chiba, Japan,* [2]*Hospital of Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, Chiba, Japan*

Purpose
Tumor microenvironment possesses extreamly important role for tumor progression and metastasis. Cytokines have autocrine and paracrine functions, and they are also secreted by normal and cancerous cells. Herewith we investigated an indicator for the efficacy of radiotherapy in cervical cancers (CC) using microarray analysis and immunohistochemical analysis.
Patients and methods
One hundred and four patients with CC were recruited and divided into two groups (research set: n=35, and validation set: n=69). Microarray analysis was performed in research set and further immunohistochemical analysis (IHA) was performed for all patients to detect candidate radioresponsive markers using pre-radiotherapy and mid-radiotherapy biopsy samples, which were taken one week after initiation of radiotherapy.
Results
FGF2 in tumor cells (FGF2–T) significantly increased in midtreatment samples compared with pretreatment samples in research set of patients, and the ratio change of FGF2-T was significantly related with better prognosis. This evidence was confirmed in validation set. Next using all 104 patimets we found IHA positive FGF2 in stromal cells (FGF2-S) in 85 patients, and the radiotherapy-induced increase of FGF-S in 23 patients. Though positive FGF2-S in pretreatment samples was significantly related with increased expression change of VEGF, it was not related with poor prognosis.
Conclusion
Radiation causes severing the normal or cancerous associations with adjacent cells and changes the extracellular matrix environment. Therefore, we need to investigate not only pretreatment status of tumors, but also modified tumor structures during fractionated radiotherapy. In this study, we found FGF2-T expression change as a monitoring marker for the effectiveness of radiotherapy, and found the relationship between FGF2-S in pretreatment status and VEGF expression change in a subgroup of patients.

Poster No. 14

The Membrane Mucin MUC4 and Its Partner Oncogenic Receptor ErbB2 Alter in Vitro and in Vivo Biological Properties of Human Pancreatic Tumor Cells

Nicolas Jonckheere[1], Nicolas Skrypek[1], Nathalie Saint-Laurent[2], Nicole Porchet[1], Christiane Susini[2], Isabelle van Seuningen[1]
[1]*Inserm U837/Jean-Pierre Aubert Research Center/Team 5 "Mucins, Epithelial Differentiation and Carcinogenesis", Lille, France,* [2]*Inserm U858/Institut de Médecine Moléculaire de Rangueil, Toulouse, France*

Rationale:
Pancreatic cancer is one of the most deadly cancers in the world with a very low (5%) survival rate at 5 years. Identification of new therapeutic targets and new biomarkers remains mandatory and will allow a better understanding of molecular mechanisms responsible for pancreatic tumor progression. The MUC4 membrane mucin is one marker candidate as it is not expressed in normal pancreas whereas it is neo-expressed as early as precursor stage of pancreatic intraepithelial neoplasia (PanIN) and constanttly increases during the carcinogenetic sequence. Moreover, as an ErbB2 partner and target of TGF-b pathway, MUC4 actively participates in signalling pathways associated with tumor progression.
Aim:
To define the roles of both MUC4 and ErbB2 in pancreatic carcinogenesis in vitro and in vivo.
Material and Methods:
The human pancreatic adenocarcinomatous cell line CAPAN-2 was used to establish stable knocked-down (KD) cellular clones by a shRNA approach.

Results:
CAPAN-2 MUC4-KD clones have a proliferation defect compared to CAPAN-2 Mock clones expressing MUC4. Decrease of proliferation is correlated to a decrease in cyclin D1 expression whereas cell cycle inhibitor p27kip1 is not affected. CAPAN-2 MUC4-KD migration properties were reduced. Invasive properties were not altered. CAPAN-2 ErbB2-KD cellular clones have reduced proliferative and invasion properties. Moreover, we show that CAPAN-2 lacking MUC4 are more sensitive to chemotherapeutic drug gemcitabine. Transcriptomic analysis on 44 K Agilent microarrays allowed us to establish a molecular signature of pancreatic cancer related to MUC4 expression. Finally, subcutaneous xenografts of MUC4-KD cellular clones in nude mice lead to decreased tumor formation and size.
Conclusion:
These results indicate that MUC4 and ErbB2 play major roles in biological properties of pancreatic tumor cells suggesting their important function in tumor progression and confirm potential of MUC4 as a therapeutic target.

Poster No. 15

The Cytoplasmic Localization and Subsequent Degradation of RUNX3 by Shh Signaling are Correlated with the Development of TGF-β Resistance in Gastric Cancer

Jung-Lim Kim[1], Myoung-Hee Kang[1], Han-Na Kang[1], Jun-Suk Kim[2], Sang-Cheul Oh[2], Young A. Yoo[3]
[1]*Graduate School of Medicine, Korea University Colledge of Medicine, Korea University, Seoul, Korea Republic,* [2]*Division of Oncology/Hematology, Department of Internal Medicine, Korea University College of Medicine, Korea University, Seoul, Korea Republic,* [3]*Brain Korea 21 Program for Biomedical Science, Korea University College of Medicine,, Korea University, Seoul, Korea Republic*

RUNX3 that belongs to the RUNX family of transcription factors acts as a tumor suppressor in gastric cancer. RUNX3 is also a functionally important component in transforming growth factor-β (TGF-β) mediated signaling pathway. Our previous studies demonstrated that TGF-β was implicated in Sonic hedgehog (Shh)-induced cellular signaling in gastric cancer. Herein, we investigated the involvement of RUNX3 in the modulation of Shh-mediated tumorigenic process in gastric cancer cell. To elucidate the role of TGF-β signaling in Shh-mediated proliferation of gastric cancer cells, we transfected gastric cancer cells with the Shh expression plasmid pcDNA3.1/Shh or with the vector pcDNA3.1 as a control. We found that higher concentrations of TGF-β significantly decreased cell proliferation in control gastric cancer cells, whereas no inhibition was observed in Shh transfectants that were treated with TGF-β. As TGF-β signaling can be affected by either stability or the subcellular localization of the RUNX3, we attempted to determine whether Shh increases RUNX3 expression. RT-PCR analysis showed that in the Shh transfectants, the RUNX3 expression was enhanced, but not transcription. In addition, treatment with MG132, a specific inhibitor of proteasomes, led to reduction of RUNX3 proteins in AGS cells transfected with Shh. The expression of RUNX3 is frequently inactivated by DNA methylation or its protein mislocalized in many cancer types, including gastric and breast cancer. Importantly, we found that overexpression of Shh facilitated nuclear export of RUNX3. Moreover, RUNX3 sequestered in the cytoplasm is rapidly degraded through a proteasome-mediated pathway. On the contrary, blockade of the Shh pathway by KAAD-Cyclopamine (a Shh signaling inhibitor) or Shh blocking antibody led to decreased nuclear export and degradation of RUNX3. In summary, these results indicate that the cytoplasmic localization and subsequent degradation of RUNX3 mediated by Shh signaling, which are correlated with the development of TGF-β resistance in gastric cancer.

Poster No. 16

Tumor Margin as a Unique Zone, which can be Molecularly Distinguished, in TME

Baek Gil Kim[1], Suki Kang[2], Nam Hoon Cho[1,2,3]
[1]*Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea Republic,* [2]*Department of Pathology, Yonsei University College of Medicine, Seoul, Korea Republic,* [3]*Global 5-5-10 System Biology, Yonsei University, Seoul, Korea Republic*

It is very important to distinguish between tumor and normal tissue for accurate pathology diagnosis and effective cancer treatments. Particularly after surgical removal of cancer, residual tumor tissue often causes high recurrence and mortality rate as well as poor prognosis. For this reason, the demand for defining clear tumor tissue margin at a molecular level has been raising. We therefore suggest that molecular tumor margin must be considered in tumor microenvironment (TME), especially in the aspect of extracellular matrix (ECM) which is a main component of TME, as well as tumor cells. Defining the portraits of tumor margin facing normal tissue can be a prerequisite step for further application of molecular margin to eliminate the chance of tumor recurrence.
Breast cancer is the best model for TME remodeling study because of frequent accompanied desmoplasia and clinical requirement for minimized operation. In our study, we made a tissue classification as follows, rear tumor burden, tumor margin predominantly facing the normal tissue, and normal tissue remote from the tumor burden. Differential ECM expression in each tissue was compared by using ECM array based on real-time RT-PCR, and further validated by western blot.
On analysis of ECM transcript gene array, LAMA3, which is a subunit of laminin332, was significantly overexpressed in tumor margin in comparison with adjacent tumor burden or normal tissue in 6 breast cancer samples. Fibronectin 1 and SPARC (osteonectin) were shown to be downregulated in tumor margin. E-cadherin was downregulated in the tumor margin in contrast to upregulated N-cadherin.
In conclusion, tumor margin could be independently unique zone differentiated from rear tumor burden and remote normal tissue, which appears dynamic and functionally most active zone during TME remodeling.

Poster No. 17

The Human *L3MBTL4* Gene, a Tumor Suppressor Gene Involved in Breast Cancer Development

Lynda Klouche[1,2], Soraya Moulessehoul[2], Max Chaffanet[1], Daniel Birnbaum[1]
[1]*Laboratoire d'oncologie Moleculaire, Centre de Recherche en Cancerologie. Institut Paoli-Calmettes.Umr 891, Marseille, France,* [2]*Laboratoire de Biotoxicologie, Universite Djillali Liabes, Sidi-Bel-Abbes, Algeria*

L3MBTL4 gene, a human homolog of *Drosophila* lethal(3) malignant brain tumor(*D-l(3)mbt)*, lies in a region of chromosome arm 18p that is frequently deleted in breast cancer cells. The D-l (3)mbt protein is required for the negative control of cell proliferation; it is considered as a tumor suppressor. The loss of function of D-l(3)mbt causes hyperplasia and transformation of the neural cells resulting in brain tumors in *Drososophila*. *L3MBTL1* the human paralog of *L3MBTL4* has been proposed as a target gene in the myeloid malignancies associated with 20q deletions. The four human L3MBTL proteins shares MBT repeats involved in transcriptional repression and chromatin remodeling. The MBT repeat is capable of methyl-lysine histone recognition. The presence of MBT repeats in L3MBTL4 suggest that it could also interact with chromatin.
We hypothesized that L3MBTL4 loss-of-function could play a role in cellular transformation. We established genomic profiles by array comparative genomic hybridization and search for mutations by sequencing analysis on large set of primary breast tumors. Our results demonstrate that *L3MBTL4* is targeted by losses and mutations suggesting that it could be a tumor suppressor gene.

Poster No. 18

PTPIP51 is Expressed in Human Keratinocyte Carcinoma, Prostate Carcinoma and Glioblastoma

Philipp Koch[1], Meike Petri[1], Albrecht Stenzinger[1], Agnieszka Paradowska[2], Monika Wimmer[1]
[1]*Institute of Anatomy and Cell Biology, Justus-Liebig-University Giessen, Giessen, Germany,* [2]*Department of Urology and Pediatric Urology, Justus-Liebig-University Giessen, Giessen, Germany*

The novel protein PTPIP51 (protein tyrosine phosphatase interacting protein 51) shows a tissue-specific expression pattern and is associated with cellular differentiation and apoptosis in several mammalian tissues. Overexpression of the full-length protein enhances apoptosis. PTPIP51 is a positive regulator of the MAPK on Raf level. Various carcinoma express PTPIP51. Here we demonstrate the expression profile of PTPIP51 in human keratinocyte carcinoma (KC), prostate carcinoma (PCa) and in glioblastoma multiforme (GBM).
Paraffin embedded sections of KC, PCa and GBM were analyzed by immunohistochemistry and in situ hybridization. RT-PCR was performed on cryo samples. For PCa, and benign prostate hyperplasia (BPH) as reference, bisulfite DNA treatment, followed by sequencing of PCR products was performed in order to analyze CpG methylation within the promoter region on the ptpip51 gene. PTPIP51 mRNA and protein was detected in all investigated tumor tissues.
Basal cell carcinoma (BCC), squamous cell carcinoma (SCC), Bowen's disease (BD) and keratoacanthoma (KA) displayed a specific localization pattern of PTPIP51 in malignant keratinocytes. For SCC, BD and KA a mainly membranous localization was investigated, whereas BCC showed an either cytoplasmic or predominantly membranous expression.
Tumor cells of the PCa express PTPIP51, however a stronger expression of PTPIP51 is present in nerve fibres, immune cells and in smooth muscle and endothelial cells of vessels. Methylation experiments revealed that at least 70% of methylated CpGs in the CpG islands of the ptpip51 gene promoter region was identified in BPH samples. A loss of methylation has been found in the PCa group.
Glioblastoma cells showed a mainly nuclear but also cytoplasmic expression of PTPIP51. These cells displayed a co-expression of PTPIP51 with its in-vitro interaction partners, PTP1B and 14-3-3β.
For all tumor tissues, PTPIP51 could also be traced in the surrounding stromal microenvironment. Infiltrating immune cells of both the innate and the adaptive immune system and endothelial cells lining arterial and venous vessels strongly expressed PTPIP51.
We suggest PTPIP51 to play a role as a cellular signaling partner for processes mandatory for tumor development and progression.

Poster No. 19

Dual Impact of Insulin-Like Growth Factor (IGF)-binding Protein 3 in IGF Action and Lung Cancer Development

Woo-Young Kim[1], Ho-Jin Moon[2], Mi-Jung Kim[3], Jong-Kyu Woo[1], Guangcheng Zhang[1], Lei Feng[4], Carolyn Van Pelt[5], Jack Lee[4,6], Waun-Ki Hong[1], **Ho-Young Lee[1,6]**
[1]*Thoracic/Head & Neck Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA,* [2]*Department of Mathematics and Statistics, California State University at Long Beach, Long Beach, CA, USA,* [3]*Department of Pathology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea Republic,* [4]*Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA,* [5]*Veterinary Medicine and Surgery, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA,* [6]*The University of Texas Graduate School of Biomedical Sciences, Houston, TX, USA*

The IGF axis has been associated with risk of developing various types of human cancer. However, the role of circulating IGF-1 and IGFBP-3 in lung cancer is still elusive, probably due to the nature of IGFBP-3 that could either suppress or enhance the IGF action. In this study, we determined the role of IGFBP-3 in the IGF action

and lung cancer development by analyzing a mouse model that convey lung-specific human *IGF-1* transgene (IGF^{Tg}), germline-null mutations of *IGFBP-3,* or both ($BP3^{+/-}$, $BP3^{-/-}$, IGF^{Tg} ; $BP3^{+/+}$, IGF^{Tg} ; $BP3^{+/-}$, IGF^{Tg} ; $BP3^{-/-}$). Serum IGFBP3 levels of $BP3^{+/-}$ and $BP3^{-/-}$ mice were 50% of the wild-type (WT) ($BP3^{+/+}$) mice and undetectable, respectively, leading to 20% and 50% decrease in serum murine IGF-1. Compared to WT mice, the mice with genetic changes in IGF-1 and/or IGFBP-3 showed significantly increased spontaneous lung tumor formation and progression to adenocarcinomas (AC) with the greatest pathogenesis in IGF^{Tg};$BP3^{+/-}$ mice. The severity of this phenotype correlated with activation of IGF-1R. The IGF^{Tg}; $BP3^{+/-}$ mice exhibited the greatest incidence and number of ACs following exposure to the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone while the overall tumor incidence was similar among the lines. These findings suggest the importance tissue-derived IGF-1 in lung cancer and the dual impact of IGFBP-3 in locally available IGF-1, activation of IGF-1R, and lung cancer development.

Poster No. 20

Apigenin: A Phytochemical that Modulates the Cell-Surface Expression of the Multifunctional Protein CD26 on Human Colorectal Carcinoma Cells

Emilie Lefort[1], Jonathan Blay[2]
[1]*Department of Pathology, Dalhousie University, Halifax, NS, Canada,* [2]*Departments of Pathology, Pharmacology and Biology, Dalhousie University, Halifax, NS, Canada*

Background:
CD26, also known as dipeptidyl peptidase IV (DPPIV), is present at the cell surface of a variety of tissues, including the epithelial lining of the normal human colon. CD26 expression is decreased in a number of malignancies and in the instance of colon cancer this decrease in level is believed to facilitate the process of metastasis to distant organs including lymph nodes and liver. CD26 itself is a multifunctional anchor protein that serves as the major cellular binding protein for the ecto-enzyme adenosine deaminase (ADA) and also interacts with proteins of the extracellular matrix, principally collagen and fibronectin. CD26 also possesses an intrinsic dipeptidyl peptidase enzymatic activity, allowing it to cleave and inactivate peptides like CXCL12, the ligand for the chemokine receptor CXCR4. To further explore the potential anticancer properties of the bioflavonoid compound apigenin, we investigated its effects on the cellular expression of CD26 on human colorectal carcinoma cells.
Methods:
Cell-surface expression of functional CD26 protein on HT-29 colorectal carcinoma was quantified using a cell-based radio-immunoassay. The multiple functions of the CD26 molecule were explored through measurements of DPPIV enzymatic activity, binding of exogenous ADA and adhesion to cellular fibronectin. Cell viability was assessed through MTT assay and by trypan blue exclusion.
Results:
Apigenin significantly up-regulated CD26 cell-surface expression and functions. This would be predicted to act to oppose the metastatic process. When apigenin was combined with chemo-therapeutic agents utilized in the treatment of colorectal cancer, the increase in CD26 expression was further enhanced.
Conclusion:
By increasing the expression of CD26 and its functions to normal levels, apigenin could be of benefit in restraining the tendency of colon cancer cells to metastasize, and enhancing the action of chemotherapeutic agents.
Supported by NSERC of Canada and by studentship award from Dalhousie CRTP.

Poster No. 21

Modulation Of Angiopoietin Expression By Platelet-Derived Endothelial Cell Growth Factor/Thymidine Phosphorylase In Human Glioblastoma Cells

Sandra Liekens[1], Annelies Bronckaers[1], Cindy Schiepers[1], Jan Balzarini[1]
[1]*Department of Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium*

Thymidine phosphorylase (TP), also known as platelet-derived endothelial cell growth factor (PD-ECGF), catalyzes the conversion of thymidine to thymine and 2-deoxy-D-ribose-1-phosphate. In many tumors, elevated levels of TP are associated with increased angiogenesis, metastasis and poor prognosis. In particular, TP was found to increase the expression and secretion of angiogenic factors, such as vascular endothelial growth factor (VEGF), matrix metal-loproteinases (MMP) and interleukins (IL). The enzymatic activity of TP was found to be crucial for its angiogenic properties.
In human glioblastomas, which are highly vascularized tumors, TP expression was found to correlate with angiogenesis. In order to identify angiogenesis mediators of TP in glioblastomas, we transfected U87 human glioblastoma cells with TP cDNA (U87/TP) or with an empty vector (U87/EV). Three clones of U87/TP with a different expression level of TP were obtained. Using a human angiogenesis antibody array the secretion of 42 (anti-)angiogenic proteins was compared in TP- and mock-transfected cells. Angiopoietin-2 (Ang-2) secretion was found to be significantly (10-fold) reduced in U87/TP cells, compared to mock-transfected cells. Further analysis showed that also the intracellular Ang-2 protein level was significantly lower in U87/TP cells than in U87/EV cells, although Ang-2 transcription was not affected by TP. In contrast, Ang-1 mRNA and Ang-1 secretion were significantly (4-fold) increased in TP-expressing U87 cells. Addition of thymidine (substrate for the TP enzymatic reaction) or an inhibitor of TP did not affect the changes in Ang-1/2 secretion, indicating that the enzymatic activity of TP is not important for the observed effects. Our findings indicate that increased TP expression in the tumor microenvironment may significantly increase the Ang-1/Ang-2 ratio, leading to increased Tie-2 receptor activation. The latter is currently under investigation.

Poster No. 22

Human Breast Organotipic Culture: Identification of Vitamin D Regulated Genes in Tumor Microenvironment

Cintia Milani[1], JoEllen Welsh[2], Maria Lúcia Hirata Katayama[1], Eduardo Carneiro Lyra[3], Maria do Socorro Maciel[4], Maria Mitzi Brentani[1], Maria A. Azevedo Koike Folgueira[1]
[1]*Departamento de Radiologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil,* [2]*Biomedical Sciences, State University of New York at Albany, Rensselaer, New York, USA,* [3]*, Instituto Brasileiro de Controle do Câncer, São Paulo, São Paulo, Brazil,* [4]*Hospital A.C.Camargo, São Paulo, São Paulo, Brazil*

Background:
Vitamin D (VD) effects on stromal-epithelium interactions may interfere with breast cancer (BC) development. We have previously identified some regulated genes in a BC organ culture model, which preserves epithelial mesenchymal interactions. Our present aim was to specifically evaluate the epithelial component behavior and determine whether candidate genes were directly modulated by VD in breast cell lines or indirectly regulated through stromal interactions in MCF7 xenograft.
Methods:
Human BC samples were sliced, cultivated and VD treated (24 h). Affymetrix gene expression profile was obtained. Mammary epithelial model of cell transformation HME, HME^{LT}, HME^{LT+Ras} and also MCF7 cells (in vitro or xenograft) were used to follow-up VD regulated genes by qPCR, western blotting, confocal microscopy and ELISA assays.
Results:
Seven up-regulated genes were confirmed modulated (RT-qPCR analysis) in the cell transformation model after VD treatment (24 h), including BMP6 and DPP4. Among them, CD14, IL1RL1 and SHE were also modulated in MCF7 cells. Despite constant levels of CD14 protein in all cells, a significant increase in sCD14 was seen. Conversely, detectable CA2 protein levels were present only in HME and HME^{LT} VD treated cells. Conclusion: Novel VD regulated genes were identified in this model, some of them probably influenced by the stromal compartment. Supported by FAPESP 2007/04799-2, CAPES, NIH CA69700.

Poster No. 23

Siah2 Controls Breast Cancer Progression through Tumor Epithelial Cell Mediated Cytokine Release and Stromal Infiltration

Christina Wong[1], Colin House[1], Mira Liu[1], Izhak Haviv[3], David Bowtell[1,2], **Andreas Moeller[1,2]**
[1]*Department of Research, Cancer Genomics and Biochemistry Laboratory, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia,* [2]*Department of Biochemistry and Molecular Biology, University of Melbourne, Parkville, VIC, Australia,* [3]*Department of Research, Baker IDI Heart and Diabetes Institute, Prahran, VIC, Australia*

In estrogen receptor positive breast cancer, one of the most significantly upregulated genes is the ubiquitin ligase Siah2. Knocking out Siah2 significantly delays the onset of breast cancer in the PyMTAg-derived breast cancer mouse model. Mammary epithelial cells from Siah2 knockout mice produce and secrete elevated levels of cytokines, including CXCL10 and GM-CSF. On a molecular level, this is caused by constant nuclear NFkB localisation and a higher sensitivity to TNFalpha-mediated activation, identifying Siah2 as a novel negative regulator of this tumor progression pathway. The elevated cytokine secretion in turn results in increased immune cell infiltrate in the mammary glands, suggesting increased immune surveillance. Siah2 knockout tumor cells from mice with tumors at advanced stage have strongly reduced stroma. This is caused by the inability of the Siah2 knockout host stromal cells to respond to attraction signals derived from the tumor epithelium. Further evidence for this is supported by data from *in vitro* work and in transplanted tumor models showing that Siah2 knockout tumor cells can recruit stroma to the tumour in wildtype mice, whereas wildtype tumor cells growing in Siah2 knockout mice are not associated with stromal infiltration.

Poster No. 24

Evaluation of Periostin Isoforms in the Tumor Microenvironment of Lung and Kidney Cancer

Laura Morra[1], Peter Schraml[1], Holger Moch[1], Alex Soltermann[1]
[1]*Department of Pathology, University Hospital, Zurich, Switzerland*

Periostin (POSTN) is an extracellular matrix N-glycoprotein of 93 kDa. Six different splice isoforms were reported, but only four of them sequenced. Their functional significance is unknown. The protein is expressed in normal tissues like the periosteum and overexpressed in many cancerous tissues, including lung and kidney cancer. In cancer, its role is tumor promoting, whereby conferring increased invasion, survival and angiogenesis in the context of epithelial-to-mesenchymal transition via integrin-activated Akt signaling. We previously reported that high protein expression correlates with decreased survival in non-small cell lung cancer (NSCLC). This study aims at further analysis of expression and localization of periostin isoforms in lung and renal cell carcinoma (RCC) and at their functional characterization. We performed isoform-specific RT-PCR, immunohistochemistry and immunoblot analysis on frozen tissues of 30 patients each with NSCLC and kidney carcinoma and their matched non-neoplastic controls. Furthermore we cloned and sequenced the region of periostin mRNA that undergoes alternative splicing (exons 17–21), giving rise to different isoforms. We identified four periostin isoforms in the lung and three in the kidney; each co-expressed in both tumor and matched non-neoplastic control. Cloning analysis of one patient with clear cell RCC revealed a new isoform of periostin. High expression of periostin was found in both the stroma as well as in the tumor cell cytoplasm of NSCLC and RCC and correlated with higher pT. On immunohistochemistry, protein expression was regularly accentuated at the tumor-stroma interface. These results suggest potential novel tissue-specific functions of periostin isoforms in RCC and NSCLC and open up the possibility of organ-specific targeted therapy against the desmoplastic stroma of the tumor microenvironment.

Poster No. 25

p53 Functions as a Non-Cell-Autonomous Tumor Suppressor by Suppressing Stromal SDF-1 Expression

Neta Moskovits[1], Yoseph Addadi[2], Alexander Kalinkovich[3], Jair Bar[4], Tsvee Lapidot[3], Michal Neeman[2], Moshe Oren[1]
[1]*Departments of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot, Israel,* [2]*Departments of Biological Regulation, The Weizmann Institute of Science, Rehovot, Israel,* [3]*Departments of Immunology, The Weizmann Institute of Science, Rehovot, Israel,* [4]*Department of Oncology, Sheba Medical Center, Tel Hashomer, Israel*

The p53 tumor suppressor acts as a major barrier against cancer. To a large extent, this is due to its ability to maintain genome stability and to eliminate cancer cells from the replicative pool through cell-autonomous mechanisms. However, in addition to its well-documented functions within the malignant cancer cell, p53 can also exert non-cell-autonomous effects that contribute to tumor suppression. We now report that p53 can repress the production of the chemokine SDF-1 by cultured human and mouse fibroblasts, due to transcriptional repression of the *SDF-1* gene. Interestingly, mutant p53 exerts a gain-of-function effect on SDF-1 transcription, showing an opposite effect to the WT p53.
We found that this effect of p53 on stromal SDF-1 production might impact on different aspects of tumor development. Specifically, using conditioned media (CM) of the fibroblasts, we show that p53-mediated repression of SDF-1 expression can attenuate tumor cell migration and invasion triggered by such CM. In addition, CM of p53-deificent fibroblasts is more capable of stimulating the proliferation of tumor cells. The extent of suppression of SDF-1 expression increases with p53 activity, as shown by Nutlin treatment, suggesting that the biological effect of this phenomenon may become more pronounced under physiologic and pathologic conditions that entail extended triggering of the p53 pathway. Finally, we show that repression of SDF-1 by p53 in stromal cells attenuates tumor growth in mice.
In recent years, several publications have suggested that stromal p53 has an inhibitory effect on tumor development, thereby creating a selective pressure on the tumor cells to down-regulate its activity. However, no specific molecular mechanism has been proposed so far. Our findings suggest that stromal p53 can exert at least some of its inhibitory effects on tumor growth via repression of SDF-1 expression within the stromal compartment.

Poster No. 26

Expression Pattern of the Pro-Apoptotic Genes PHLDA1 and PAWR during the Morphogenesis of MCF-10A Human Mammary Epithelial Cells

Simone A. de Bessa Garcia[1], Michelly Christiny Pereira[1], **Maria A. Nagai[1]**
[1]*Department of Radiology, Medical School, University of São Paulo, São Paulo, São Paulo, Brazil*

The histhological organization of the mammary gland reflects a spatial interaction of epithelial and myoepithelial cells with the specialized basement membrane (BM) composed by the extracellular matrix (ECM) proteins, which is disrupted during the tumorigenic process. In a previous study we identified the pro-apoptotic genes PAWR (PKC apoptosis WT1 regulator; also named PAR-4, prostate apoptosis response-4) and PHLDA1 (pleckstrin homology-like domain, family A, member 1; also named TDAG51) as differentially expressed in breast tumors. Next, using IHC on TMA containing a large series of primary breast tumors we provide evidence that PAWR and PHLDA1 reduced expression are frequent events associated with a more aggressive phenotype. Three-dimensional (3D) cell culture of the spontaneously immortalized cell line MCF10A is a well-established model system to study breast epithelial cell biology and morphogenesis. MCF10A cells grown in 3D form spheroids, acquire apicobasal polarization and lumen formation that resemble acini structures, process that involves cell death. Here, using this system with growth factor reduced matrigel, we evaluated the expression pattern of PAWR and PHLDA1 and activated caspase 3 by immunofluorescence on day 3, 5, 7 and 10 of morphogenesis of MCF10A cells. We provide preliminary results showing that the PHLDA1 was reduced during MCF10A morphogenesis and was highly expresssed in the cells in contact with the matrigel, suggesting its role on acinar polarity and cell-extracellular matrix contact. On the other hand, PAWR was highly expressed in the MCF10A cells inside the acini structure, suggesting that PAWR might have a role for the lumen acini formation. During the morphogenesis of MCF10A cells in 3D cell culture, the cells within the lumen show apoptotic activity evidenced by caspase-3 activation. PAWR expression on this cells was only partially co-expressed with activated caspase-3. Although preliminary our results suggest that PHLDA1 and PAWR may have a role in the process of the mammary gland morphogenesis.
Supported by FAPESP and CNPq.

Poster No. 27

The Stem Cell Niche / Microenvironment Connectome: Mapping Transcription Factors and Signalling Networks in Normal and Pathological Conditions

Rajesh Natarajan[1]
[1]*Department of Science and Informatics, Hogent and Ghent University, Gent, East-vlanderen, Belgium*

Our realisation is that the stem-cell niche or microenvironment plays more than just a supporting role in tumour progression represented a radical shift in the study of stem-cell biology.
To introduce briefly, in the bone marrow, osteoblasts and endothelial cells constitutes the major cellular components contributing to the endosteal and vascular niches that serve as the microenvironment for maintaining haematopoietic stem cells (HSCs). The niche is also likely comprised of osteoclasts and endothelial cells, fibroblasts and cancer-associated fibroblasts (CAFs), as well as adipocytes and macrophages.

Although the profound influence of the stroma on tumorogenesis is now widely accepted, a full understanding of the cross talk between stem cells and the niche (which translates into changes in transcriptional networks and chromatin modifications), microenvironment role on heterogeneity of embryonic and adult stem cells as well as role in development of leukaemia (LSCs) and cancer stem-cells (CSCs), remains a nascent field. In this scenario, there is an urgency to map transcriptional factors and cell signalling networks from different niches in one place, in order to exploit stem-cell niche for potential therapeutic benefits.

To accomplish this goal, we are trying to apply an multidisciplinary approach to address and document molecular networks that involves in normal and in disease conditions, which is including the role of tumor initiating genes in tumor microenvironment during metastasis, small nonprotein-coding RNAs (such as microRNA pathway that differentiate LSCs from CSCs, for an example), signalling by morphogens and growth-factors (IGF1R is expressed exclusively in the hESCs, for an example) as well as functional assays (to distinguish normal HSCs from cells that have undergone some degree of neoplastic progression) and novel imaging methodologies.

Hope our advanced 'connectome- review' initiative will eventually help us to increase quality of life for survivors of various cancers.

Poster No. 28

Analysis of Expression of the miR-200 Family in Colorectal Adenocarcinoma by *In Situ* Hybridisation

Emily L. Paterson[1,2], Andrew G. Bert[1], Philip A. Gregory[1,2], Andrew Ruskiewicz[3], Yeesim Khew-Goodall[1,4], Gregory J. Goodall[1,2]
[1]*Centre for Cancer Biology, Hanson Institute, Adelaide, SA, Australia,* [2]*School of Medicine, The University of Adelaide, Adelaide, SA, Australia,* [3]*Division of Tissue Pathology, SA Pathology, Adelaide, SA, Australia,* [4]*School of Molecular and Biomedical Sciences, The University of Adelaide, Adelaide, SA, Australia*

The progression of metastasis is a complex event thought to incorporate the reversible developmental process of epithelial-mesenchymal transition (EMT). We are interested in the role of microRNAs in EMT and are focused on expression of the miR-200 family in *in vitro* and *in vivo* examples of this process. Madin-Darby Canine Kidney (MDCK) cells induced to undergo EMT with either TGF-β or the protein tyrosine phosphatase Pez, exhibited a strong downregulation of miR-200.[1,2] We have shown miR-200 is essential for the maintenance of the epithelial phenotype and sustains E-cadherin expression by post-transcriptionally inhibiting ZEB1 and ZEB2, which are E-cadherin transcriptional repressors that contain multiple miR-200 binding sites in their 3′UTRs.[2] *In vivo*, qPCR analysis of miR-200 expression in human ductal (epithelial) and metaplastic (mesenchymal) breast cancers showed ductal tumours had high levels of E-cadherin and miR-200, whereas invasive metaplastic tumours lacked both, indicating loss of miR-200 may increase tumour aggressiveness.[2] We developed a method for *in situ* hybridisation of miRNAs to screen formalin-fixed paraffin-embedded tumours. We are using this technique, along with immunofluorescence and immunohistochemistry, to assess expression of miR-200 and EMT markers in human colon adenocarcinomas. We hypothesise miR-200 will be downregulated in budding cells, which have detached from the primary tumour and display typical features of EMT including translocation of β-catenin to the nucleus and loss of E-cadherin.[3] We are also conducting laser capture microdissection to quantitate and compare levels of miR-200 in normal epithelium, tumour core and the invasive front.

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Poster No. 29

Regulation of Osteopontin in Senescence

Ermira Pazolli[1], Xianmin Luo[1], Sarah Brehm[1], Kelly Carbery[1], Jun-Jae Chung[2], Julie L. Prior[3], Jason Doherty[4], Shadmehr Demehri[5], Lorena Salavaggione[2], David Piwnica-Worms[3,5], Sheila A. Stewart[1,6]
[1]*Department of Cell Biology and Physiology, Washington University School of Medicine, Saint Louis, MO, USA,* [2]*Immunology and Pathology,* [3]*Mallinckrodt Insititute of Radiology,* [4]*Biology and Biomedical Sciences,* [5]*Developmental Biology,* [6]*Medicine, Washington University School of Medicine, Saint Louis, MO, USA*

Alterations in the tissue microenvironment collaborate with cell autonomous genetic changes to contribute to neoplastic progression. Senescent fibroblasts, similar to cancer-associated fibroblasts (CAFs), have a unique expression profile and promote preneoplastic cell growth *in vitro* and *in vivo*. Because senescent cells accumulate with age, their presence is hypothesized to facilitate preneoplastic cell growth and tumor formation in older individuals. We have previously identified osteopontin (OPN) as one of the differentially secreted proteins in senescent fibroblasts. Furthermore, we demonstrated that targeting OPN by RNAi, had no impact on senescence induction; however, it dramatically reduced the growth-promoting activities of senescent fibroblasts *in vitro* and *in vivo*. OPN's role as a paracrine stimulator of preneoplastic growth was further corroborated by its early expression in senescent stroma present in preneoplastic lesions that arise following DMBA/TPA treatment of murine skin. To further understand the importance of OPN and the associated senescence

secretome, we are investiating its regulation in senescence. We confirmed that senescence triggers a robust DNA damage response (DDR) represented by activation of ATM. Inhibition of ATM, but not p53, leads to a significant decline in OPN levels. In addition, analysis of human OPN promoter luciferase constructs revealed a distinct pattern of upregulation in response to senescence induction, suggesting binding of putative transcription factors. Together, our results demonstrate that OPN is a critical senescent stromal-derived factor and that specific mechanisms control its regulation in senescence.

Poster No. 30

Involvement of the Extracellular Protease ADAMTS1 in a Process of Tumor Cell Plasticity

Carmen Casal[1], Antoni Xavier Torres-Collado[2], María del Carmen Plaza-Calonge[1], Estefanía Martino[1], Arjan W. Griffioen[3], **Juan Carlos Rodriguez-Manzaneque[1,2]**
[1]*Oncology and Molecular Pathology, GENYO (Pfizer-University of Granada-Andalusian Government Centre for Genomics and Oncological Research), Armilla, Granada, Spain,* [2]*Medical Oncology Research Program, Vall d'Hebron University Hospital Research Institute, Barcelona, Spain,* [3]*Research Institute for Growth and Development, Maastrich University & University Hospital Maastricht, Maastricht, The Netherlands*

ADAMTS1 (a disintegrin and metalloprotease with thrombospondin motifs) is an extracellular metalloproteinase known to participate in a variety of biological processes including inflammation, angiogenesis and development. Its role in cancer has also been highlighted although the specific mechanisms have not been fully disclosed. Using distinct methods we have identified various factors on the extracellular milieu as targets of the action of this protease, including the inhibitor TFPI-2, the proteoglycan syndecan-4, and the basement membrane glycoproteins nidogens. Our studies in cellular and xenograft models showed the relevance of these substrate-protease partners for the development of vasculogenic-like networks, rich in matrix components. This phenomenon, first characterized for aggressive melanoma cells and named vasculogenic mimicry, illustrates a paradigm of tumor cell plasticity. Accordingly, our main objective was to study the implication of ADAMTS1 in the formation of pseudo-vascular channels in both melanoma and sarcoma settings. We demonstrated its mRNA and protein expression in aggressive Ewing sarcoma and melanoma cell lines that formed vascular-like structures in 3D-cultures. We also studied the presence of specific substrates of ADAMTS1 in these cell lines. In addition we approached xenograft assays using HT1080 fibrosarcoma cells, negative for ADAMTS1, which were properly modified to study the functional role of this protease. After the subcutaneous injection of these cells in Nu/Nu Balb/c mice, we observed that ADAMTS1 overexpression altered tumor growth rate and induced the appearance of vascular-like structures together with the overexpression of endothelial-specific genes, such as VE-Cadherin. Currently we are characterizing the phenotypic properties of both sarcoma and melanoma cells and its alteration by the protease ADAMTS1.
Our work appears in accordance with recent reports that suggest the essential role of extracellular matrix remodeling for tumor plasticity and it provides new insights behind the concept of cancer stem cells.

Poster No. 31

Analysis of Transcriptome of Breast Epithelial and Stromal Matched Components Isolated by Laser Capture Microdissection

Patricia Bortman Rozenchan[1], Rosimeire Aparecida Roela[1], Maria Lúcia Hirata Katayama[1], Dirce Maria Carraro[2], Elisa Napolitano e Ferreira[2], Cynthia Aparecida Bueno de Toledo[2], Fernando Augusto Soares[2], Maria Aparecida Azevedo Koike Folgueira[1], Maria Mitzi Brentani[1]
[1]*Laboratório de Oncologia Experimental, Departamento de Radiologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil,* [2]*Centro de Ensino e Pesquisa, Hospital A.C. Camargo, São Paulo, SP, Brazil*

The microenvironment on which tumors grow is complex consisting mainly of tumor epithelial cells and associated fibroblasts as well as non transformed epithelial cells, normal fibroblasts and also endothelial and immune cells. The exact role of these cell types, interacting with each other, in the progression of breast cancer has yet to be fully understood. One approach to study this interaction is to determine changes in gene expression profiles between fibroblasts and non-malignant or malignant breast epithelial cells, evaluated separately. Previously, we have demonstrated changes in differential expression profiles of mammary epithelial cells and fibroblasts in a co-culture model; herein we attempt to show these interactions by removing each cell type directly from the respective tissue. For this purpose, we have compared gene expression profiles between tumoral and matched normal epithelial and also stromal *versus* tumoral breast epithelial tissues from patients diagnosed with clinical stage II, luminal subtype, invasive ductal carcinoma, all obtained through Laser Capture Microdissection (LCM). After each tissue type removal, i.e., tumoral and normal epithelium and stromal tissue (avoiding capturing endothelial and immune cells), total RNA was extracted, amplified and hybridized onto Affymetrix GeneChip U133 X3P arrays. Genes differentially

expressed between groups were identified using Limma algorithm ($p<0.01$) of the Bioconductor software suite and further assessed using gene ontology analysis, performed using the GO Tree Machine tool. When compared to epithelial tumoral cells, stromal cells presented enriched categories related to "T cell receptor signaling pathway" ($p=0.004$); "protein folding" ($p=0.008$); and "chemotaxis" ($p=0.006$). The most prominently enriched category in tumoral *versus* normal breast epithelium were "inflammatory response" ($p=0.002$) and "response to stress" ($p=0.009$). The evaluation of components separately resulted in distinct signatures that should help to better understand some of the molecular mechanisms involved in the complex heterotypic signaling between epithelial cells and fibroblasts.
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Poster No. 32

HIF2alpha Overexpression Drastically Reduces HIF1alpha Protein Amounts in Melanoma Cells under Hypoxia

Anne-Lise Steunou[1], Laurence Nieto[1], Eric Clottes[1]
[1]*Department of "Biologie du Cancer", Institut de Pharmacologie et de Biologie Structurale-UMR 5089, Toulouse, France*

Hypoxia inducible transcription factors (HIF) are key regulators of cellular adaptation to hypoxia in normal but also in pathologic conditions such as cancer development. They are involved in melanocyte transformation, tumour progression and metastasis of melanoma cells. HIF is a heterodimeric protein composed of an alpha subunit regulated by oxygen pressure and a beta subunit constitutively expressed. In melanoma, HIF1a and HIF2a subunits are recovered. Although both HIFa subunits are structurally homologous, they exhibit different roles sometimes antagonist in the tumoral development. In order to understand these different behaviours, stable human melanoma cell lines overexpressing HIF2a protein were constructed. Surprisingly, in these cells, a decrease in HIF1a protein expression was monitored under hypoxia. HIF1a protein underexpression was inversely correlated with HIF2a protein amount. To explain this observation, transcript concentrations of HIF1a and aHIF were measured using a qRT-PCR assay. aHIF is a natural antisense of HIF1a transcript complementary to HIF1a mRNA 3′untranslated region, suspected to negatively regulate HIF1a mRNA amounts. Under hypoxia, aHIF RNA quantity was strongly increased in control transfected melanoma cells (empty vector) whereas aHIF induction was totally lost in stable cell lines overexpressing HIF2a. To confirm that the loss of aHIF induction was correlated with HIFa subunit pattern, we selected renal carcinoma cell lines naturally exhibiting high levels of both HIF-a proteins or high amounts of HIF2a protein and low quantities of HIF1a protein. Disappearance of aHIF induction under hypoxia was only confirmed in the cell lines expressing high levels of HIF2a protein and low amounts of HIF1a protein. In conclusion, we have observed that, in the cell lines studied, a high HIF2a protein expression could be correlated with a decrease of HIF1a expression and a loss of aHIF induction under hypoxia. Experiments are currently in progress to elucidate molecular mechanisms explaining these observations.

Poster No. 33

Elevated Claudin-2 Expression is Associated with Breast Cancer Metastasis to the Liver

Sébastien Tabariès[1,6], Zhifeng Dong[1,6], François Pépin[2,3,6], Véronique Ouellet[1,6], Atilla Omeroglu[4], Mazen Hassanain[5], Peter Metrakos[5], Michael Hallett[3,6], Peter Siegel[1,2,6]
[1]*Department of Medicine, McGill University, Montreal, QC, Canada,* [2]*Department of Biochemistry, McGill University, Montreal, QC, Canada,* [3]*McGill Centre for Bioinformatics, McGill University, Montreal, QC, Canada,* [4]*Department of Pathology, McGill University, Royal Victoria Hospital, Montreal, QC, Canada,* [5]*Department of Surgery, McGill University, Royal Victoria Hospital, Montreal, QC, Canada,* [6]*Goodman Cancer Centre, McGill University, Montreal, QC, Canada*

Breast cancer is the most commonly diagnosed cancer affecting Canadian women and is the second leading cause of cancer deaths in these patients. The acquisition of metastatic abilities by breast cancer cells is the most deadly aspect of disease progression. Upon dissemination from the primary tumor, breast cancer cells display preferences for specific metastatic sites. The liver represents the third most frequent site for breast cancer metastasis, following the bone and lung. Despite the evidence that hepatic metastases are associated with poor clinical outcome in breast cancer patients, little is known about the molecular mechanisms governing the spread and growth of breast cancer cells in the liver.
We have utilized 4 T1 breast cancer cells to identify genes that confer the ability of breast cancer cells to metastasize to the liver. *In vivo* selection of parental cells resulted in the isolation of independent, aggressively liver metastatic breast cancer populations. The expression of genes encoding tight-junctional proteins were elevated (Claudin-2) or lost (Claudin-3, -4, -5 and -7) in highly liver aggressive *in vivo* selected cell populations. We demonstrate that loss of claudin expression, in conjunction with high levels of Claudin-2, is associated with migratory and invasive phenotypes of breast cancer cells. Furthermore, overexpression of Claudin-2 is sufficient to promote the ability of breast cancer cells to colonize and grow out in the liver. Finally, examination of clinical samples revealed that Claudin-2 expression is evident in liver metastases from patients with breast cancer.

The identification and functional validation of candidate genes important for the ability of breast cancer cells will provide basic insights into the pathways required for breast cancer cells to metastasize to the liver. Our results suggest that claudin-2 may play an important role in enabling breast cancer cells to metastasize to the liver.

Poster No. 34

Metastasis Genes Expression Profile in Cholangiocarcinoma Cell Induced by External Estrogenic Agent in associate with TFF1 Trefoil Protein

Peti Thuwajit[1,2,3], Chanitra Thuwajit[1,2,3]
[1]*Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand,* [2]*Division of Medical Molecular Biology, Office for Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand,* [3]*Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*

Cholangiocarcinoma is the carcinoma generated from bile duct epithelium. The prevalence of cholangiocarcinoma is low among worldwide, however it was raised each year. In Thailand cholangiocarcinoma is endemic especially in northeastern part and associated with a liver fluke *Opisthorchis viverrini* infection. The prognosis of cholangiocarcinoma is quite poor because it has high metastasis rate. Previous study showed that cholangiocarcinoma had impairment of estrogen metabolizing enzyme that could leading to the accumulation of estrogen in plasma as we found in our preliminary study. Estrogen itself could induce tumor progression include tumor growth and invasion. TFF1 trefoil protein, an estrogen responsive protein, is a secreted protein that has motogenic effect and can promote cell migration and invasion. In this study we tested the effects of 17b-estradiol, the most potent natural estrogenic substance, on invasion and metastasis genes expression of cholangiocarcinoma cell lines *in vitro*. To test the role of TFF1 trefoil protein in estrogen-stimulated invasion, the permanent knockdown cholangiocarcinoma cell line and mock cell were generated and treated with 17b-estradiol. The results showed that 17b-estradiol could stimulate the invasion of cholangiocarcinoma cell but not in TFF1 knockdown cell compared to both negative control and mock control. Eighty-four tumor metastasis genes expression of estrogen treated cholangiocarcinoma cells (normal control, mock and TFF1 knockdown cell) was measured by RT^2 Prolifiler^{TM} PCR array system. By compared between 3 cell groups, the result indicated 14 genes (CHD4, COL4A2, CST7, CTBP1, KISS1R, IL18, MET, MMP10, NF2, NME1, PTEN, TIMP2, TIMP4 and TRPM1) associated with invasive property induced by estrogen and TFF1 trefoil protein. The pathway of estrogen induced metastasis genes should be analyzed and the results should indicate the mechanism and control of cholangiocarcinoma metastasis for development of new therapeutic method.

Poster No. 35

Influences of the Tumour Microenvironment on Proteins Involved in the Migration and Invasion of Colorectal Carcinoma Cell Lines

Kristin Tweel[1], Jonathan Blay[2]
[1]*Department of Pharmacology, Dalhousie University, Halifax, NS, Canada,* [2]*Department of Pharmacology, Pathology and Biology, Dalhousie University, Halifax, NS, Canada*

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that play a role in extracellular matrix (ECM) remodeling. While MMP activity is normally tightly regulated, both at the expression level and by endogenous tissue inhibitors of metalloproteinases (TIMPs), dysregulation of MMP activity has been linked to many pathological conditions, including cancer progression and metastasis. The expression of MMPs in colorectal carcinoma (CRC), including MMPs-1,2,7,9 and 13, has been correlated with disease prognosis.

We have previously shown that tumour microenvironmental factors regulate the cell-surface levels of CD26 and CXCR4, two proteins involved in the migration and invasion of CRC cells. While there is evidence linking the expression of MMPs to cell regulation through CXCR4, no information is available to address whether MMPs are important in the overall response of CXCR4 and CD26 to the cellular microenvironment, or whether there is a link to CD26 regulatory pathways. In this work we examined whether different factors, or stressors, found in the tumour microenvironment were able to regulate MMP-7,9,13 and TIMP-1-3 mRNA expression and protein secretion.

We show that such tumour microenvironmental stressors, including adenosine and its metabolites, are able to enhance mRNA expression of MMP-7,9 and 13 as determined by quantitative RT-PCR. Additionally, Western blot analysis indicated that these microenvironment stressors are not only able to increase gene expression, but also enhance MMP protein secretion.

Together, these data suggest that factors in the tumour microenvironment are able to regulate changes in protein expression, possibly playing a role in the migratory phenotype of the CRC cells in a local context. These changes may work alongside with, and possibly be mechanistically linked to, the down-regulation of CD26 and up-regulation of CXCR4 that occurs under the same conditions.

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Poster No. 36

The Contribution of the Immune System to Initiation and Progression of Pancreatic Ductal Adenocarcinoma

Renee Vander Laan[1], Geraldine Bienvenu[1], Matthias Hebrok[1]
[1]*Diabetes Center, Department of Medicine, University of California, San Francisco, San Francisco, CA, USA*

In many cancers, the inflammatory response has been shown play a role in tumor formation, progression and metastasis. Although the immune microenvironment has been characterized during the preneoplastic and invasive stages in a mouse model of pancreatic ductal adenocarcinoma (PDA) (Clark et al 2007), the inflammatory response involved in initiation of preneoplastic lesions called pancreatic intraepithelial neoplasias (PanINs) is unknown. Additionally, the functional involvement of immune cells in tumor development and the progression of PDA is unclear. In this study we use mouse models of pancreatitis and PDA to explore to the contributions of the immune response to tumor initiation and progression. We use flow cytometry to carefully monitor the inflammatory response during the initiation of PanIN formation. Additionally, we show that components of the immune system are significantly involved in acinar cell damage that occurs during a mouse model of pancreatitis. This damage, along with a genetic activation of Kras, leads to the development of preneoplastic lesions and promotes tumor development (Carriere et al 2009, Morris et al, in revision). Our study also indicates an important role for the inflammatory response in promoting progression of neoplastic lesions to invasive disease.

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Carriere, C, Young, AL, Gunn, JR, Longnecker, DS and Korc M. Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras. Biochem. Biophys. Res. Commun, 2009 May 8;382(3):561–5.

Poster No. 37

Modulation of Telomerase by *Scutellaria barbata* at Transcriptional Level: An *in vitro* and *in vivo* Study

Christine MN Yow[1], Ellie SM Chu[1], Stephen CW Sze[2]
[1]*Department of Health Technoloy & Informatics, The Hong Kong Polytechnic University, Hong Kong, Hong Kong,* [2]*School of Chinese Medicine, LKS Faculty of Medicine, University of Hong Kong, Hong Kong, Hong Kong*

Traditional Chinese Medicine (TCM) has long been practiced in China over thousands of years. Currently, TCM medications are gaining much attention from modern pharmaceutical institutes and have been studied systematically. Recent studies illustrated that *Scutellaria barbata* (SB) is one of the potential herbs exhibiting anti-tumor efficacy on several tumors, such as head and neck carcinoma, lung cancer and ovarian cancer. Human telomerase reverse transcriptase (*hTERT*), a human catalytic subunit of telomerase, which highly expressed in over 80% human cancers, is an indicative marker for treatment efficacy and therapeutic monitoring. In Hong Kong, colorectal cancer ranks the second of the leading cause of cancer death.

This study aimed to comparatively study the modulation of *hTERT* mRNA expression by *Scutellaria barbata* (SB) at transcriptional level in colorectal cancer cell (HT-29) and the HT-29 immunized BALB/c nude mice models. The efficacy of SB on HT-29 cancer cells was determined by MTT assay; whereas the size of the colon cancer in xenografts was monitored by magnetic resonance interference (MRI) at pre- and post-SB treatment. The modulation of *hTERT* mRNA expression in HT-29 cells and the excised tumors were determined by real-time PCR using TaqMan probe at pre- and post-SB treatment with untreated control cells/tumors and the internal control of *GAPDH*.

The efficacy of *SB* on HT-29 cells was in a dose- and time-dependent manner with the LD_{50} achieved at 550 μg/mL and 72-hour incubation. A 50% reduction in size of the excised tumors was determined in SB-treated xenografts (10 mg/kg/day) for 21 days when compared with that of the untreated control tumors. For the *hTERT* mRNA expression, a 1.3-fold down-regulation was quantitated in HT-29 cells at LD_{50}; whereas a 0.6-fold reduction was quantitated in SB-treated excised tumors at Day 21.

In summary, SB was effective not only to inhibit HT-29 colon cells, but also reduce the tumor size of the colon cancer xenografts. The *hTERT* mRNA expression was down-regulated by *SB* in both in vitro and in vivo models. Thus, *hTERT* could be an effective marker for monitoring SB treatment for colon cancer.

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Poster No. 38

Evidence for a Role of MAGI1 in Colon Carcinoma Invasion

Jelena Zaric[1], Curzio Ruegg[1,2]
[1]*Division of Experimental Oncology, Centre Pluridisciplinaire d'Oncologie, Lausanne University Hospital, University of Lausanne, Epalinges, Switzerland,* [2]*National Center for Competence in Research, Molecular Oncology, ISREC-EPFL, Lausanne, Switzerland*

Colorectal cancer (CRC) is the second most common type of malignancy in the Western world. COX-2 derived PGE_2 promotes CRC progression. However, increased cardiovascular risks of selective COX-2 inhibitors limit their use in chemoprevention. We have observed that Celebrex induces a scaffolding protein MAGI1 (Membrane Associated Guanylate Kinase with Inverted domain structure -1) in COX-2 positive colon carcinoma-derived cell lines (e.g. SW480, HCT116, HT29). MAGI1 appears to function as scaffold that assemble multimolecular complexes at functionally relevant subcellular sites in polarized epithelial cells. When overexpressed, this inner membrane associated protein completely inhibited both migration and invasion of colon carcinoma cells in vitro.

Moreover, MAGI1 enabled colon cancer cells to re-establish cell-to-cell contacts leading to epithelial-like phenotype and increased adhesion on different extracellular matrix proteins. Conversely, stable MAGI1 knock-down though an shRNA approach, favored anchorage independent cell growth. One of the reported MAGI1 binding partners in cell junction complexes is beta-catenin. MAGI1 overexpression induced increased beta-catenin membrane localization while its activity as transcription factor was decreased. The opposite effects were observed in cells in which MAGI1 was knock-down. We are currently testing the effect of of MAGI1 modulation in tumour growth, local invasion and distant metastasis formation. The screening for MAGI1 expression in colon carcinoma tissue is also in progress. This work demonstrates that molecular scaffolds are critical in organizing signaling complexes that control cell adhesion/migration and that a deeper understanding of the effector systems regulating junctional complexes and cell scattering may open new avenues for development of specific therapies targeting invasion and dissemination of CRC cells.

Poster No. 39

FGF-Mediated Suppression of RIG-I Contributes to the Low Responsiveness of Human Hepatocellular Carcinoma to IFN Treatment

Yuanyuan Zheng[1], Qiuyan Liu[1], Ying Chen[1], Yi Zhao[1], Zhenzhen Zhan[1], Xuetao Cao[1]
[1]*National Key Laboratory of Medical Immunology and Institute of Immunology, Second Military Medical University, Shanghai, China*

Retinoic acid-inducible gene I (RIG-I), as a sensor of viral RNA, plays important roles in the induction of virus-mediated type I IFN production and antiviral responses. Recently, identification of negative regulator of RIG-I in the regulation of antiviral innate immune response has attracted much attention and many negative regulators of RIG-I have been discovered. However, the role of RIG-I in tumor development or treatment remain unclear. With tissue array, we find that the expression of RIG-I is reduced significantly in hepatocellular carcinoma (HCC) and some other tumors, such as bladder cancer, renal clear cell carcinoma, endometrial carcinoma and esophagus cancer. Basis FGF, a member of the FGF family, is expressed in many kinds of cancer cells and can stimulate the proliferation of cancer cells of mesodermal, neuroectodermal, ectodermal and endodermal origin. As a mitogenic factor, basic FGF has a close relation with cancer development. Interestingly, we demonstrate that basic FGF can inhibit the mRNA expression of RIG-I in a time-dependent manner in SMMC-7721 HCC cells which highly express FGFR1 and FGFR3. PD173034, the specific inhibitor of basic FGF, can reverse the inhibition of RIG-I expression by basic FGF. Furthermore, inhibitors of PI3K/Akt and ERK pathways (LY294002 or U0126) can also reverse the inhibition of RIG-I expression by basic FGF. Importantly, overexpression of RIG-I enhances the suppression of SMMC-7721 cell growth by interferon a (IFNa), which is attributed to more cell arrest at G2/M phase and the promotion of apoptosis of SMMC-7721 cells. These results demonstrate that FGF-mediated suppression of RIG-I in HCC cells contributes to the low responsiveness of HCC to IFNa treatment.

Poster No. 40

Emerging Role of the RAB25 GTPase in Head and Neck Cancer Metastasis

Panomwat Amornphimoltham[1], Kantima Leelahavanishkul[1], J. Silvio Gutkind[1], Roberto Weigert[1]
[1]*Oral and Phryngeal Cancer Branch, National Institutues of Dental and Craniofacial Research/ National Institutes of Health, Bethesda, MD, USA*

Invasion and metastasis of tumor cells from primary site into stroma and the metastatic organ is a key step in cancer progression with poor prognosis. The 5-year survival rate of head and neck cancer patients, the sixth most common cancer in the developed world, is approximately 50%, despite the recent advances in treatment modalities. Even favorable prognosis in patients that respond to available treatments is often confounded by recurrences and secondary neoplasm, which are generally less sensitive to therapy. Indeed, understanding the biology of the metastatic and invasive cell motility in the tumor microenvironment is critical for developing novel strategies for treatment and prevention in oral cancer patients. Recently, we have established human head and neck primary cell lines panel composed of cells acquired the tumorigenicity and metastasis in tongue tumor xenograft model in immunodeficiency mice. High throughput gene array analysis in these cells against the normal human oral keratinocytes demonstrates the differential expression of a number of molecules involved membrane trafficking process. Among them, RAB25, member of RAB11 small GTPases family essential for membrane protein recycling and translocation of proteins from trans-Golgi network to plasma membrane. Loss of RAB25 expression in metastatic cells has been confirmed by RT-PCR and Western blot analysis compared to both non-metastatic and normal cells. Indeed, expression of RAB25 in the metastatic cells displayed significant arrest of cell invasion and metastatic both in vitro and in vivo model compared to parental cells. Furthermore, intravital imaging technique in tongue tumor xenograft with the genetically modified both to express a fluorescent marker and to either express (or ablate) RAB25 in metastatic and non-metastatic cells, respectively, allow us to investigate the interaction of the tumor and the tumor microenvironment that contribute to the metastatic invasion of this cancer in the physiologic condition.

Poster No. 41

Evidence for a Functional Interaction between CAIX, CAII, and a Bicarbonate Transporter in the Regulation of pH in MDA-MB-231 Breast Cancer Cells

Susan Frost[1], Hai Wang[1], Ying Li[1], Chingkuang Tu[2], David Silverman[2]
[1]*Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL, USA,* [2]*Department of Pharmcology and Therapeutics, University of Florida, Gainesville, FL, USA*

Carbonic anhydrase IX (CAIX), like other members of the carbonic anhydrase family, catalyzes the reversible hydration of CO_2. CAIX is normally expressed only in the epithelial cells of the gut, but is frequently upregulated in cancer cells. CAIX has now been shown to be a marker for hypoxic regions of breast tumors, is associated with poor prognosis, and is linked to acidification of the tumor microenvironment which favors cancer cells survival and resistance to chemotherapeutic agents. CAIX expression has also been linked to the basal B, triple-negative phenotype, an aggressive breast cancer for which there are few treatment options. It has been proposed that CAIX reduces extracellullar pH (pH_e) and increases intracellular pH (pH_i) through functional interactions with one or more of the bicarbonate transporters and CAII, one of the cytosolic CAs. We have utilized a novel technique to provide evidence that these relationships exist. We have shown previously that MDA-MB-231 breast cancer cells express only one membrane-associated form of the CA....i.e., CAIX. Thus, cell surface activity measurements reflect the activity of only this isoform. This form is induced by hypoxia, and we show here using the ^{18}O-exchange technique that membranes isolated from hypoxic cells have a substantial increase in CA activity. We then utilized this technique in whole cells. These data demonstrated that the activity of CAIX can be distinguished from that of CAII and infers a role for the bicarbonate transporter in their individual catalytic activities. Application of an impermeant sulfonamide, which selectively blocks CAIX activity, confirmed its specific contribution to cell-surface CA activity. Further, inhibition of bicarbonate transport demonstrated the requirement of this component in the cross-talk between the two CAs. A model predicted by these studies will be presented.

Poster No. 42

Cathepsin D Binds to the Extracellular Domain of the Beta Chain of LRP1 and Inhibits LRP1 Regulated Intramembrane Proteolysis, Stimulating LRP1-dependent Fibroblast Invasive Growth

Mélanie Beaujouin[1], Christine Prébois[1], Danielle Derocq[1], Valérie Laurent-Matha[1], Olivier Masson[1], Peter Coopman[2], Nadir Bettache[2], Hongyu Zhang[3], Bradley Hyman[4], Peter van Der Geer[5], Gary Smith[6], **Emmanuelle Liaudet-Coopman[1]**
[1]*Inserm U896, IRCM, Montpellier, France,* [2]*CNRS UMR5237, CRBM, montellier, France,* [3]*University of Ottawa, Ottawa, ON, Canada,* [4]*Alzheimer Disease Research Laboratory, Harvard Medical School, Charlestown, MA, USA,* [5]*San Diego University, San Diego, CA, USA,* [6]*Glaxosmithkline, NC, USA*

The protease cathepsin-D (cath-D) is secreted at high levels by breast cancer cells and triggers fibroblast outgrowth *via* a paracrine loop (Laurent-Matha et al., 2005). Here, we evidence that cath-D interacts with the extracellular domain of the beta chain of the LDL receptor-related protein-1, LRP1, in fibroblasts. LRP1 is composed of a 515 kDa extracellular alpha chain and an 85 kDa beta chain. The beta chain contains an extracellular domain, a trans-membrane region and a cytoplasmic tail. LRP1 originally identified as an endocytosis receptor, is also involved in signal transduction by tyrosine phosphorylation of its cytoplasmic NPXY motifs. LRP1 was then shown to participate in cell signalling by regulated intramembrane proteolysis (RIP). In the RIP process, LRP1betae chain undergoes ectodomain shedding, generating the membrane-associated LRP1 fragment, that becomes a substrate for constitutive intramembrane cleavage by gamma-secretases, producing the LRP1 cytoplasmic intracellular domain that acts as a transcriptional modulator. In this study, we show that cath-D binds to residues 349–394 of LRP1beta and this binding is not competed by the chaperone protein RAP. Interaction occurs in lipid rafts, as well as in vesicular-like structures and secreted cath-D is partially endocytosed by LRP1 in human breast fibroblasts. We demonstrate that the ability of secreted cath-D to promote fibroblast invasive growth depends on the presence of LRP1. Interestingly, the gamma-secretase inhibitor, DAPT, that inhibits the release of LRP1beta intracellular domain, also triggers fibroblast outgrowth, suggesting involvement of LRP1 RIP. We further show that both LRP1beta intracellular domain and membrane-associated LRP1beta fragment production are decreased in presence of wild-type or catalytically-inactive cath-D, suggesting a cath-D-mediated inactivation of RIP signalling by competition with the first cleavage event. In summary, our results indicate that cath-D hypersecreted by cancer cells triggers the fibroblastic outgrowth in the breast tumor micro-environment in an LRP1-dependent paracrine manner by inhibiting LRP1 RIP.

Poster No. 43

Early Diagnosis of Breast Cancer through the Analysis of the Breast Intraductal Microenvironment: Identification of Cellular and Metabolic Biomarkers in Nipple-Aspirate Fluids

Ferdinando Mannello[1], Virginia Medda[1], Alessandra Smaniotto[1], Gaetana A. Tonti[1]
[1]*Department of Biomolecular Sciences, Section of Clinical Biochemistry, University "Carlo Bo", Urbino, PU, Italy*

Breast cancer, a complex and multifactorial disease, is the most commonly diagnosed malignancy affecting women; its aetiology may include diet and xenobiotic compounds that influence breast microenvironment (1). Currently available methods of breast cancer detection have well-described limitations (2); in this respect, the biological intraductal approaches directly assess the microenvironment of the breast (3). Breast nipple aspirate fluids (NAF) can be non-invasively obtained from the breast in almost all women (4), thus representing a promising biological tool to assess metabolic and molecular changes occurring in cells lining the ducts from which breast cancer arises. The analyses of NAF collected from healthy and breast cancer patients allows to identify

biomolecular characteristics (1) assessing morphological (5,6), protein (7) and hormonal (8) changes in the breast ductal microenvironment. The NAF studies set the basis for biomarker discovery useful for the early detection and prevention of breast cancer, improving the identification of women with increased breast cancer risk analyzing directly the breast intraductal microenvironment.

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Poster No. 44

A DNA Damage Response (DDR) Involving the DNA Dependent Protein Kinase (DNA-PK) Contributes to the Adaptation of Tumours Cells to Hypoxic Conditions

Marielle Ousset[1], Fanny Bouquet[1], Frederique Fallone[1], Denis Biard[2], Bernard Salles[1], Catherine Muller[1]
[1]*IPBS CNRS UMR 5089 - Université de Toulouse, Toulouse, France,* [2]*CEA-DSV-iRCM/INSERM U935, Institut A. Lwoff-CNRS, Villejuif, France*

The phosphoinositide 3-kinase related kinases (PIKKs) family mainly comprised the ATR ATM and DNA-PK proteins. These large proteins initiate cellular stress responses when genome integrity is compromised. Emerging evidence suggest that hypoxia led to activation of these stress kinases in severe hypoxic conditions. For example, stalled replication forks contribute to ATR activation. ATM is also activated in severe hypoxia (less than 0.1% O2) through alternate mechanisms that do not involve DNA breaks. However, the role of this DDR –like response on hypoxia tolerance remains unknown. We first demonstrated here that the third member of the PI3KK family, DNA-PK (that comprises a DNA binding sub-unit Ku and a catalytic sub-unit DNA-PKcs) is activated by mild hypoxia conditions (0.1 to 1% O2). This was shown by Ku/DNA-PK mobilization from a soluble nucleoplasmic compartment to a less extractable nuclear fraction and its autophosphorylation on serine 2056. This activation was independent of DNA double strand breaks (DSBs) and probably relies on the chromatin modification observed in hypoxic cells according to our preliminary results. Importantly, DNA-PK nuclear activation positively regulates HIF-1α accumulation and its subsequent target gene expression as shown using DNA-PK deficient cells. This effect is dependent of the kinase activity of the whole DNA-PK complex since a strong decrease in HIF-1α expression was observed in cells deficient in its regulatory sub-unit Ku and in presence of a selective inhibitor of the kinase activity of DNA-PK, Nu7026. Finally, the reduced half-life of HIF-1α in DNA-PK deficient cells upon hypoxia provided a mechanistic explanation for the observed effects. In conclusion, our results demonstrate that a new nuclear and DNA dependent stress response pathway contributes to the adaptative response of hypoxic tumours cells and shed a new light on the interest of DNA-PK inhibitors to down-regulate HIF-1α expression in human tumours.

Poster No. 45

***Nuphar lutea* Thioalkaloids Inhibit the Nuclear Factor-kB Pathway, Potentiate Apoptosis, are Synergistic with Chemotherapy and have Antimetastatic Activity**

Janet Ozer[1], Nadav Eisner [1], Elena Ostrozhenkova[2], Adelbert Bacher[2], Wolfgang Eisenreich[2], Daniel Benharroch[4], Avi Golan-Goldhirsh[3], Jacob Gopas[5]
[1]*Deptartment of Microbiology and Immunology, Ben-Gurion University of the Negev, Beer-Sheva, Israel,* [2]*Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München, Garching, Germany,* [3]*Albert Katz Department of Dryland Biotechnologies, The Jacob Blaustein Institutes for Desert Research Ben-Gurion University of the Negev, Sede Boqer Campus, Israel,* [4]*Deptartment of Pathology, Ben-Gurion University of the Negev, Beer-Sheva, Israel,* [5]*Deptartment of Oncology, Ben-Gurion University of the Negev, Beer-Sheva, Israel*

We screened thirty-four methanolic plant extracts for inhibition of constitutive nuclear factor kB (NF-kB) activity by a NF-kB-luciferase reporter gene assay. Strong inhibition of NF-kB activity was found in extracts of leaf and rhizome from *Nuphar lutea* L. SM. (Nuphar). The inhibitory action was narrowed down to a mixture of thionupharidines and/or thionuphlutidines that were identified in chromatography fractions by one- and two-dimensional NMR analysis. Dimeric sesquiterpene thioalkaloids were identified as the major components of the mixture. The Nuphar alkaloids mixture (NUP) showed a dose dependent inhibition of NF-kB activity in a luciferase reporter gene assay as well as reduction of nuclear NF-kB subunits expression as tested by western blots and immunohistochemistry. Decreased DNA binding was demonstrated in Electro Mobility Shift Assays (EMSA). NUP inhibited both inducible and constitutive NF-kB activation and affected the canonical and alternative pathways. Suppression of NF-kB was not cell type specific. Induction of apoptosis by the alkaloid mixture was demonstrated by time-dependent and dose-dependent cleavage of procaspase-9 and PARP. Synergistic cytotoxicity of the active mixture with cisplatin and etoposide was demonstrated. In addition, NUP partially protected mice from LPS- induced septic shock and from experimental B16 melanoma lung metastasis.

Overall, our results show that NUP inhibits the NF-kB pathway and acts as a sensitizer to conventional chemotherapy, enabling the search for its specific target and its application against cancer and inflammation.

Poster No. 46

Molecular Dissection of the Pro-metastatic Effects of ASAP1

Anna Poletti[1], Thomas Müller[2], Ulrike Stein[3], Nicoletta Tata[4], Livia Garzia[4], Massimo Zollo[4], Jonathan Sleeman[1,2]
[1]*Department of Microvascular Biology, Universitätsmedizin Mannheim, University of Heidelberg, Mannheim, Germany,* [2]*Department of Toxicology and Genetics, Forschungszentrum Karlsruhe, Karlsruhe, Germany,* [3]*Department of Surgery and Surgical Oncology, Gene Therapy Group, Max-Delbrück-Center for Molecular Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany,* [4]*CEINGE, Centro di Ingegneria Genetica e Biotecnologia, Naples, Italy*

To understand the molecular mechanisms that underlie the metastatic process is of pivotal importance in cancer research. In an unbiased genetic screen for genes that are involved in metastasis formation we identified ASAP1 (Arf-GAP with SH3-domains, Ankyrin-repeats and PH-domains), and subsequently showed that it promotes tumor cell motility and invasiveness. Loss and gain of function experiments in a pancreatic carcinoma model demonstrate a functional role for ASAP1 in regulating metastasis. In human colorectal cancer patients we found that ASAP1 expression strongly correlates with short metastasis-free survival and poor prognosis. At the molecular level, preliminary co-immunoprecipitation experiments have shown that ASAP1 binds to both h-prune and nm23-H1 (Non-Metastatic protein 23-H1), proteins that are also involved in the metastatic process. Using the highly metastatic breast cell line MDA-MB-231 that endogenously expresses ASAP1, nm-23H1 and h-prune as well as their interaction partners c-src and GSK3-_, we have begun to characterize the putative ternary complex by addressing the following issues: a) the influence of the complex's components on each other's activities; b) further possible interaction partners that may modulate the complex's activity; c) effects of the complex in terms of cellular motility and metastasis formation both *in vivo* and *in vitro*.

Poster No. 47

Targeting Tumour Hypoxia Enhances Castration Effects in a Rat Prostate Cancer Model

Stina Rudolfsson[1], Anna Johansson[2], Sigrid Kilter[2], Anders Bergh[2]
[1]*Department of Surgical and Perioperative Sciences, Urology and Andrology, Umeå University, Umeå, Sweden,* [2]*Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden*

Background: Castration therapy is the standard treatment for advanced prostate cancer, but for reasons largely unknown the effect is only moderate and temporary in comparison with that in non-malignant prostate tissue. In non-malignant prostate tissue castration-induced epithelial cell death is, in part, initiated by vascular regression and tissue hypoxia. Prostate tumours are however hypoxic already prior to treatment and it is unknown whether castration results in an additional drop in tissue oxygen, and if so whether it is of importance for the therapeutic response. In this study we therefore started to explore the effects of castration therapy in relation to tumour hypoxia.
Methods: For this purpose we used the androgen sensitive rat Dunning H prostate tumour model that transiently responds to castration treatment followed by a subsequent relapse, much like the scenario in human patients. Tumour tissues from three different groups; intact, one day, and seven days post castration therapy, were analysed using stereological methods.
Results: We found that hypoxia was transiently up-regulated following castration therapy and correlated with the induction of tumour cell apoptosis. When castration therapy was combined with tirapazamine (TPZ), a drug that targets hypoxic cells and the vasculature, the effects on tumour cell apoptosis and tumour volume were enhanced compared to either castration or TPZ alone.
Conclusions: This study suggests that castration - induced tumour hypoxia could be a novel target for therapy.

Poster No. 48

Nemosis, a Novel Type of Fibroblast Activation, is Associated with Autophagy and Markers of Cellular Senescence

Pertteli Salmenperä[1], Kati Räsänen[1], Anna Enzerink[1], Antti Vaheri[1]
[1]*Virology, Haartman Institute, Helsinki, Finland*

Cells acquire different phenotypes and responses depending on their growth environment and signals derived from it. When mesenchymal cells are grown as multicellular spheroids, a massive proinflammatory, proteolytic and growth factor response promoting tumor invasiveness is induced. Genome-wide microarray analysis revealed three major phenotypic changes in fibroblast spheroids compared to standard 2-dimensional culture; arrest in cell cycle, downregulation of cytoskeleton and induction of secreted proteins (chemokines, proinflammatory cytokines and growth factors). In addition to downregulation of cell cycle proteins, the list of upregulated genes resembled remarkably those reported to be induced during cellular senescence. Furthermore, fibroblast spheroids stained positively to senescence associated ß-galactosidase. Interestingly, classical senescence pathways, p53-p21 and retinoblastoma, were downregulated. Furthermore, the cell cycle arrest was reversible, indicating a mechanism different from that in cellular senescence. A mechanism to leading to this activation (now named as nemosis) and cell cycle arrest is still largely uncharacterized, but one of the first processes seen in nemosis is autophagy. Keeping in mind the important role of autophagy in cellular senescence, it might be that autophagy has a major role in regulation this kind of fibroblast activation.
Since senescent fibroblasts have been shown to stimulate growth of non-invasive cells in vivo and convert them to invasive, we tested whether fibroblast spheroids are able to modulate growth of metastatic keratinocytes in xenograft model. Interestingly, fibroblast spheroids were able to inhibit growth of tumor cells in vivo.

Our results show an important and interesting function of fibroblasts. Furthermore, targeting mechanisms leading to nemotic activation may function as a new therapeutic approach in cancer treatment.
This work was supported by the Helsinki Graduate School in Biotechnology and Molecular Biology, Finnish Cancer Societies, and Academy of Finland.

Poster No. 49

Inhibitory Effects of Tumor-derived 5′- Deoxy- 5′-Methylthioadenosine (MTA) on Human T Cells

Katrin Singer[1], Axel Stevens[2], Christine Hammerschmied[3], Michael Aigner[1], Katja Dettmer[2], Anja Bosserhoff[4], Peter Oefner[2], Marina Kreutz[5], Arndt Hartmann[3], Andreas Mackensen[1]
[1]*Department of Internal Medicine 5, Haematology/Oncology, University of Erlangen, Erlangen, Germany,* [2]*Institute of Functional Genomics, University of Regensburg, Regensburg, Germany,* [3]*Institute of Pathology, University of Erlangen, Erlangen, Germany,* [4]*Institute of Pathology, University of Regensburg, Regensburg, Germany,* [5]*Department of Haematology/Oncology, University of Regensburg, Regensburg, Germany*

Tumor cells develop multiple mechanisms including a dysregulated metabolism to escape T-cell mediated immune recognition. Tumor-derived metabolites are known to modulate cellular components of stromal cells, like immune effector cells and antigen-presenting cells. Studies on malignant tumors, such as malignant melanoma, have revealed loss of methylthioadenosine phosphorylase (MTAP) expression in vitro and in vivo. In this context it has also been shown that MTAP deficient tumor cells secrete 5′-deoxy-5′-methylthioadenosine (MTA). Recent in vitro data have revealed that MTA by modulating melanoma cells as well as tumor infiltrating fibroblasts leads to tumor progression.
In our studies we have demonstrated that MTAP deficiency plays an important role also in renal cell carcinoma (RCC). We have analysed 240 tissue microarrays of RCC including different subtypes (clear cell, papillary, chromophobic and oncocytoma). We have found that 55% of all tumors are deficient in MTAP expression while corresponding normal tissues exhibit significantly higher expression of MTAP. Additionally, RCC cell lines showing loss of MTAP expression on mRNA and protein levels displayed an accumulation of MTA in the cell culture medium as measured by mass spectrometry.
Furthermore we have analysed the effects of MTA on human CD4+ and CD8+ T cells in vitro. Here we show that MTA suppresses proliferation of T lymphocytes in a reversible manner. We further demonstrate that in vitro induction of Ag-specific immune responses is completely abrogated by small amounts of MTA. Also effector functions of highly activated cytotoxic CD8+ T cells, like secretion of IFN-gamma and cytotoxicity against antigen presenting target cells, are diminished greatly in the presence of MTA.
In summary, loss of MTAP expression in malignant tumors results in the secretion of MTA causing direct inhibition of the functional activity of human T cells. Inhibition of specific metabolic pathways in malignant tumors may provide a promising approach to improve the immunotherapy of cancer.

Poster No. 50

Changes in the Expression of HSP27 in Response to the Tumour Microenvironment, and Relationship to Human Breast Cancer Cell Migration

Julia Tufts[1], Robert Douglas[2], Thomas H. MacRae[1], Jonathan Blay[2]
[1]*Department of Biology, Dalhousie University, Halifax, NS, Canada,* [2]*Department of Pharmacology and Pathology, Dalhousie University, Halifax, NS, Canada*

Tumour cells exist in a hostile environment in which they are exposed to many stresses including hypoxia. One consequence of the hypoxic conditions is an increase in extracellular levels of the purine nucleoside adenosine, which has many effects on tumour cells including enhanced migration. This is achieved through an increase in the levels of the chemokine receptor CXCR4 which, along with its ligand CXCL12, is a key player in breast cancer metastasis.
The cellular response to stress is mediated by a family of proteins alternatively known as heat-shock proteins (HSPs), molecular chaperones, or stress proteins. One such chaperone, the small heat shock protein HSP27, has been implicated in changes in cancer cell migration. We have therefore studied the regulation of HSP27 in human breast cancer cells by conditions that normally exist in the stressful environment of a tumour. We aim ultimately to establish whether changes in HSP27 may be linked to hypoxia, adenosine levels and alterations in the CXCL12-CXCR4 migratory pathway.
As revealed by western immunoblotting and immunofluorescence staining, the abundance of HSP27 protein in breast cancer cells increased beginning 3–6 h after initiation of exposure either to hypoxia or to the purine nucleoside adenosine, with a maximal effect after 24–48 h. Further studies detail the signaling pathways and important features of the HSP27 response. These data represent the first stage in our exploration of the link between physiological stress and the capacity for migration in breast cancer cells.
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Poster No. 51

The Impact of Obesity on Angiogenesis in Colon Cancer Patients

Ekaterina Volkova[1], Jinny A. Willis[2], Bridget A. Robinson[1,3], Gabi U. Dachs[1], Margaret J. Currie[1]
[1]*Angiogenesis and Cancer Research Group, University of Otago, Christchurch, New Zealand,* [2]*Lipids and Diabetes Research Group, Christchurch Hospital, Christchurch, New Zealand,* [3]*Oncology Services, Christchurch Hospital, Christchurch, New Zealand*

Obesity is associated with increased risk and mortality in colon cancer, and epidemiological and clinical evidence point to insulin resistance as playing a central role in the underlying molecular pathways. Inflammatory cytokines and growth factors elevated by insulin resistance are potential drivers of tumour blood vessel formation (angiogenesis). Therefore, the purpose of this study was to investigate correlations between markers of obesity, insulin resistance, angiogenesis, tumour pathology and patient survival in colon cancer patients.

Immunoassays were used to measure levels of adiponectin, C-reactive protein (CRP), insulin, insulin-like growth factor-1 (IGF-1), C-peptide, vascular endothelial growth factor-A (VEGF-A) and angiopoietin-2 (Ang-2) in colon cancer patient serum samples (n= 400). Levels of these markers were analysed together with clinicopathological parameters including patient age, gender and tumour characteristics (from Cancer Society Tissue Bank, Christchurch), and Body Mass Index (BMI) and survival data obtained from medical records.

In serum, levels of adiponectin were inversely correlated with patient BMI and IGF-1 protein levels ($p<0.0001$). CRP levels were positively correlated with the levels of VEGF-A and Ang-2, tumour stage, size, depth, and necrosis (all $p<0.001$). Levels of both VEGF-A and Ang-2 were also positively correlated with tumour size, depth and lymph/vascular invasion. In addition, VEGF-A levels were positively correlated with tumour stage, and Ang-2 protein levels with tumour necrosis (all $p<0.05$). Preliminary analysis of survival data show better outcome for patients with serum adiponectin levels in the highest quartile, and worse outcome for patients with serum VEGF-A, Ang-2 ($p<0.05$) and CRP ($p<0.05$) levels in the top quartile.

Our clinical data show colon cancer patients with high BMI and low serum adiponectin have high circulating levels of pro-angiogenic and inflammatory factors that are correlated with adverse tumour characteristics and reduced patient survival.

Poster No. 52

Archazolid B, a New V-ATPase-Inhibitor of Myxobacterial Origin, Exhibits Anti-Metastatic Potential

Romina Wiedmann[1], Ingrid Chen[2], Dirk Trauner[2], Anita Rudy[1], Angelika M. Vollmar[1]

[1]*Department of Pharmacy, Center for Drug Research, Pharmaceutical Biology, Ludwig-Maximilians-University, Munich, Munich, Germany,* [2]*Department of Chemistry and Biochemistry, Ludwig-Maximilians-University, Munich, Munich, Germany*

Resistance of chemotherapy and the rapid formation of metastasis are the main problems in the treatment of highly invasive cancers. Growing evidence suggests that V-ATPase, which is highly overexpressed in metastatic cancer cells, contributes to an acidic tumor environment, promoting cancer progression and metastasis. Archazolid B is a V-ATPase-inhibitor, isolated originally from the myxobacterium *Archangium gephyra*.

We therefore hypothesize that Archazolid B could be a potent compound to inhibit the metastatic process in highly invasive cancer cells and to overcome chemoresistance by directly regulating the pH gradient within the tumor microenvironment.

We could show that Archazolid B changes the intra-and extracellular pH of tumor cells and potently inhibits the proliferation of highly metastatic cancer cells (L3.6pl: IC_{50} ~ 80 pM; SK-BR-3: IC_{50} ~ 500 pM). Interestingly, Archazolid B has only a moderate apoptotic effect (about 20 % apoptosis at 1 nM, 48 h) accompanied by the activation of Caspase 8 and 9 and the downregulation of anti-apoptotic proteins. Along with a strong inhibition of the clonogenic tumor cell growth, our most recent data shows that Archazolid B potently inhibits the migration of highly metastatic cancer cells.

Taken together, Archazolid B inhibits the growth and survival of highly proliferating cancer cells as well as their migration. Ongoing experiments will investigate molecular mechanisms and targets involved other than V-ATPase. Since V-ATPase, targeted by Archazolid B, controls the cancer microenvironment this experimental drug opens up the opportunity to increase the efficiency of different chemotherapeutics and therefore to overcome drug resistance of highly invasive cancer cells.

Poster No. 53

Kynurenine Induce Tolerogenic Dendritic Cell Maturation

Claudia Zavadil[1], Michael Unger[1], Marina Pargger[1], Markus Stoeger[1], Karin Joehrer[1], Richard Greil[2], Raimund Margreiter[1], Albert Amberger[1]

[1]*Gastrointestinal Unit, Tyrolean Cancer Research Institute, Innsbruck, Austria,* [2]*III Medical Department, Private Medical University Hospital, Salzburg, Austria*

In the progression of cancer, malignant cells evolve strategies to avoid an immune response probably through induction of immune tolerance. It is proposed that dendritic cells (DC) have a dramatic impact on tumor immune tolerance and that the tumor microenvironment determine differentiation of DC into a tolerogenic phenotype. Indoleamine 2,3 dioxygenase catalyzes the rate limiting step in the breakdown of L-tryptophan and has been shown to be involved in immune tolerance and cancer progression. However, nothing is known about metabolites of the tryptophan catabolism on DC function.

$CD14^+$ cells were isolated from periperal blood and activated to fully mature DC in vitro. In parallel cultures, DCs were generated in the presence of different concentrations of kynurenine and quinolinic acid. These mature DC were used to analyse expression of differentiation markers by FACS, to stimmulate naïve T-cells to proliferation, and to induce Th-1 T-cell response.

Kynurenine, but not quinolinic acid, had a dramatic effect on the expression of the DC maturation marker CD83, suggesting that kynurenine has an impact on DC maturation. The expression of MHC-class I molecules, the co-stimulatory receptors CD80/CD86

and CCR7 on DC was not affected by kynurenine or quinolinic acid. In further analysis we found that kynurenine treated DC dramatically decrease the ability of T-cells to produce INF-gamma a key cytokine indicating a Th-1 immune response. Subsequently T-cell subpopulations were analysed and found that the portion of $CD4^{+}CD25^{+}$ T-cells was significantly increased in the T-cell population generated by kynurenine treated DC, which indicate an increase in a suppressor T-cell population.
In summary, these data suggest that kynurenine "primed" mDC induce generation of suppressor T-cells. Based on the data presented above we hypothesize that metabolites of the kynurenine pathway are important determinants in turning the immune system especially DC to a tolerogenic phenotype.

Poster No. 54

Impact of Hypoxia on Furin Trafficking and the Formation of Invadopodia

Dominique Arsenault[1], Sébastien GrandMont[1], Martine Charbonneau[1], Kelly Harper[1], Claire M. Dubois[1]
[1]*Department of Pediatric, Immunology Division, Université de Sherbrooke, Sherbrooke, QC, Canada*

Recent studies indicate that tumoral invasion and metastasis, triggered by the hypoxic microenvironment, involves strategic relocalization of convertases, adhesion molecules, and metalloproteases. We used the highly invasive human fibrosarcoma cells HT-1080, stably transfected with eGFP-tagged-furin in order to study the impact of hypoxia on the cellular localization of the convertase furin. Our results indicate that in hypoxic cells, furin is relocalized at the plasma membrane and is internalized via both clathrin- and caveolin/raft dependent endocytosis. Using furin trafficking mutants, we demonstrate that filamin-A, a cytoskeletal tethering protein, is essential for the membrane localization of furin under hypoxia. We further demonstrate that in hypoxic cells, furin and its substrate MT1-MMP relocalize to specific pericellular compartments and this relocalisation is associated with an increased cell ability to convert pro-MT1-MMP into its active form. Because MT1-MMP is known to be involved in ECM degradation at site of invadopodia, we further looked at the implication of cell-surface furin in the formation and functions of these structures. Using a matrix degradation assay, we found that furin colocalize at invadopodia sites with its substrate MT1-MMP under hypoxic conditions. This is associated with an increase in both formation and functions of invadopodia. To better characterize the impact of hypoxia on the invadopodia formation, we next demonstrate that overexpression of furin increases the number of invadopodia and their capacity to degrade ECM. Furthermore, the inhibition of furin with PDX or the MT1-MMP inhibitor GM6001 decreases invadopodia numbers and functions. This is correlated with a decrease in cell invasion in a 3D assay. Our results suggest that hypoxia promotes the formation of a peripheral processing compartment in which furin is concentrated for enhanced processing of substrate involved in the formation of invadopodia leading to cell invasion.

Poster No. 55

Insulin-like Growth Factor II (IGF-II) Enhances Tumor Progression and Stroma Activation in a Model of Skin Squamous Cell Carcinoma (SCC)

Renate Becker[1], Martina Oehme[1], Carolin Bürck[1], Margareta M. Mueller[1]
[1]*Tumor and Microenvironment, German Cancer Research Center, Heidelberg, Germany*

The loss of growth control is one important characteristic of tumor progression. This can be a consequence of a reduced dependence of the tumor cells on growth-stimulatory factors and/or of a decreased sensitivity to growth-inhibitory factors and can be caused by an aberrant expression of growth factors and their receptors.
A progression model for human skin squamous cell carcinoma (SCC) based on the keratinocyte cell line HaCaT was used to elucidate the molecular basis of this increasing environment-independent tumor growth. This model system includes ras-transfected and *in vivo* passaged cells forming tumors of all stages of tumor progression, ranging from benign to late stage malignant and metastasizing tumors. Using a cDNA array comparing the transcriptome of the benign HaCaT-ras A-5 and the high-grade malignant HaCaT-ras A-5RT3 cells, 67 differentially regulated cytokines, growth factors and receptors were identified. Among these differentially expressed genes, Insulin-like Growth Factor II (IGF-II) was shown to be up-regulated associated with increasing tumor malignancy. Stimulation of the benign HaCaT-ras A-5 cells with recombinant IGF-II resulted in increased proliferation and migration/invasion in cell monolayer and in 3-D skin organotypic culture (OTC). The stable IGF-II over-expressing HaCaT-ras A-5 transfectant E2 (A-5E2) demonstrated a proliferation stimulating phenotype leading to a highly increased epithelial growth and differentiation in comparison to the control transfected HaCaT-ras A-5 clone SV3 (A-5SV3) in skin OTCs *in vitro* as well as in transplantation assays *in vivo*. Additionally, IGF-II over-expressing A-5E2 cells induced an earlier and stronger recruitment of neutrophils but not macrophages and an enhanced angiogenesis in *in vivo* transplantation assays compared to the control A-5SV3 cells. In summary, the strong proliferation stimulating function and the additional pro-angiogenic, pro-migratory and stroma-inducing characteristics of IGF-II have an important effect on tumor progression and tumor-stroma interaction.

Poster No. 56

TRAF Family Member Associated NF-KB activatior (TANK) Mediates TGFbeta Resistance in Breast Cancer

Mahaveer Swaroop Bhojani[1], Rajesh Ranga[1], Swathi Pasupulati[1], Brian Ross[1], Alnawaz Rehemtulla[1]
[1]*Radiation Oncology, University of Michigan, Ann Arbor, MI, USA*

TGF-beta and their receptors are key regulators of many aspects of cell growth, differentiation, and function. Regulation of TGF-beta expression and activation is crucial for normal development and growth control. The loss of responsiveness of different tumor cells to the antiproliferative effects and a novel nexus between TGF-beta expression and increased tumorigenicity, invasion and drug resistance is a common feature in carcinogenesis. Here we show, by in silico meta-analysis of breast cancer microarray data that TRAF Family-Associated NF-KappaB Activator (TANK), a signaling adaptor protein reported to be involved in regulating NF-κB activity, is upregulated in metastatic breast cancer and grade 3 tumors. Further, upregulation of TANK was seen in 67% of invasive breast cancers (n=148) by immunohistochemistry based tissue microarray analysis and by western blotting in a number of human breast cancer cells lines. In BT474, breast cancer cells that are refractory to TGF-beta, targeted down regulation of TANK using either siRNA or shRNA lead to increased sensitization to TGF-beta and chemotherapeutic agents. Further, disruption of SMAD2 and NF-κB transcriptional activity was monitored by promoter assay, western blotting and functional ELISAs in TANK siRNA transfected cells when compared to non silencing siRNA or vector control. Taken together, these results suggest a link between NF-κB and TGFβ signaling and that loss of responsiveness to TGF-beta may be mediated by the over-expression of TANK.

Poster No. 57

Stromal PDGFR-α Expression, in Normal Mucosa and Lymph Nodes, Predicts Prognosis in Colorectal Cancer

Maja Bradic Lindh [1], Helgi Birgisson[2], Janna Paulsson[1], Bengt Glimelius[2], Arne Östman [1]
[1]*Oncology-Pathology Department, Cancer Center Karolinska, Karolinska Institutet, Karolinska Sjukhuset, Stockholm, Sweden,* [2]*Department of Surgical Sciences, Colorectal Surgery, Akademiska Sjukhuset, Institutionen för Kirurgiska Vetenskaper, Uppsala, Sweden*

To characterize the prognostic significance of stromal PDGFR-alpha expression in colorectal cancer (CRC), we evaluated the expression of PDGFR-alpha using a tissue micro array (TMA) of a population-based CFRC cohort of having undergone standardized treatment.
The TMA was composed of more than 300 primary tumors, more than 60 lymph node metastases and 114 samples from normal colon. Samples from lymph nodes and normal mucosa were derived from patients whose primary tumors were also part of the TMA. PDGFR-alpha expression was analyzed by immunohistochemistry, and expression was scored separately for epithelial cells, stroma fibroblasts and perivascular cells.
In general, PDGFR-alpha expression was frequently seen in perivascular cells and fibroblasts, but not in epithelial cells. Fibroblast expression was up-regulated in tumors as compared to normal tissue. PDGFR-alpha expression was higher in colon cancer fibroblasts than in rectal cancer fibroblasts. PDGFR-alpha expression in primary tumor CAFs was correlated with more advanced N stage.
Several associations were observed between PDGFR-alpha expression in lymph node metastases and survival. Increased expression of PDGFR-alpha in lymph node fibroblasts was associated with worse survival in the whole patient cohort. High PDGFR-alpha expression in fibroblasts or pericytes in lymph nodes was associated with increased recurrence risk in curatively treated patients. The associations between survival and stromal PDGFR-alpha lymph node expression were also significant in a multivariate analysis. Interestingly, also high expression of PDGFR-alpha in fibroblasts of normal mucosa was associated with worse over-all survival.
These findings thus highlight the prognostic potential of tumor stroma and specifically demonstrate novel prognostic significance of stromal PDGFR-alpha in CRC. The associations between PDGFR-alpha status of normal mucosa and survival also points to the importance of "host factors" in tumor progression.

Poster No. 58

Serum Levels of Dermcidin Increase with Progression of Mammary Carcinogenesis

Heather Ann Brauer[1,2], Tanya E. Libby[1], Yutaka Yasui[3], Anne McTiernan[1], Henry J. Thompson[4], Paul D. Lampe[1,2]
[1]*Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA,* [2]*Molecular and Cellular Biology Program, University of Washington, Seattle, WA, USA,* [3]*Department of Public Health Sciences, University of Alberta, Edmonton, AB, Canada,* [4]*Cancer Prevention Laboratory, Colorado State University, Fort Collins, CO, USA*

Early detection and prognostic profiling of cancers has the potential to increase lifespan and quality of life. The "field effect" hypothesis that motivated this investigation suggests that there are cellular changes that occur both within and around tumor cells that could be detectable in serum. These changes may be detectable before the disease is histologically identifiable using the current testing methods. This valuable information could potentially come from serum where early stages of tumorigenesis lead to changes in the serum peptidome. An experiment testing this idea was carried out using a rat model of mammary carcinoma. Samples were collected at different stages of progression and abundant proteins depleted to determine if MALDI-TOF mass spectrometry could provide a proteomic profile that could identify disease. MALDI-TOF spectra were obtained on an Applied Biosystems Voyager-DE PRO Biospectrometer and analyzed using peak picking computer algorithms and logistic regression models. MALDI-TOF analysis was performed on sera taken from control and carcinogen-treated at each necropsy time point. The peak 4253 m/z revealed a monotone change in the intensity difference that was statistically significant between the treated and untreated rats over weeks 2, 3, 4, and 5. The corresponding band was excised from a gel and possible identifications were determined via electrospray ionization (ESI). One biologically plausible candidate for this band was Dermcidin, a protein previously linked to breast cancer.

We have found Dermcidin levels to increase in serum during disease progression, gaining significance as tumor size increases. We are currently characterizing the role that Dermcidin plays in rat mammary carcinogenesis and investigating a potential correlation with human breast carcinogenesis.

Poster No. 59

Role of CD24 in Gene Regulation and Cancer Invasion

Niko Bretz[1], Mina Fogel[2], Steffen Runz[1], Peter Altevogt[1]
[1]*Department of Translational Immunology D015, German Cancer Research Center, Heidelberg, Germany,* [2]*Institute of Pathology, Kaplan Medical Center, Rehovot, Israel*

CD24 is a mucin-like, highly O- and N-glycosylated, glycosyl-phosphatidylinositol (GPI-) anchored membrane protein. It is expressed in maturing B-cells, neutrophils, epithelial cells and neuronal tissue. CD24 is also overexpressed in various types of human cancers such as lung, stomach, colorectal, prostate, breast and ovarian. In tumors, CD24 has been shown to affect cell proliferation and migration, tumor growth and invasion. However, the cellular downstream events of CD24 remain completely unclear.
Here, we investigated CD24-dependent gene regulation in RNAi and overexpression systems *in vitro*. RNA-microarray based chip-analysis verified by quantitative real-time PCR, identified a small number of genes that were regulated by CD24 expression. One of the most promising target genes is tissue factor pathway inhibitor-2 (TFPI-2). This member of the Kunitz-type serin proteinase inhibitor family functions in the maintenance and the stability of the tumor microenvironment. TFPI-2 is secreted into the extracellular matrix (ECM) and acts as an inhibitor of matrix metalloproteases (MMP) or plasmin-mediated ECM proteolysis. Downregulation of TFPI-2 protein enhances cancer cell ability to degrade ECM due to the lack of this potent inhibitor function. Using ovarian carcinoma cells and CD24-transfected cell lines, we provide evidence that CD24 promoted effects on tumor cell invasion and MMP-activity could be mediated by TFPI-2 levels.

Poster No. 60

Extracellular Matrix Niches Characterization in the Bone Marrow Microenvironment: "Characterizing the Soil"

Ana Sofia Cachaço[1], Ana Cristina Santos[2], Sérgio Dias[1]
[1]*Centro de Investigação em Patobiologia Molecular/Instituto Português de Oncologia de Francisco Gentil de Lisboa, Lisbon, Portugal,* [2]*Laboratório de Anatomia Patológica, Faculdade de Medicina de Lisboa, Lisbon, Portugal*

Bone marrow (BM) is not only the organ where hematopoiesis takes place, but also a target for metastasis from different solid and hematologic cancers.The extracelular matrix (ECM) meshwork envolving BM cells, creates well defined niches where cells must receive appropriate signs for hematopoiesis to take place. We believe thatHere we attempt to identify a role for ECM niche interactions with invading cancer cells are important for the success of the metastatic process. Therefore we started to characterize the ECM and integrin receptor expression patterns in murine BM, using RQ-PCR, FACS and immunofluorescence. We observed that fibronectin is widely distributed within BM, while laminins and collagen IV are predominantly associated with basement membranes. Megakaryocytes and endothelial cells express important amounts of these ECM molecules, megakaryocytes being the major fibronectin producers. Integrin receptors are expressed, generally, by hematopoietic and endothelial cells. Our in vitro data indicate that hematopoietic progenitor cells prefer fibronectin matrices in terms of adhesion and survival, so we want to scrutinize possible cell-ECM interactions in vivo. For that we developed a new immunostainning technique where whole BM are isolated, extensivly permealised, stainned for different cell types and molecular markers and analysed by confocal microscopy. Our protocol overcame frequent immunostainning-related problems like bone decalcification, section damage, antigen masking and loss of the three-dimensional structure. We found that mature BM cells are distributed close or within fibronectin-rich areas and this association increases during BM remodeling following irradiation. Hematopoietic progenitor cells reside in close association with fibronectin, becoming clustered within fibronectin niches; such interactions are impeded in the presence of integrin neutralizing antibodies. Our ongoing work concerns the putative role of BM microenvironment in creating favorable conditions for circulating tumor cells spreding and survival within BM. Using our in vivo 3-dimensional model we are currently analysing the behaviour of metastatic tumor cells in an altered fibronectin BM environment.

Poster No. 61

The Functional Role of ADAM23 Splicing Isoforms on the Modulation of avb3 Integrin Expression and Activation

Felicia Cavalher[1], Érico Costa[1], Anamaria Camargo[1]
[1]*Laboratory of Molecular Biology and Genomics, Ludwig Institute for Cancer Research, Sao Paulo, SP, Brazil*

The ADAMs (a disintegrin and metalloprotease domain) are membrane-anchored glycoproteins characterized by a multi-domain structure, which includes a metalloprotease and a disintegrin domain. Because of their proteolytic and cell-adhesion activity, the ADAMs are involved in various biological process, including fertilization, neurogenesis, angiogenesis and inflammation. ADAM23 exhibits the typical structure of ADAM family members, although its metalloprotease domain is inactive (1). Loss of ADAM23 expression is observed in different types of tumors and, in breast tumors, silencing by promoter hypermethylation is associated with the development of distant metastasis and a worse disease outcome (2–3). Analysis of ADAM23 binding to integrins revealed a specific interaction with avb3 integrin mediated by the disintegrin domain (4). Recently, we demonstrated that ADAM23 negatively modulates avb3 integrin activation during metastasis (3). Ablation of ADAM23 expression using shRNA

enhanced integrin activation by 2–4 fold and ADAM23 knock-down cells showed enhanced migration and adhesion to classical avb3 integrin ligands. Three ADAM23 splicing isoforms have been described so far, two of them (alpha and beta) contain a transmembrane domain that differ in their aminoacid sequence, and the third one (gama) does not encode a transmembrane domain, suggesting to be a secreted protein (5). In the present work, we analyzed by Real-Time PCR the expression pattern of ADAM23 splicing isoforms and found that they are differentially expressed in tumor cell lines. Moreover, using siRNA to specifically knock-down the expression of each splicing isoform, we found that they play different roles on the modulation of avb3 activity, affecting migration and adhesion to classic avb3 ligands.

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Poster No. 62

Triggering of TLR3, 4, 7 and 8 on Human Lung Cancer Regulates Cell Survival and Apoptosis

Julien Cherfils-Vicini[1], Sophia Platonova[1], Liana Ghazarian[1], Pierre Validire[1], Fathia Mami-Chouaib[2], Wolf Herman Fridman[1], Diane Damotte[1,3], Catherine Sautes-Fridman[1], Isabelle Cremer[1]
[1]*INSERM U872, Team 13 Immune Microenvironment and cancer, Centre de Recherche des Cordeliers, Paris, France,* [2]*INSERM U753, Institut Gustave Roussy, Villejuif, France,* [3]*Service d'Anatomo-pathologie, Hôpital Hôtel Dieu, Paris, France*

Compelling evidence support a link between inflammation, cell survival, and cancer, with a central role played by NF-κB, a master switch of inflammation. Recent studies implicate some TLRs in tumor development or regression, and immune escape. However, mechanisms leading to tumor growth or apoptosis induced by TLR stimulation are not fully understood. Several studies strongly suggest that chronic inflammation in lungs induced by chronic bronchitis, chronic obstructive diseases or tobacco smoke, increases the risk of carcinogenesis. We hypothesized that TLRs can contribute to lung inflammation and tumor development.

TLR expression in lung cancer was assayed by immunohistochemistry or flow cytometry. NFκB activation was determined by western blot and nuclear translocation assay. Clonogenicity of stimulated cells was analyzed by colony assay. Transcriptomic analysis were performed by Taqman LDA technology. Tumor growth in vivo was analyzed in NOD/SCID mice.

We have observed that primary human lung tumors express TLR3, TLR4, TLR7 and TLR8 and that stimulation of these receptors in lung tumor cell lines by Poly I:C, LPS, Loxoribine or Poly U induces NFκB activation through atypical signaling pathway, with phosphorylation of IκBα without its degradation and nuclear translocation of p50 and p65 NFκB subunits. Interestingly, we observed that TLR3 stimulation induces apoptosis. On the contrary TLR4, TLR7 and TLR8 stimulation induces cell survival and increases clonogenicity. Moreover, despite a common atypical activation of NFκB, our transcriptomic analysis revealed major differences in gene modulation after triggering of TLR3, TLR4, TLR7 and TLR8. Finally, in vivo TLR7 stimulation of human lung tumor cells dramatically increases tumor growth.

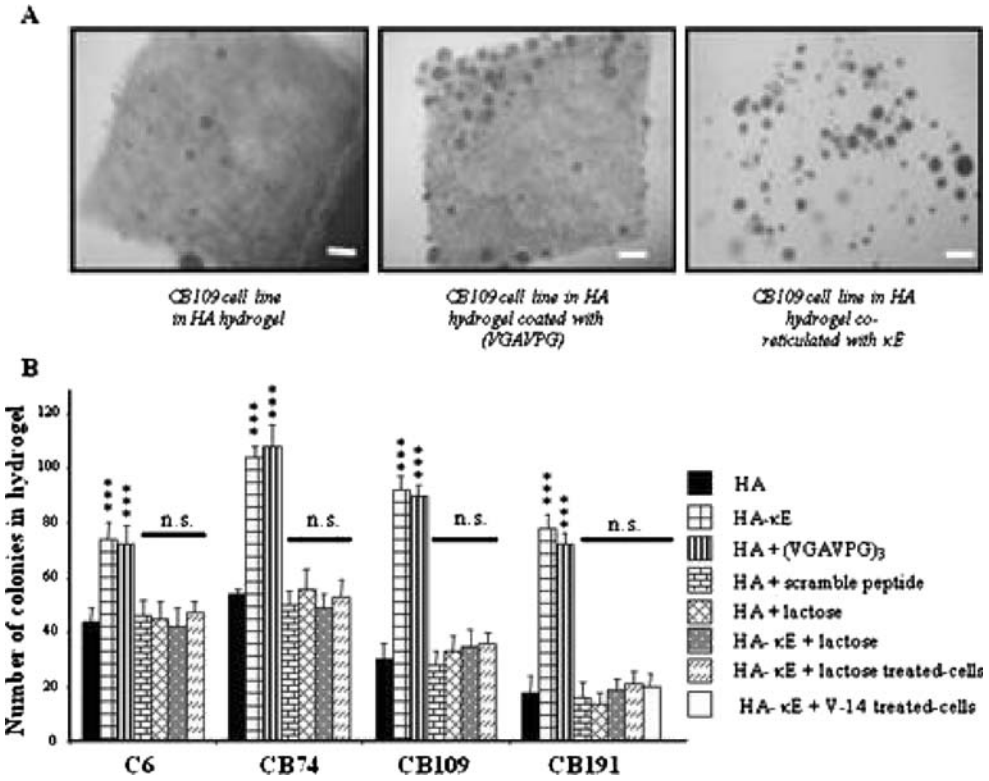
Altogether, these data emphasize that TLR4, TLR7 or TLR8 triggering can directly favor tumor development whereas TLR3 signaling can induce tumor cell death. These data suggest that anticancer immunotherapy using TLR adjuvants should take into account the expression of these TLRs in lung tumor cells.

Poster No. 63

Elastin-Derived Peptides: Matrikines Critical for Glioblastoma Cell Aggressiveness in a 3-D System

Berenice Coquerel[1], Francois Proust[2], Georges Bellon[3], Jean-Pierre Vannier[1]
[1]*Faculté de Médecine de Rouen, Laboratoire MERCI UPRES EA 3829, Rouen, France,* [2]*Department of Neurosurgery, CHU de Rouen, Rouen, France,* [3]*Faculté de Médecine de Reims, Laboratoire de Biochimie et Biologie Moléculaire, UMR 6237 CNRS, Reims, France*

In the most common primary brain tumors, malignant glioma cells invade the extra-cellular matrix (ECM) and proliferate rapidly in the cerebral tissue which is mainly composed of hyaluronan (HA) along with the elastin present in the basement membrane of blood vessels. To determine the role of ECM components in the invasive capacity of glioma cell lines, we developed a 3-D cell culture system, based on a hydrogel in which HA can be co-reticulated with kappa-Elastin (HA-kE). Using this system, the invasiveness of cells from four glioma cell lines was dramatically increased by the presence of kE and a related, specific peptide $(VGVAPG)_3$ (see figure 1 A and B). In addition, MMP-2 secretion increased and MMP-12 synthesis occurred. Extracellular injections of kE or $(VGVAPG)_3$ provoked a pronounced, and dose-dependent increase in $[Ca^{2+}]_i$. kE significantly enhanced expression of the genes encoding elastin-receptor and tropoelastin, the migration (see figure 2 A and B), the adhesion and the proliferation of the glioma cells. We propose the existence of a positive feedback loop in which degradation of elastin generates fragments that stimulate synthesis of tropoelastin followed by further degradation as well as migration and proliferation of the very cells responsible for degradation. All steps in this ECM-based loop could be blocked by addition of either of the EBP antagonists, lactose and V-14 peptide, suggesting that the loop itself should be considered a new therapeutic target. We are currently confirming our findings by studying the correlation between the sensitivity of patients' glioblastoma cells and the patient's survival.



Poster No. 64

Development of a New Brain Metastasis Model in the Nude Rat

Jian Wang[1], **Inderjit Kaur Daphu[1],** Paal-Henning Pedersen[2], Hrvoje Miletic[1], Randi Hovland[3], Sverre Mørk[4], Rolf Bjerkvig[1], Frits Thorsen[1]

[1]*Department of Biomedicine, University of Bergen, Bergen, Norway,* [2]*Department of Neurosurgery, Haukeland University Hospital, Bergen, Norway,* [3]*Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway,* [4]*Department of Pathology, Haukeland University Hospital, Bergen, Norway*

Brain metastasis is a common cause of mortality in cancer patients, and associated with poor prognosis. In order to better understand the complex metastatic process and the interaction between metastases and the microenvironment, we developed a new animal model, where human brain metastases were xenografted into the brains of immunodeficient rats.

Tumor take was achieved in 7 out of 9 human brain metastases implanted. By MR imaging, the animal brain metastases showed similar radiological features as observed clinically. Histological comparisons between the primary tumors from the patients, the patient brain metastases and the xenografted brain metastases showed similar growth patterns. An immunohistochemical study showed similar marker expressions between the patient tumors and the corresponding animal brain tumors. A DNA copy number analysis showed several chromosomal deletions and amplifications, but only one change, gain of 2q, was exclusively found in the animal brain metastases. In conclusion, we have developed a representative *in vivo* model for studying metastatic brain cancer, which will be used to assess responses to treatment.

This model was refined by establishing a cell line (H1) from one of the brain metastases (primary: melanoma). In order to follow systemic spread of the cell line *in vivo*, we generated two new cell lines by transfecting with either dsRed or H1 GFP-Luc reporter genes. The transgene-positive cells were selected by fluorescence activated cell sorting to obtain homogenously fluorescent cell lines. A pilot study showed that the H1/dsRed cells were tumorigenic when implanted intracranially and subcutaneously in matrigel, in nod/SCID eGFP positive mice. A bioluminescence assay using optical imaging on H1/GFP-Luc cells was done *in vitro*, which showed a strong luciferase activity in the cells. Currently the H1/GFP-Luc cells is injected intracardially, to study the ability of systemic homing of these cells into the brain of nod/SCID mice.

Poster No. 65

Role of Extacellular Matrix and their Receptors in the Progression of Colorectal Inflammation and Carcinogenesis

Adele De Arcangelis[1], Fabien Alpy[2], Olivier Lefebvre [2], Sophie Rodius[1], Agnès Méchine-Neuville[3], Stéphanie Siebert[1], Sylvie Robine[4], Michèle Kedinger[2], Patricia Simon-Assmann[2], Elisabeth Georges-Labouesse[1]
[1]*Department of Cell Biology and Development, IGBMC, UDS, Inserm, U964, CNRS, UMR7104, ILLKIRCH, France,* [2]*Inserm U682, Strasbourg, France,* [3]*CHRU Strasbourg, Hôpital de Hautepierre, Strasbourg, France,* [4]*Institut Curie, CNRS, UMR144, Paris, France*

Integrins are transmembrane receptors which mediate interactions of cells with the extracellular matrix. Among integrin receptors, several bind to laminins, major components of the basal lamina. In particular, integrin alpha6 beta1 and alpha6 beta4 can bind to laminins 111, 332 and 511. A specific feature of integrin alpha6 beta4 is its participation to hemidesmosomes, anchorage junctions found in epithelia (skin, intestine), which are the devices by which epithelial cells attach to the basal lamina. In the cells, molecular interactions of alpha6 beta4 with plakins results ultimately with the establishment of a connection with the keratin intermediate filament network. Hemidesmosomes provide cells with resistance against mechanical stress, and it has been largely documented that molecular alterations of hemidesmosomal composition leads to tissue integrity defects such as epidermolysis bullosa. In addition to this structural role, hemidesmosomes are also signalling entities since plakins or integrin cytoplasmic tails recruit signalling molecules. By regulating cell fundamental behaviours (adhesion, migration, proliferation, survival), integrin signalling pathways contribute to the control of tissue integrity and homeostasis. To be able to analyze the functions and signalling of integrin alpha6 beta4 *in vivo* in different tissues, we have generated a conditional integrin alpha6-floxed mutant line. We are using this mouse model to study the functional role of integrin alpha6 beta4 in intestinal physiology and pathology.

Poster No. 66

CD151 Expression and Prostate Cancer Progression

Sujitra Detchokul[1], Bradley Newell[1], Jian Ang[1], Michael W. Parker[2], Elizabeth D. Williams[3], Albert G. Frauman[1]
[1]*Department of Medicine (Austin Health/Northern Health), The University of Melbourne, Heidelberg, Victoria, Australia,* [2]*Structural Biology Laboratory, St. Vincent's Institute of Medical Research, Melbourne, Victoria, Australia,* [3]*Monash Institute of Medical Research, Monash University, Clayton, Victoria, Australia*

Despite improvement in earlier detection and treatment, prostate cancer (PCa) still remains a leading cause of death in most Western countries. CD151, a member of the tetraspanin superfamily is involved in cell signaling, cell motility, cell adhesion, and tumour metastasis by acting as a molecular facilitator recruiting groups of specific cell-surface proteins and thus stabilizing functional signaling complexes[1]. CD151 was identified to be the first tetraspanin member to be linked as a promoter of metastasis[2].
We have previously shown that CD151 has prognostic value in PCa; patients with lower expression of CD151 protein have better prognosis than patients with higher levels of expression[3]. We are now interested in CD151's role in PCa as a motility and metastasis promoter. Human PCa cell lines LNCaP and PC3 were used in cell migration and invasion assays (Matrigel membrane; BD). The motility and invasiveness of wild-type LNCaP (low endogenous level of CD151) vs. CD151 transfected LNCaP cells and PC3 (high endogenous level of CD151) vs. CD151 knock-down PC3 cells (KD PC3) was analyzed. LNCaPs transfected with CD151 showed increased cell motility and invasion compared to control LNCaPs ($P<0.05$), while KD PC3 cells demonstrated reduced cell motility and invasion compared to control PC3s ($P<0.05$). Currently, paired primary and secondary PCa tumors generated using a SCID mouse model bearing implanted human PCa cell lines are being examined for expression of CD151, and its relationship to the density of blood and lymphatic vasculature markers assessed using immunohistochemistry.
Although its mechanism in tumor progression is still unknown, CD151 could be a valuable biological marker for the prognosis of PCa.

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[2] Testa JE et al. Cancer Research (1999) 59: 3812–3820
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Poster No. 67 - Cancelled

Poster No. 68

Bone Marrow Mesenchymal Stem Cells are Altered in B-Cell Chronic Lymphocytic Leukemia

Frédérique Dubois-Galopin[1], Richard Veyrat-Masson[1], Céline Pebrel-Richard[1], Jean-Jacques Guérin[1], Laurent Guillouard[1], Jacques Chassagne[1], Jacques-Olivier Bay[2], Olivier Tournilhac[2], Karin Tarte[3], Marc Berger[1]
[1]*Hematoly Biology, CHU Clermont-Ferrand, Clermont-Ferrand, France,* [2]*Hematology, CHU Clermont-Ferrand, Clermont-Ferrand, France,* [3]*INSERM U917-MICA, Faculté de médecine, Rennes, France*

In B-cell chronic lymphocytic leukemia (B-CLL), malignant cells are not susceptible to apoptosis *in vivo*, while they die rapidly *in vitro* in the absence of specialized non-hematopoietic feeder cells, such as mesenchymal stem cells (MSC). Recent observations have suggested that there is a functional relationship between B cell clone and the bone marrow (BM) stroma. We have thus compared BM-MSC obtained from B-CLL patients and healthy subjects.
We found that most BM-MSC cultures from B-CLL patients failed under standard culture conditions, in contrast with normal BM. In agreement, $CD45^{neg}CD14^{neg}CD73^{pos}$ cells in unmanipulated BM

samples (subset previously shown to contain CFU-F (Veyrat-Masson *et al*., BJH, 2007)), were under the threshold of detection in most of B-CLL BM samples. In productive cultures, we found more CFU-F from B-CLL formed by large, polygonal MSC. These cells proliferated poorly and in most cases could not be further amplified. The use of soluble factors such as bFGF enabled us to detect CFU-F in most malignant samples and to amplify MSC but their frequency remained lower than in control BM. By ELISA, we observed that CLL-MSC release higher amounts of IL-6, IL-8, VEGF and MCP-1. Finally, among 384 genes tested by RQ-PCR (TLDAs, Applied Biosystem) for 9 expanded BM-MSC (5 untreated B-CLL ; 4 normal), we identified 16 statistically up-regulated genes and 41 down-regulated genes. Up-regulated genes included several growth and angiogenic factors as well as key players of the stroma - tumor cell crosstalk. Most down-regulated genes were involved in differentiation pathways.

These results show that CLL-MSC were quantitatively and functionally altered and could be involved in the B-CLL specific stromal cell alterations previously reported (dysregulation of cytokine secretion, angiogenesis, host-tumor relationships). These findings also suggest the possible permissive role of MSC on B-cell clone progression.

Poster No. 69

CReMEC Initiative: Creation and Characterization of New *in vivo* Models of Human Colorectal Cancers

Diane Goéré[2,6], Pascale Mariani[3,6], Marc Pocard[4,6], Ludovic Bigot[2,6], Fariba Nemati[3,6], Denis Lantuas[4,6], Loïc Morgand[1], Ludovic Lacroix[2,6], Sylvia Julien[9], Grégoire Prévost[9], Patrick Gonin[2,6], Virginie Dangles-Marie[3,4,6], Alain Pierré[8], Alain Bruno[8], Hugues De Thé[5,6], Hany Soliman[5,6], Ana Merino-Trigo[7], Guillaume Lardier[7], Hervé Rique[7], Brigitte Demers[7], Cyril Berthet[1], **Olivier Duchamp[1]**

[1]*Oncodesign, Dijon, France,* [2]*Institut Gustave Roussy, Villejuif, France,* [3]*Institut Curie, Paris, France,* [4]*Hôpital Lariboisière, Paris, France,* [5]*Hôpital Saint Louis, Paris, France,* [6]*Canceropole d'Ile de France, Paris, France,* [7]*Sanofi-aventis, Vitry-sur-Seine, France,* [8]*Institut de recherche Servier, Croissy sur Seine, France,* [9]*Ipsen, Paris, France*

New well characterized models representing the heterogeneity of human colorectal cancers (CRC) are needed to develop effective therapeutic agents for that indication; establishment of such tools will allow a better prediction of the clinical outcome, taking into account the diversity of each patient tumor phenotype and genotype. For this purpose and with the financial support of the French Ministry of Industry, we have associated efforts from hospitals, academic groups, biotech and private pharmaceutical companies. From May 2007 to October 2008, 63 surgical specimens [primary tumors (44) and /or metastasis (19)] were collected from CRC patients after obtaining informed consent and confirmation of negative HBV, HCV, and HIVs serologies. Tumor samples were subcutaneously xenografted in *Nude* and *SCID* mice. Thirty-five transplantable tumors were passed at least once in animals, indicating a high take rate (55%). The established models are being evaluated for *ex vivo* and *in vivo* sensitivities to relevant anticancer drugs (5-FU, oxaliplatin and irinotecan), histological and molecular characteristics. Initial molecular characterization includes determination of the MSI status, genetic annotation of *APC*, *KRAS*, *BRAF*, *TP53*, *CTNNB1*, *PI3KCA*, *FBXW7*, and determination of gene copy number using CGH technology. Preliminary molecular and histological characterizations indicate a 35% *KRAS* mutation rate on clinical samples, which is in accordance with the mutation frequency described in the literature for CRC, and a high degree of histological similarity between early passages of xenografts and the original clinical tumor samples. All model characteristics are being compiled in a web-based database for efficient features search and interconnection. We will present the first characterized models and will discuss their usefulness and chance to bring benefit to patients via novel therapeutic strategies.

Poster No. 70

Circulating Endothelial Cells and Microparticles as Potential Surrogate Biomarkers in Multiple Myeloma Management

Hélène Duval[1], Frédéric Dugay[1], Mikael Roussel[2], Karin Tarte[3], Thierry Fest[2], Benoît Guillet[1]

[1]*Pôle Cellules et Tissus, Service d'Hémostase Bioclinique, CHU Pontchaillou, Rennes, France,* [2]*Pôle Cellules et Tissus, Laboratoire d'Hématologie Biologique, CHU Pontchaillou, Rennes, France,* [3]*INSERM U917 - MICA, Faculté de Médecine de Rennes, Rennes, France*

New blood vessel development is an important process in tumor progression. In multiple myeloma (MM), the growth of neoplastic plasma cells is directly regulated by neoangiogenesis. Evidence is emerging that angiogenesis not only relies on the sprouting of resident endothelial cells from preexisting vessels. Circulating endothelial progenitors (CEP) derived from the bone marrow and blood circulating endothelial cells detached from mature vessels (CEC) may also contribute to postnatal angiogenesis. Upon cell activation, procoagulant microparticles (MP) derived from platelets, leukocytes, endothelial cells or erythrocytes are also found in circulating blood. Besides their potential implication in cancer-associated thrombosis, MPs are able to trigger an angiogenic program. Interestingly, MM is characterized by an increased incidence of deep venous thrombosis. In this context, we aimed to test the potential usefulness of studying angiogenic markers (levels of CEP, CEC, VEGF, Endostatin) and MP in circulation but also directly in the bone marrow, as potential biomarkers for the prognostic and the follow-up of myeloma patients. DNA+CD45- CD31+ CD146+ CD34+ circulating endothelial cells were enumerated using a flow cytometer dedicated to the study of rare events (Cyan^{TM} ADP Analyser). Phenotypic specifications were shown to be partly shared with plasma cells. Endothelial cell phenotype was confirmed by immunocytochemistry using anti-von Willebrand Factor staining and UEA-1 lectin binding. In parallel, annexinV+CD41+ platelets-derived microparticles were quantitated. Quantification and kinetics of occurrence of CEC, CEP and MP should reflect vascular injury or malignancy and would be therefore useful to optimize therapeutic options. This project aim to develop a less invasive method to improve the patient management.

Poster No. 71

Genes Associated with Neuroblastoma Lung Metastasis

Liat Edry-Botzer[1], Ido Nevo[1], Shelly Maman[1], Orit Sagi-Assif[1], Tsipi Meshel[1], Ilana Yron[1], Isaac P. Witz[1]
[1]*Department of Cell Research and Immunology, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel*

We developed a human to mouse xenograft model of Neuroblastoma (NB) consisting of local and metastatic variants from each of 2 NB cell lines (MHH-NB11 and SH-SY5Y). The local and metastatic variants derived from each of these tumors had the same genetic background and could thus serve as an unlimited source for the identification of specific NB metastasis biomarkers. A NB-specific oligonucleotide-array detected 4 genes that were differentially expressed both by stage 1 and stage 4 NB patients as well as, correspondingly, by the local and metastatic MHH variants.
These genes are: Damage Specific DNA Binding Protein 2 (DDB2) participating in DNA damage repair; Thymidine Kinase 1 (TK1) a cytosolic enzyme involved in DNAsynthesis; Hexokinase 2 (HK2) an enzyme that participates in the glycolytic pathway; Cingulin like 1 (CNGL1) a homologue of the tight junction component Cingulin.
Protein level validation of the differentially expressed genes revealed a similar pattern to that indicated by the microarray analysis. Furthermore, HK2 and CGNL1 showed the same expression pattern both in the SY5Y and MHH systems. We hypothesize that a differential expression of these proteins by local and metastatic NB variants is a general feature of NB metastasis. In addition to an increased expression of HK2, the metastatic NB variants exhibited also an increased activity of this enzyme.
Inhibition of HK's activity by 3-bromopyruvic (3-BrPa), a specific HK's inhibitor, decreased the enzymatic HK activity in the local variants, whereas the metastatic variants were resistant to the effects of this compound. Furthermore, inhibitor-treated metastatic variants manifested an increased enzymatic activity.
3-BrPa selectively killed the MHH and the SY5Y metastatic variants compared to the local ones suggesting a dependence of such cells on this enzyme.
This study was supported by grant from: Bonnie and Steven Stern, New York, NY, USA.

Poster No. 72

Involvement of Microenvironment Vitronectin and Fibronectin in Human Ovarian Cancer Cell Dissemination: Cell Aggregates Formation and Extracellular Matrix Remodelling

Julien Fernandes[1], Sabrina Kellouche[2], Loraine Heyman [1], Ludovic Carduner[1], Olivier Gallet[1], Johanne Leroy-Dudal [1], Franck Carreiras[1]
[1]*ERRMECe, University of Cergy Pontoise, Cergy Pontoise, Val d'Oise (95), France,* [2] *INSERM 12, University of Picardie-Jules-Verne, Amiens, Somme (80), France*

Ovarian cancer is the most common fatal gynaecological malignancy in western country and is diagnosed at an advanced stage. Epithelial ovarian cancer cells frequently metastasize by *i)* formation of cell aggregates or spheroids in malignant ascite which functions as a permissive microenvironment *ii)* implantation of these cell clusters onto the mesothelial surface of the abdominal cavity (peritoneum) *iii)* invasion of the mesothelial extracellular matrix (ECM) environment.
The aim of our study was to investigate adhesive and remodelling events underlining these processes. Our previous studies[a,b,c] incite us to focus on vitronectin (Vn) and fibronectin (Fn), two ECM proteins widely founded in ovarian cancer microenvironment, especially in peritoneal mesothelium. We developed *in vitro* cell culture method based on the inhibition of cell adhesion to a substratum to generate multicellular suspension aggregates. In these conditions IGROV1 ovarian cancer cells generate viable cell clusters in suspension. Thus, we first studied the implication of Vn and its main receptors (αv integrins) in the initiation of cancer cell aggregates formation and second the Fn remodelling during aggregates adhesion. In cells clusters, Vn and alpha-v integrins are localized at cell-cell contacts. Addition of anti-Vn, anti-αv integrins or cyclic peptide cRGDfV to cell culture inhibited initial aggregates formation. Moreover, the remodelling of coated plasma Vn and Fn was studied in the presence of IGROV1 cell aggregates. Whereas Vn was weakly remodelled, Fn was drastically dislocated. In this context, proteolytic activities are investigated by Vn or Fn zymography.
These results suggest that Vn and its receptors contribute to the formation of spheroids in ascite and that Fn dislocation could facilitate ovarian adenocarcinoma cells dissemination through peritoneal mesothelium.
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[b] Leroy-Dudal *et al.* Bull. Cancer, 95(9), 829–839, Review, 2008
[c] Heyman *et al.*, Tumor Biology, 29, 231–244, 2008

Poster No. 73

Structure-Function Approach Identifies a C-Terminal Domain that Mediates Heparanase Signaling

Liat Fux[1], Nir Feibish[1], Victoria Cohen-Kaplan[1], Svetlana Gingis-Velitski[1], Sari Feld[1], Chen Geffen[1], Neta Ilan[1], Israel Vlodavsky[1]
[1]*Cancer and Vascular Biology Research Center, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Background:
Heparanase is an endo-β-D-glucuronidase capable of cleaving heparan sulfate, activity that is strongly implicated in cellular invasion associated with tumor metastasis, angiogenesis, and inflammation. Heparanase up-regulation was documented in an increasing number of human carcinomas and hematological malignancies, induction that was associated with increased tumor metastasis, vascular density and shorter post operative survival rate. These studies provide compelling evidence and a strong clinical support for the pro-metastatic and pro-angiogenic func-

tions of the enzyme, positioning heparanase as an attractive target for the development of anti-cancer drugs. In addition, heparanase was noted to exert biological functions apparently independent of its enzymatic activity, enhancing the phosphorylation of selected protein kinases and inducing gene transcription. Protein domains that mediate enzymatic activity-independent functions of heparanase have not been so far elucidated.

Principle findings:

We utilized structure prediction server (http://www.robetta.org) to predict the three dimensional structure of active heparanase. The structure obtained clearly delineates a TIM-barrel fold previously anticipated for the enzyme. Interestingly, the model also revealed the existence of a C-terminal domain (C-domain) apparently not being an integral part of the TIM-barrel fold. We provide evidence that the C-domain is critical for heparanase enzymatic activity and secretion. Moreover, the C-domain was found to mediate non-enzymatic functions of heparanase, facilitating Akt phosphorylation, cell proliferation, and tumor xenografts progression. Binding experiments indicate the existence of high affinity, low abundant cell surface receptor, and cross-linking experiments revealed the existence of two major cell surface binding protein(s)/receptor(s) complexes, exhibiting molecular weights of ~ 130 and ~ 170 kDa that interact with heparanase C-domain.

Conclusions:

These findings support the notion that heparanase exert enzymatic activity-independent function, and identifies, for the first time, protein domains responsible for heparanase-mediated signaling. Inhibitors directed against the C-domain, combined with inhibitors of heparanase enzymatic activity, are expected to neutralize heparanase function and to profoundly affect tumor progression and metastasis.

Poster No. 74

Polarization of Macrophages in Lung Metastasis Formation

Annamaria Gal[1], Thomas Tapmeier[1], Ruth J. Muschel[1]
[1]*Gray Institute for Radiation Oncology & Biology, University of Oxford, Oxford, UK*

Tumor associated macrophages have been described in primary tumors. They polarize towards the alternatively activated phenotype (M2) with a distinct receptor and cytokine pattern and support tumor growth. Less is known however about macrophage polarization and the pro-tumoral macrophages in metastasis formation.

In a mouse model of experimental metastasis, we i.v. injected B16F10 melanoma cells into C57BL/6 syngeneic mice and monitored lung colony formation. In a time course of tumor cell challenge, we analysed immune cell infiltration and cytokine expression in order to characterize the metastatic lung environment. Shortly after tumor cell injection (30 min), we found an inflammatory response, involving Gr-1+, CD11b+, Ly6C+neutrophil and monocyte infiltration that ceased within 24 h. After 24 h, we observed CD68+, CD11b+monocyte/macrophage recruitment that lasted no longer than up to 48 h of tumor cell challenge. The recruited macrophages displayed a cytokine pattern resembling the M1 macrophage subpopulation predominantly with IL-12 expression. Although we did not find macrophage recruitment after 48 h of tumor cell injection onwards, the cytokine pattern of the macrophages in the metastasis bearing lung shifted in favour of the M2 phenotype with typically higher IL-10 than IL-12 expression.

Co-culture of *in vitro* polarized bone marrow derived macrophages and B16F10 cells helps reveal the mechanism driving the pro-tumoral function of M2 macrophages in melanoma. In order to investigate the involvement of macrophage receptors in the establishment of a metastatic environment, we used macrophage receptor deficient mice. Preliminary results show that scavenger and mannose receptors might be involved in lung metastasis formation in a tumor cell specific manner. The effect of macrophage receptor deficiency on macrophage polarization will be discussed.

Poster No. 75

An Extracellular Hsp90α-LRP1 Signaling Axis is Required for EphA2 Signaling and Cell Migration in Glioblastoma

Udhayakumar Gopal[1], Venkatesababa Samanna[1], Jennifer S. Isaacs[1]
[1]*Department of Cell and Molecular Pharmacology, Medical University of South Carolina, Charleston, SC, USA*

Glioblastoma multiforme (GBM), the most aggressive type of brain tumor, robustly infiltrates into normal brain parenchyma. This diffuse infiltration precludes complete tumor removal, and contributes to treatment failure and death. Therefore, approaches that target cell migration would be expected to provide a therapeutic benefit. The receptor tyrosine kinase EphA2 is highly overexpressed in GBM tumor cells and its expression serves as a negative prognostic factor. Functionally, EphA2 plays an essential role in regulating GBM cell motility. We have found that GBM cells secrete the intracellular chaperone protein heat shock protein 90 (Hsp90). Extracellular (eHsp90) possess distinct cellular functions from the intracellular Hsp90 chaperone, and has been implicated in promoting cell motility. Importantly, we now identify a unique relationship between eHsp90-dependent signaling and EphA2 activity. Interference with extracellular Hsp90 (eHsp90) suppresses EphA2 signaling and dramatically inhibits GBM motility. eHsp90 has been proposed to signal via LRP1, a multi-functional endocytic receptor. LRP1 is upregulated in GBM cells and its expression correlates with cell migration and invasion. Silencing of LRP1 also suppressed EphA2 signaling and dramatically reduced cell motility, implicating an eHsp90-LRP1 signaling axis in regulation of EphA2 activity. EphA2 is phosphorylated by src and we show that perturbation of src signaling mimics the effects of eHsp90 targeting or LRP1 silencing, thereby implicating Src as a critical effector in EphA2 signaling. We propose that eHsp90-LRP1 signaling crosstalks with EphA2 signaling via src. Our results identify a novel mechanism by which GBM tumors secrete Hsp90, which acts in a paracrine manner to induce motility. We anticipate that interference with the eHsp90-LRP1 signaling axis will attenuate GBM infiltration in vivo. Experiments are underway to elucidate whether other components of the brain parenchyma may secrete eHsp90, thereby further contributing to GBM aggressiveness.

Poster No. 76

Maspin Inhibits the Invasion and Metastasis of Human Tumor Cells

Brigitte Goulet[1], Wendy Kennette[1], Amber Ablack[1], Joe Mymryk[1,3,4], Alan B. Tuck[1,2,4], Ann F. Chambers[1,2,4], John D. Lewis[1,3,4]
[1]*London Regional Cancer Program, LHSC, London, ON, Canada,* [2]*Pathology, University of Western Ontario, London, ON, Canada,* [3]*Surgery, University of Western Ontario, London, ON, Canada,* [4]*Oncology, University of Western Ontario, London, ON, Canada*

Maspin (Serpin B5) is a tumor suppressor that promotes apoptosis and inhibits angiogenesis, tumor formation and metastasis of breast cancer. A number of early clinical studies found that increased levels of Maspin were associated with a worse prognosis, while others found decreased Maspin expression in the primary tumor and undetectable levels in metastases. In subsequent studies, it was found that nuclear localization correlated with a well-differentiated phenotype, chemo-responsiveness and improved survival. These clinical data suggest that the anti-metastatic activity of Maspin resides in the nucleus. However, the exact mechanism by which Maspin prevents metastasis is unknown.

To investigate this, we assessed the effect of Maspin over-expression in two human cancer cell lines that do not normally express Maspin; MDA-MB-231-luc-D3H2LN, a lymph node-tropic breast cancer cell line, compared to HEp3, a (head and neck) squamous cell carcinoma. Over-expression of Maspin inhibited invasion of both cell lines in the Boyden chamber assay, but did not inhibit cell spreading of cells grown in Matrigel. *In vivo*, it was observed that while Maspin expression did not affect migration velocity, there was a 40% decrease in average displacement compared to control cells. Over-expression of Maspin in both cell lines resulted in diminished lung metastasis using a spontaneous metastasis assay in chick embryos. However, in an experimental metastasis model, the ability to seed secondary sites and establish metastases was comparable to that of vector control cells. These data indicate that Maspin expression inhibits an early step in metastasis from a primary tumor.

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Poster No. 77

Bone Marrow-derived Cells are Critical Mediators of Tumor Lymphangiogenesis and Promote Lymph Node Metastasis

Selena Granitto[1], Hannah Lederman[2], Till-Martin Theilen[4], Jared Wels[1], John Lawrence[2], Rosandra Kaplan[2,3], David Lyden[1,2,3]
[1]*Department of Cell and Developmental Biology, Weill Cornell Medical College, New York, NY, USA,* [2]*Department of Pediatric Hematology-Oncology, Weill Cornell Medical College, New York, NY, USA,* [3]*Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [4]*Department of Pediatric Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

Tumor lymph vessels are a key component required for tumor growth and metastatic progression. However, controversy exists as to the origin of these vessels and the specific cells that comprise them. In this study, the orthotopic B16 melanoma model was used to study the role of bone marrow-derived cells (BMDCs) in lymphangiogenesis and lymph node metastasis. In mice transplanted with bone marrow from GFP transgenic mice, $LYVE1^+/GFP^+$ BMDCs were seen at the invasive edge of growing tumors as early as seven days post tumor implantation and were seen incorporating into developing lymph vessels by day 10. Many of these cells were also $CD11b^+$, suggesting a new role for myeloid cells in lymphangiogenesis. $CD45^+/CD11b^+/LYVE1^+$ cells were also detectable in the circulation by flow cytometry of peripheral blood and this population increased with tumor progression. The myeloid cell contribution in lymphangiogenesis was further investigated by examining the function of vascular endothelial growth factor receptor 1 (VEGFR1). VEGFR1 was co-localized with LYVE1-expressing lymph vessels in the primary tumor throughout the course of tumor progression. Targeting VEGFR1 by neutralizing antibody resulted in decreased LYVE1 expression at the primary tumor, decreased lumen size and an overall delay in lymph vessel maturity at the invasive edge of the primary tumor. A decrease in circulating $CD45^+/CD11b^+/LYVE1^+$ cells was seen with VEGFR1 antibody treatment, as well as decreased lymph vessel diameter and decreased metastases in local and draining lymph nodes. Together these results demonstrate that VEGFR1-expressing myeloid cells are important in the process of lymph vessel development that is, in turn, necessary for local lymph node metastasis and subsequent distant dissemination of tumor cells. Early targeting of BMDCs via VEGFR1 blockade can hinder lymph vessel maturation and thereby inhibit lymphatic metastasis, thus serving as a novel therapeutic strategy in the treatment of metastatic disease.

Poster No. 78

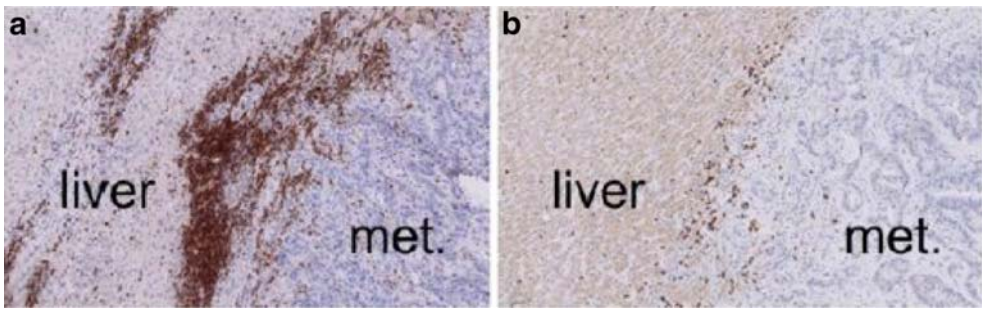
Response to Chemotherapy in Patients with Metastasized Colorectal Cancer is Associated with Densities of Immune Cells at the Invasive Margin of Liver Metastases

Niels Halama[1,2], Sara Michel[3], Matthias Kloor[3], Inka Zoernig[1], Thora Pommerencke[2], Magnus von Knebel-Doeberitz[3], Nadine Feyen[6], Uwe Martens[6], Peter Schirmacher[4], Juergen Weitz[5], Niels Grabe[2], Dirk Jaeger[1]
[1]*Medical Oncology, National Center for Tumor Diseases, University of Heidelberg, Heidelberg, Germany,* [2]*Tissue Imaging and Analysis Center (TIGA), Institute for Medical Biometry and*

Informatics, University of Heidelberg, Heidelberg, Germany, [3]*Applied Tumor Biology, Institute of Pathology, University of Heidelberg, Heidelberg, Germany,* [4]*Institute of Pathology, University of Heidelberg, Heidelberg, Germany,* [5]*Surgical Department, University Clinic Heidelberg, University of Heidelberg, Heidelberg, Germany,* [6]*Hematology and Oncology, SLK Kliniken Heilbronn, Klinikum am Gesundbrunnen, Heilbronn, Germany*

In primary colorectal cancer (CRC) high densities of tumor infiltrating lymphocytes (TIL) were shown to be correlated with improved survival. TILs therefore represent a prognostic tool in the treatment of CRC, a high density of immune cells being associated with good outcome independently of other established prognostic markers. We investigated the relation between infiltrates of immune cells in liver metastases of CRC and response to chemotherapy using immunohistochemical staining. Liver samples from 33 patients with metastasized CRC (samples from 22 patients were used as training set and samples from 11 patients as validation set) were analyzed. Patients underwent surgery after the initial workup appeared to warrant complete surgical removal of the liver metastases. In these patients, only partial resections were possible and these patients received palliative chemotherapy afterwards. Statistically significant differences within the training set allowed prediction of response to chemotherapy by evaluation of the invasive margin of the liver metastasis. Complete sections were examined using an automated high-resolution microscope.

The observed differences (see figure, CD3 positive cells stain dark red, panel A shows a sample with high infiltrate density, panel B shows a sample from another patient with low density) in TIL densities also translated into differences in the time to progression under chemotherapy, where higher numbers of positively stained cells were associated with longer intervals. The difference between the groups with either response or no response to chemotherapy in time to progression was statistically significant (Mann-Whitney-U, $p<0.001$, two-tailed, $z=-3{,}961$, $n=33$). Our results suggest that the immune system influences efficacy of chemotherapy. We have first evidence that the impact of the local immune response on the clinical course is a general phenomenon, not limited to the primary tumor but also present in metastatic lesions. This might have implications for the assessment of therapy options.



Poster No. 79

Association of an Extracellular Matrix Gene Cluster with Breast Cancer Prognosis and Endocrine Therapy Response

Jozien Helleman[1], Maurice P.H.M. Jansen[1], Kirsten Ruigrok-Ritstier[1], Iris L. van Staveren[1], Maxime P. Look[1], Marion E. Meijer-van Gelder[1], Anieta M. Sieuwerts[1], Stefan Sleijfer[1], Jan G.M. Klijn[1], John A. Foekens[1], Els M.J.J. Berns[1]

[1]*Medical Oncology, Erasmus MC, Rotterdam, The Netherlands*

Therapy resistance is a major problem in the treatment of breast and ovarian cancer. We observed in our expression profiling study in breast cancer a gene cluster of ECM related genes, with a similar expression pattern, that was associated with first-line tamoxifen response in advanced breast cancer (Jansen et al. J Clin Oncol 2005). We subsequently validated these ECM genes (COL1A1, FN1, LOX, SPARC, TIMP3, TNC) in 1286 breast carcinomas using qPCR.

High TIMP3, FN1, LOX and SPARC expression is associated with a worse prognosis for 680 untreated lymph node negative patients ($p<0.03$) that is independent of traditional prognostic factors for FN1, LOX and SPARC. Interestingly, only high TNC expression was associated with resistance to tamoxifen treatment in the adjuvant ($n=145$, $HR=1.42$, $p=0.004$) as well as the advanced setting ($n=298$, $HR=1.20$, $p<0.001$). This association is independent of traditional prognostic and predictive factors.

Moreover, in ovarian cancer we also identified a gene cluster of ECM related genes with a similar expression pattern that was

associated with platin-based chemotherapy resistance (Helleman et al. Int J Cancer2006). Pathway analysis of both ECM gene clusters using Ingenuity Pathway Analysis (IPA) showed that both clusters form one gene network with transforming growth factor beta (TGFB) as the key gene. This suggests that TGFB is involved in the regulation of these ECM genes.

We hypothesize that binding of cancer cells to different ECM proteins could result in a similar growth stimulus via integrins possibly together with growth factor receptors. This growth stimulus could overrule the apoptotic signal generated by chemotherapy or could make breast cancer cells independent of the estrogen growth signalling. By analyzing publicly available data we currently investigate whether the ECM, TGFB and related miRNAs, play a general role in therapy resistance (e.g. endocrine, chemo-, radiotherapy) in different tumor types.

Poster No. 80

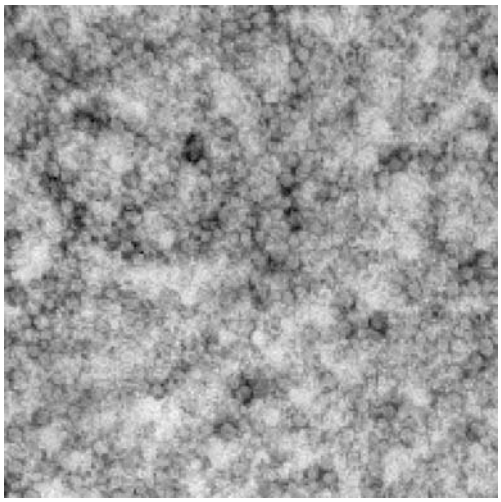
Investigation into the Impact of Xenobiotics on Membrane Mediated Processes, Prostasome Formation and Steroidogensis during Prostate Cancer Progression

Elham Hosseini-Beheshti[1], Jennifer A. Locke[1], Emma S. Guns[1]
[1]*Department of Experimental Medicine, University of British Columbia-The Prostate Centre, Vancouver, BC, Canada*

Prostate cancer (PCa) progression after androgen deprivation therapy resulting from up-regulation of lipogenesis pathways and increased intra-tumoral production of androgen from cholesterol has been previously reported by us. We are interested in the role of cholesterol-trafficking triggering androgen synthesis and the ability of xenobiotics to alter this. Presence of lipid rafts (LR) in cholesterol-rich prostasomes are the communication entities that act within the tumoral microenvironment (Fig1). We recently demonstrated presence of steroidogenesis enzymes in circulating prostasomes. The current study was designed to establish cell line models for use in evaluation of the effects of xenobiotics on LR signalling involved in prostasome formation and the role of prostasomes as steroidogenesis enzyme transporters.

We evaluated a panel of human PCa cell lines to determine their ability to undergo steroidogenesis as compared to that previously determined in LNCaP cells *in vitro*. We used both a 'snapshot' approach using tandem LCMS/MS analysis to profile the presence of steroids in PC3, DU145, LNCaP, VCaP and C4-2 cells in an androgen-deficient environment, as well as treatment with a radiolabelled androgen precursor to monitor downstream androgen production using radiomatic HPLC in conjunction with LCMS. Prostasomes isolated from PC-3 and VCaP cells were imaged using transmission electron microscopy.

Cell lines known to express androgen receptor, including the androgen-resistant C4-2 cells, are efficient at producing androgens while PC-3 and DU145 cells do not produce androgens. The use of these model systems is important for studying the effects of xenobiotics on LR signalling involved in prostasome formation as well as the potential role of prostasomes as steroidogenesis enzyme transporters.



Poster No. 81

A Colorectal Cancer Model Initiated from Freshly Harvested Patient Biopsies Orthotopically Xenografted in GFP-scid Mice

Hege Jacobsen[1], Aly Dicko[2], Jian Wang[1], Kristian Storli[3], Frits Thorsen[1], Karl Søndenaa[3], Donald Gullberg[1], Per Øyvind Enger[1]
[1]*Department of Biomedicine, University of Bergen, Bergen, Norway,* [2]*Department of Surgery, Haukeland University Hospital, Bergen, Norway,* [3]*Department of Surgery, Haraldsplass University Hospital, Bergen, Norway*

Most animal models typically involve ectopically implanted cancer cell lines. Since tumor-stroma interactions are organ specific, and cancer cells undergo profound changes during *in vitro* culture, the resulting tumors have a limited relevance to the patient tumor. To address this issue, we inserted human colorectal tumor biopsies onto the ceacal wall of scid mice, and used a mice strain expressing the green fluorescent protein (GFP) to enable separation of the tumor and host compartments.

Biopsy specimens from 8 histologically verified colorectal cancers (CRC) were minced into pieces that were xenografted in 20 GFP-scid mice. The animals were palpated for tumors, of which some were subsequently monitored *in vivo*, using a small animal, 7 Tesla, Magnetic Resonance Imager. Tumor imaging parameters such as tumor size, vascularity and presence of metastatic sites were assessed. At this stage, 9 animals have been sacrificed due to prominent disease, and tumor growth was histopathologically confirmed in all cases. However, the remaining 11 animals have considerable palpable tumour masses, suggesting a 100% tumor take rate. Preliminary analysis suggests that the pathological

staging and TNM of the patient tumors does not impact survival times, ranging from 42 to 448 days.
The tumors demonstrate a histoarchitecture similar to the parent tumors. These studies will be extended to include immunohistochemical staining for markers of stromal activation. Moreover, tumors have been dissociated and FACS sorted into GFP cancer and GFP^+ stromal cell populations of more than 95% purity, providing a valuable tool for *in vitro* experiments.
We conclude that this model mimics the histopathological features of human CRCs, and provide reproducible high take rates. Furthermore, the fluorescent mouse phenotype is useful for separation of tumor and host compartments, allowing further studies of tumor-stroma interactions.

Poster No. 82

The Role of Notch1 in Tumor Progression and Metastasis

Katarzyna Jesien[1], Xiujie Li[1], Paul Jolicoeur[1]
[1]*Laboratory of Molecular Biology, Clinical Research Institute of Montreal, Montreal, QC, Canada*

Notch signaling plays critical roles in the progression of human malignancies, however the precise role and mechanism of Notch1 in tumor invasion and metastasis remains unclear. In an earlier report, our group demonstrated that Notch1 truncation occurs frequently in retrovirus-induced thymomas in MMTV/c-myc transgenic mice producing the overexpression of a distinct secreted Notch1 mutant product. It was hypothesized that this Notch1 mutant plays a role in neoplastic progression. In order to assess this, transgenic mice were generated to overexpress the mutated form of Notch1 in T cells and the myeloid lineage.
Recently, it was found that tumor progression is facilitated in transgenic mice treated with chemical carcinogen. In addition, increased pulmonary metastasis was observed when syngeneic breast tumor cells were inoculated in these mice. Transplantation studies reveal that the observed increase in metastasis in our model is due to hematopoietic cells, and further inoculation studies demonstrate that this is occurring through a paracrine loop. Additionally, transgenic primary subcutaneous tumors have increased microvascular density and are highly necrotic compared to wild-type controls. Early findings from preliminary experiments suggest increased tumor permeability within primary tumors, as well as increased intravasation in tumor-bearing transgenic mice.
A major barrier to successful long-term cancer treatment is recurrence and metastatic spread. The outcome of these studies will allow us to determine a clear functional role for Notch1 involvement in tumor microenvironment and metastasis, as well as lead us to the identification of mechanisms involved in this novel pathway of cancer spread. From this, we can form a basis from which we can identify potential new molecular targets for the development of rational cancer therapies in the future.

Poster No. 83

An eGFP-Expressing Immunodeficient Mouse Model with dsRed Expressed Mammary Tumors and the Effect of Hyperbaric Oxygen

Alison Charlotte Jevne[1], Ingrid Moen[1], Rolf K. Reed[1], Rolf Bjerkvig[1], Linda Stuhr[1]
[1]*Department of Biomedicine, University of Bergen, Bergen, Norway*

Background: A NOD/Scid mouse expressing enhanced green fluorescent protein (eGFP) has been developed and established with different transfected dsRed cell lines (1). We wanted to develop a mice mammary tumor model (4 T1) in these eGFP mice and use this model to further explore our previous observations of a significant decrease in tumor growth in DMBA induced mammary tumors in rats after hyperbaric oxygen treatment (2–3).
Methods: We injected 3 million dsRed transfected cells into the eGFP mice, subcutaneously in the groin-area. After the tumors had become ~ 3 mm in diameter the mice were divided in two groups. One group was exposed to 2,5 atm pure oxygen (3 exposures a 90 min), whereas another was housed under normal atmospheric conditions and served as controls. Using light microscopy as well as multiphoton confocal microscopy, we investigated the tumor-host interaction in situ. The effect of the treatment on tumor volume was determined by measuring the tumor size with a caliper day 1, 4 and 8.
Results: The experiments confirmed that we have established a very aggressive dsRed mammary tumor in the eGFP mice, showing the tumor cells invading the stromal cells as well as a number of vascular elements *in situ*. Furthermore, tumor growth was significantly reduced after HBO treatment compared to control animals and a significant decrease in collagen density was also found.
Conclusion: We have established a dsRed mammary tumor in eGFP expressing mice. This model will enable us to study tumor-stroma interactions in a new and more specified way. The reduction in tumor growth and collagen density found in the HBO treated tumors will be further elucidated.
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Poster No. 84

Platycodin D inhibits VEGF-Mediated Angiogenesis through Regulating MAPKs Activation and IL-8 Expression in HUVECs

Ki-Rim Kim[1,2], Won-Yoon Chung[1,2], Ju-Ah Son[1], Yeong-Shik Kim[3], Young-Wan Ha[3], Kwang-Kyun Park[1,2]
[1]*Department of Oral Biology, Oral Cancer Research Institute, Oral Science Research Institute and Brain Korea 21 Project, Yonsei University College of Dentistry, Seoul, Korea Republic,*

[2]*Department of Applied Life Science, The Graduate School, Yonsei University, Seoul, Korea Republic,* [3]*Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul, Korea Republic*

The communication between the tumor cells and the surrounding cells helps to drive the process of tumor progression. Especially, angiogenesis by endothelial sprouting from preexisting venules facilitates solid tumor growth by providing oxygen and nutrients to proliferating cells, and acts as a physical route for metastasis transport. Therefore, detection of anti-angiogenic agents is one of the most promising approaches to control tumor progression. Vascular endothelial growth factor (VEGF), a major angiogenic factor, is produced by many tumor as well as normal cells, and induces the expression of various angiogenesis-related proteins such as interleukin-8 (IL-8). Platycodin D, the major constituent in the root of *Platycodon grandiflorum*, has been reported to have a number of pharmacologic activities including anti-inflammatory and anti-allergic activities. In this study, we examined the ability of platycodin D to interfere with the various steps of angiogenesis. Platycodin D treatment inhibited VEGF-induced adhesion, proliferation, DNA synthesis, chemotactic motility and tube formation in a dose-dependent manner in primary cultured human umbilical vein endothelial cells (HUVECs). Platycodin D reduced VEGF-induced phosphorylation of ERK1/2, p38 and JNK, closely associated with tube formation of HUVECs, and also reduced the IL-8-induced tube formation as well as VEGF-induced IL-8 expression in HUVECs. Furthermore, the anti-angiogenic activity of platycodin D was confirmed by performing the Matrigel plug assay in mice. In a mouse tumor xenograft model, platycodin D inhibited the growth of MDA-MB-231 breast carcinoma, and reduced the expression of VEGF, CD34 and IL-8. Taken together, our results indicate that platycodin D exerts anti-angiogenic action by regulating MAPKs activation and IL-8 expression. Therefore, platycodin D may beneficial for prevention and treatment of angiogenesis-dependent human diseases such as tumor.

Poster No. 85

Role of Complement in Lymphoid-Like Tumor Transformation and Invasion

Iraklis C. Kourtis[1], Jacqueline D. Shields[1], Melody A. Swartz[1]
[1]*Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Vaud, Switzerland*

Changes in the immunological equilibrium in the tumor microenvironment are critical for the progression of a developing tumor, allowing tumor escape from immune surveillance and metastases. We have identified that invasive B16 F10 melanomas naturally secrete CCL21, a ligand for CCR7, which is used by dendritic cells and naïve T cells to home to the T cell zone of the lymph node to initiate an immune response. B16 F10 melanoma cells were engineered to either knockdown, maintain or over-express CCL21. Chemokine secreting tumors, but not knockdown variants, attracted $CCR7^+$ lymphoid tissue inducer cells (LTis, $CD45^+CD3^-CD4^+IL\text{-}7Ra^+ROR\text{-}\gamma t^+$) into the tumor and drove lymphoid-like changes in the tumor microenvironment including a reticular fibroblast stromal network ($CCL21^+gp38^+$ $ERTR7^+$ $LYVE\text{-}1^-$) surrounding the tumor, HEV-like vessels ($ERTR7^+$ $PNAds^+LYVE\text{-}1^-$) inside the tumor, and, importantly, an over-expression of complement regulating receptors. This microenvironment, reminiscent of the T cell zone in the lymph node, attracted naive T cells into the tumor where, we hypothesized, they could be educated towards a tolerogenic phenotype only in a regulatory microenvironment. Recent studies have suggested a role of complement in tumor growth, and since complement can serve both immune regulatory and functional roles depending its processed form, we implanted these tumors into C3-/- mice. We found that both CCL21 expressing and knockdown tumors grew poorly, and CCL21-secreting tumors could not drive a regulatory T cell response as they did in wild type mice. These findings suggest that invasive tumors may utilize complement dependent strategies in the newly formed quasi lymph node microenvironment, to further provide a regulatory environment for in situ education of T cells shifting the host immune response from a functional to regulatory repertoire.

Poster No. 86

The Effects of Inflammation on the Metastatic Microenvironment: Peritoneal Metastasis in a Mouse Model with Abdominal Incision Wound

In Kyu Lee[1], Michael N. VanSaun[2], Lynn M. Matrisian[2], D. Lee Gorden[2]
[1]*Department of Surgery, St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea Republic,* [2]*Department of Cancer Biology, Vanderbilt University, Nashville, Tennessee, USA*

Purpose: Pro-inflammatory processes of the early postoperative states may induce peritoneal metastases in patients with advanced diseases. To identify that wound healing response after an abdominal incision leads to increased MMP-9 activity locally, therefore providing a favorable environment for peritoneal metastasis. Increased MMP9 in a post-operative injury setting increases the number and severity of peritoneal metastasis when compared to mice without wounds.
Methods: Eighteen C57bl/6 J male mice were obtained at 8 weeks of age. Metastatic tumors were initiated using a peritoneal injection model with syngeneic MC38 murine colon cancer cells. Peritoneal injections were performed into the intraperitoneum at right lower quadrant area via 25G syringe. A 1.5 cm upper midline incision was made in the abdominal wall to recapitulate the postoperative wound model. The abdominal wall was closed by a continuous 4-0 prolene suture with 5 stitches. Mice were sacrificed at various time points. And we observed the rate of the peritoneal metastasis from each group.
Results: By making incision into the abdominal wall, we induced inflammation of the mouse and observed the incidence of the peritoneal metastasis was increased(Fig.1). Early stage of wound healing process increases pro-inflammatory cytokines and number

of inflammatory cells in the peritoneum, and this leads to increase pro-MMP9 proteins. And the inflammatory process which initiated by the wound, in turn, increased the proliferation of the mesothelial cells and provoked expression of the inflammatory cells and increased parietal peritoneal metastasis.

Conclusion: stage of wound healing process increases pro-inflammatory cytokines and number of inflammatory cells in the peritoneum, and this leads to increase pro-MMP9 proteins. So the increased pro-MMP9 proteins play a key role on the growth and progressions of cancer cells in peritoneal metastasis.

Figure 1.

Poster No. 87

Cytokine-Mediated Activation of Gr-1$^+$ Inflammatory Cells and Macrophages in Squamous Cell Carcinoma towards a Tumor-Supporting Pro-Invasive and Pro-Angiogenic Phenotype

Nina Linde[1], Dennis Dauscher[1], Margareta M. Mueller[1]
[1]*Group Tumor and Microenvironment, German Cancer Research Center, Heidelberg, Germany*

Inflammatory cells have been widely accepted to contribute to tumor formation and progression. In a HaCaT model for human squamous cell carcinoma (SCC) of the skin, we have observed that infiltration of inflammatory cells does not only promote tumorigenesis but is indispensable for persisten angiogenesis and the development of malignant tumors. Analysis of the inflammatory infiltrate revealed that the majority of the inflammatory infiltrate constitutes of CD11b$^+$/Gr-1$^+$ cells. Depletion of these cells from tumor bearing nude mice resulted in a decrease in tumor growth, reduced angiogenesis and an inhibiton of tumor invasion. In order to characterize the tumor-supporting capacities of inflammatory cells we analysed the contribution of neutrophils and macrophages to tumor invasion *in vitro*. We were able to demonstrate that both cell types strongly enhance invasion of SCC tumor cells in the presence of exogenously added stimulating cytokines while they do not influence invasion without additional cytokine stimulation. This implies that inflammatory cells need stimulation by specific mediators to be activated towards a tumor supporting phenotype. In this context we are currently analysing selected stimulatory factors with respect to their influence on both neutrophils and macrophages and have identified a novel factor that activates these two cell types.

Poster No. 88

Ovarian Cancer Cells Acquire Chemoresistance through Intercellular Transfer of MSC-Derived PgP

Raphaël Lis[1,2], Pejman Mirshahi[2], Rowaida Ziad Taha[1], Mary Poupot[3], Eliane Mery[4], Jean Jacques Fournié[3], Denis Querleu[4], Massoud Mirshahi[2], Arash Rafii[1]
[1]*Stem Cells Research, Weill Cornell Medical College - Qatar, Doha, Qatar,* [2]*Tumor cells resistance, Centre de Recherche de Cordeliers - INSERM U872, Paris, France,* [3]*Oncology, Centre de Physiopathologie Toulouse Purpan - INSERM U563, Toulouse, France,* [4]*LFR 44, IFR 31, Institut Claudius Regaud, Toulouse, France*

Background:
The microenvironment plays a major role in the onset and progression of metastasis. Epithelial ovarian cancer (EOC) tends to metastasize to the peritoneal cavity where interactions within the microenvironment might lead to chemoresistance. Mesothelial cells and particularly Mesenchymal Stem Cells (MSC) are important actors of the peritoneal homeostasis; we determined their role in the acquisition of chemoresistance of ovarian tumours.
Methodology/Principal Findings:
We isolated MSC from ascitis of patients with ovarian carcinosis using limiting dilution. We studied their ability to confer chemoresistance through heterocellular interactions. These MSC derived from ascitis diplayed positive immunostaining for CD9, CD10, CD29, CD146, CD166 and Multi drug resistance protein, as described in the litterature. They preferentially interacted with epithelial ovarian cancer cells. This interaction induced chemoresistance to platin and taxans with the implication of multi-drug resistance proteins. This contact enabled EOC cells to capture patches of the MSC membrane through intercellular transfer of

membrane and proteins (also referenced as trogocytosis), therefore acquiring their functional P-gp proteins and thus developing chemoresistance. Presence of MSC in ovarian cancer tissue micro-array from patients with neo-adjuvant chemotherapy was also significantly associated to chemoresistance.

Conclusions/Significance:

This is the first report of intercellular transfer of protein occurring between a cancer cell and a stromal cell (here MSC). This interaction induced autonomous acquisition of chemoresistance. The presence of stromal cells within patient's tumour might be predictive of chemoresistance. The specific interaction between cancer cells and stromal cells might be targeted during chemotherapy.

Poster No. 89

Extracellular Matrix Regulation of EGRR Activity: Hyaluronan Alters Epidermal Growth Factor Receptor-Dependent Cell Morphology

Jeanne Louderbough[1], Joyce Schroeder[1]
[1]*Department of Molecular & Cellular Biology, University of Arizona, Tucson, AZ, USA*

EGFR is an important regulator of breast cancer progression and is capable of integrating multireceptor signaling pathways to promote metastasis. Through these interactions, EGFR is subject to extensive regulatory cues from the extracellular matrix (ECM), of which the extracellular glycoprotein hyaluronan (HA) is a major component. In mammary tumors, HA is deposited in the stromal compartment surrounding tumor epithelium where it functions in both biomechanical support and, through binding to the adhesion receptor CD44, modulates intracellular signaling. We have used a 3D collagen culture system in which HA is either polymerized into a collagen matrix to mimic epithelial-stromal interactions or provided soluble in the media (sHA). We have found that collagen-embedded HA (eHA) inhibits EGFR activation and alters cell morphology by inhibiting filopodia formation while soluble HA promotes these events. The ability of cells to spread on a collagen matrix is also impaired on eHA, demonstrating a novel function for eHA in regulating cell morphology and membrane dynamics. Inhibition of EGFR and alterations to cell morphology are due to cell-matrix interactions, as collagen polymerization is unaltered by eHA. EGFR interaction with the HA receptor, CD44, is impaired on eHA suggesting that this is a mechanism by which HA regulates EGFR activity. Furthermore, given the ability of EGFR to alter cell morphology on a matrix, we have examined the ability of erbB ligands to regulate cell morphology on diverse matrix substrates and have found that these ligands induce collagen-dependent changes indicative of EMT. These findings highlight a novel role for eHA as a protective molecule when encountered in the collagen matrix during cancer progression, while reinforcing the tumor promoting effects of sHA, and demonstrate the ability of the ECM to alter erbB-dependent EMT.

Poster No. 90

Regulation of Invadopodia Formation by Hypoxia-Induced NHE-1 Activity

Fabrice Lucien[1], Dominique Arsenault[1], Claire M. Dubois[1]
[1]*Immunology Division, University of Sherbrooke, Sherbrooke, QC, Canada*

Most tumors are characterized by an acidic and hypoxic microenvironment that promotes metastasis. The Na^+/H^+ exchanger (NHE-1) plays an important role in the regulation of pH homeostasis. It has been demonstrated that NHE-1 is constitutively active in tumor cells, promoting cell invasion, but the mechanisms are not defined. One of the ways that tumor cells are able to promote invasion is by forming invadopodia. Invadopodia are actin-rich structures that are responsible for focal concentration of matrix-metalloproteases (MMP) that degrade the extracellular matrix. Our lab has shown that hypoxia significantly increases invadopodia formation in human fibrosarcoma cells (HT-1080). Also, it has been demonstrated that MMP degrading activity is dependent on extracellular acidic pH. Therefore, the aim of our study is to identify the role of NHE-1 in hypoxia-induced invadopodia production. We observed that hypoxic stimulation increases NHE-1 mRNA and protein expression. Intracellular pH monitoring by live-cell imaging revealed that NHE-1 activity is also increased in hypoxic conditions. Using inhibitors and shRNA-mediated depletion, we demonstrated that NHE-1 participates in invadopodia formation in HT-1080 cells. Zymography assays showed that inhibition of NHE-1 activity resulted in the loss of MMP activation. Disruption of extracellular pH abolished invadopodia-mediated matrix degradation. Moreover, NHE-1 overexpression stimulated invadopodia formation and invadopodia-associated matrix degradation. Alltogether, our results indicate that NHE-1 is involved in hypoxia-dependent matrix degradation by invadopodia and suggest a mechanism by which the hypoxic and acidic tumor microenvironment promotes metastasis.

Poster No. 91

Growth Factor Mediated Deregulation of AKT3 in Multiple Myeloma

Eva Maizner[1], Thomas Möst[1], Karin Jöhrer[1], Johanna Parteli[1], Daniel Neureiter[2], Richard Greil[1,3]
[1]*Tyrolean Cancer Research Institute, Innsbruck, Austria,* [2]*Institute of Pathology at the Private Medical University Hospital Salzburg, Salzburg, Austria,* [3]*Laboratory of Immunological and Molecular Cancer Research, 3rd Medical Department of Oncology, Hematology, Hemostaseology, Rheumatology and Infectiology at the Private Medical University Hospital Salzburg, Salzburg, Austria*

Multiple myeloma is the second most common haematopoietic malignancy and remains incurable. The mechanism of survival

and proliferation of myeloma cells depends on the bone marrow microenvironment, the specific location where myeloma expansion occurs. Activation of growth factor pathways such as IGF-1 and IL-6 provide myeloma cell growth and drug-resistance. Recently, myeloma cells were shown to respond to IGF-1 and IL-6 via strong PKB/Akt activation. Although deregulation of Akt during myeloma tumorigenesis has been confirmed by many studies, it is currently unknown which Akt isoform is induced most frequently by growth factors.

In order to assess growth factor-induced upregulation of distinct isoforms we elucidated Akt isoform profile for the first time in multiple myeloma. Both Akt1 and Akt3 were the predominant active isoforms in myeloma cell lines. Interestingly, activated Akt3 was induced by IGF-1 or IL-6 in all tested myeloma cell lines, showing the highest response for strong Akt activation in a milieu that corresponds to the tumour microenvironment. Experiments using siRNA-induced knock down of the isoforms indicated a central role for Akt3 during myeloma cell migration and adhesion to stroma cells, highlighting for the first time a crucial implication for Akt3 during myeloma progression. Further analyses on bone marrow of myeloma patients are currently performed to elucidate the clinical rationale of distinct Akt isoforms for targeted therapeutic intervention.

Poster No. 92

Generation of Breast Cancer Cell Lines Stably Overexpressing EpCAM

Agnieszka Martowicz[1], Martin Wurm[1], Johanna Gostner[1], Florian Lehne[1], Dominic Fong[2], Guenther Gastl[2], Gilbert Spizzo[3]
[1]*Tyrolean Cancer Research Institute, Innsbruck, Austria,* [2]*Department of Haematology and Oncology, Medical University of Innsbruck, Innsbruck, Austria,* [3]*Oncology and Haematology Day Hospital, Franz Tappeiner Hospital, Merano, Italy*

The Epithelial cell adhesion molecule (EpCAM) is a calcium-independent homophilic cell adhesion molecule and is over expressed in a variety of tumours, such as breast and colon cancer. EpCAM, a cell surface antigen with oncogenic features can modulate cell-cell contacts by antagonizing E-cadherins and therefore support invasion and metastasis.

To gain insights into molecular changes following EpCAM overexpression, we decided to establish breast cancer cell line models stably overexpressing EpCAM. Therefore, two EpCAM negative human epithelial breast cancer cell lines, Hs578t and MDAMB-231 were selected. Both cell lines Hs578t and MDA-MB231 were transfected with the pIRESpuro3_EpCAM plasmid and after selection the resulting cell lines were named Hs_EpCAM and MDA_EpCAM. Cells were also transfected with the pIRESpuro3 empty vector and resulting cells were named Hs_control and MDA_control, respectively. After selection of stable lines, EpCAM gene expression was compared to that of the positive control breast cancer cell lines MCF-7 and SkBr-3. The localisation of EpCAM protein in Hs_EpCAM and MDA_EpCAM cell lines was analysed by immunofluorescence and confocal fluorescence microscopy. Expression was compared with positive controls MCF-7 and SkBr-3. Notably, cell density was very important for the localization of EpCAM. Highly dense cultures showed high membranous EpCAM staining, while cells lacking interactions with neighbouring cells exhibited weaker membrane but stronger cytosolic staining.

The findings obtained by analyzing EpCAM overexpressing breast cancer cell line models suggest that EpCAM tumour promoting function is specific for each distinct cell type and can be mediated by different strategies depending on the cellular microenvironment.

Poster No. 93

Alterations in Levels of Circulating Plasmacytoid and Myeloid Dendritic Cells in Colorectal Cancer Patients Pre and Post Surgery

Adriana Michielsen[1], Blathnaid Nolan[1], Elizabeth Ryan[2], John Hyland[1], Kieran Sheahan[1], Diarmuid O'Donoghue[1], Hugh Mulcahy[1], Jacintha O'Sullivan[1]
[1]*Centre for Colorectal Disease, St. Vincent's University Hospital, Dublin, Ireland,* [2]*Department of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland*

Introduction: Dendritic cells (DC's) are the most potent antigen presenting cells that play an important role in cancer immunity. The importance of DC's in governing response to therapies in colorectal cancer patients is unknown. Factors released from the tumour microenvironment may inhibit DC function, maturation and activation in the tissue. Circulating levels of myeloid and plasmacytoid DC's may also be affected.

Aims: The aim of this study is to assess the levels of circulating plasmacytoid DC's (pDC) and myeloid DC's (mDC) in colorectal cancer patients with different tumour staging pre-surgery and post surgery

Methods: Whole blood was obtained from 30 patients pre-surgery, 10 patients post-surgery and 11 healthy controls. Cells were stained with Lin1-FITC, CD1c-PE, CD303-APC and their corresponding isotype controls. Samples were analysed by flow cytometry and levels of plasmacytoid and myeloid DC's were measured as percentage of total cell number. Statistical analyses were performed using student t-test.

Results: Plasmacytoid dendritic cell populations were significantly lower in cancer patients compared to healthy controls ($p=0.0001$). Myeloid dendritic cell populations were also lower in cancer patients. A decreasing trend was observed in plasmacytoid DC levels with increasing stage, and this was statistically significant for stage II ($p=0.03$, $n=8$) and stage III ($p=0.004$, $n=12$) cancers. Myeloid DC numbers also showed a declining trend with increasing stage. 5 patients showed an increase in

post-surgery circulating pDC levels compared to pre-surgery. 4 additional patients showed a decrease in pDC levels post-surgery, and 1 patient had the same levels of pDC pre- and post-surgery. A similar trend was seen for the myeloid DC population.
Conclusion: Colorectal cancer patients have significant lower numbers of plasmacytoid DC, but not myeloid DC compared to healthy individuals, and interestingly, this is associated with severity of disease.

Poster No. 94

Elevated Stromal Expression of VEGF-A Correlates with Reactive Stroma Appearance in a Human Prostate Xenograft Model

Viviana P. Montecinos[1], Jennifer Hinklin[1], Alejandro Godoy[1], Claudio Morales[1], James Mohler[1], Gary Smith[1]
[1]*Urologic Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA*

Many similarities exist between the stroma at sites of wound repair and reactive stroma in cancer. Common features include an elevated stromal cell proliferation, altered expression of matrix components, expression of several common stromal markers, and neovascularization. Although emerging data points to the fundamental role that carcinoma associated stromal cells play in angiogenesis, little is known about specific mechanisms and key regulatory components in prostate cancer or other tumors. In this report, benign or malignant human prostate tissue harvested by radical prostatectomy, and transplanted into SCID mice implanted with testosterone pellets, demonstrate an explosive burst of angiogenesis by human blood vessels between Days 6–14 after transplantation. The nascent vessels exhibited alterations in structure and function similar to tumor blood vessels, and leaked serum components into the interstitial tissue space until the vessels matured by establishing interactions with pericytes. The wave of human angiogenesis was preceded by a striking increase in expression of VEGF-A in the human prostate stroma. The over-expression of VEGF-A during the initial days after tissue implantation, and the subsequent increase in microvessel density, was concurrent with the appearance of a reactive stroma phenotype, as determined using Masson's trichrome stain and immunohistochemistry analysis for the expression of α-SMA, Vimentin, Tenascin, Calponin and Desmin. These results suggest that the stromal present in the human prostate xenografts undergo activation potentially comparable to what occurs in a tumor microenvironment and suggest that VEGF-A is a candidate regulator of reactive stroma generation. A better understanding of the mechanism(s) of modulation of the human prostate stromal activation could have significant implications for more effective modeling of new forms of anti-angiogenic therapies for prostate cancer, and for developing targeted adjuvant therapies to improve the efficacy of androgen deprivation therapy.

Poster No. 95

CD44 Signaling Potentiates uPA Expression and Activity in Breast Cancer Cells

Nicola Montgomery[1], Ashleigh Hill[1], Suzanne McFarlane[1], David Waugh[1]
[1]*Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland, UK*

CD44 is a cell surface receptor for the glycosaminoglycan hyaluronan (HA). Overexpression of HA and CD44 in breast cancer correlate with poor prognosis and distant recurrence. In vitro, CD44 signaling underpins breast cancer cell invasion and cell adhesion. Initial experiments revealed that RNAi-mediated suppression of CD44 alone markedly attenuated the magnitude and rate of invasion demonstrated by MDA-MB-231 breast cancer cells through collagen-enriched matrices. Therefore, the objective of this study was to determine the proteolytic targets of CD44 signaling in breast cancer cells that assist in promoting localized invasion and intravasation. Urokinase plasminogen activator (uPA) is a serine protease whose increased activity has been implicated in the potentiation of cancer cell intravasation and whose elevated expression also correlates with poor prognosis in breast cancer. Our further experiments conducted in the invasive breast cancer cell line MDA-MB-231 demonstrates that HA-induced CD44 signaling increases the transcription of the uPA gene and that of its cell-surface expressed receptor (uPAR). Furthermore, immunoblotting confirms increased expression of uPA and uPAR in HA-stimulated MDA-MB-231 cells. Parallel assays studying the effects of HA upon the endogenous inhibitors of the uPA system, PAI-1 and PAI-2 confirm an increased expression of PAI-1 but not PAI-2 in the MDA-MB-231 cells. Further immunoblotting and substrate-based activity assays confirm that the resultant impact of HA-induced CD44-mediated signaling is to increase the cell-surface associated uPA activity in these breast cancer cells. Our continuing studies are aimed at demonstrating the link of this CD44-promoted uPA activity in underpinning the CD44-promoted invasion of collagen matrices and experimental models of cross-linked collagen-enriched basement membranes, and exploiting in vivo models to demonstrate the linkage of CD44 signaling and uPA activity to the enhanced rates of breast cancer cell intravasation.

Poster No. 96

Irradiation-Induced Changes in Metabolism and Metastatic Properties of Melanoma Cells

Birgit Mosch[1], Katrin Mueller[1], Joerg Steinbach[1], Jens Pietzsch[1]
[1]*Department of Radiopharmaceutical Biology, Forschungszentrum Dresden-Rossendorf, Institute of Radiopharmacy, Dresden, Germany*

As it is known that irradiation can influence cellular metabolism it is conceivable that it can induce metabolic changes which lead to a predisposition of certain cells to show enhanced survival, migratory activity and metastasis. The aim of this study was to investigate short term and long term irradiation effects on proliferation and metabolism of melanoma cells *in vitro* and their ability to form metastases *in vivo*.

B16-F10 melanoma cells were irradiated with different doses of X-ray irradiation in the range of 1 to 20 Gy. One, two, and three days (short term effects) and, furthermore, 7, 14 and 21 days (long term effects) after treatment cells were analyzed concerning cell growth, proliferation, viability, glucose and amino acid transport. Additionally, we performed *in vivo* studies in a syngeneic mouse model to analyze the capability of irradiated melanoma cells to form lung metastases.

The analysis of short term effects showed decreased cell growth, viability and arrest in the G2/M phase of the cell cycle while glucose transport is increased. Long term effects involve recovered proliferation, accompanied by increased glucose transport and decreased viability and amino acid transport. *In vivo* studies showed loss of metastasis immediately after irradiation and reduced metastasis if cells were allowed to recover proliferation before injection.

We conclude that melanoma cells as short term response to irradiation show cell cycle arrest and impairment in growth and viability. Three days after irradiation compensatory mechanisms start, leading to recovered growth within three weeks. Studies concerning metabolic properties indicate that a subpopulation of surviving melanoma cells compensate for the initial irradiation-induced damage possibly by metabolic modulations such as increase in glycolysis. As metastasis *in vivo* is impaired beyond recovered cell proliferation, the role of adjusted cell metabolism and additional extrinsic factors is strongly suggested.

Poster No. 97

Characterizing CXCL12-mediated Survival Signaling in Cancer

Morgan O'Hayre[1], Catherina Salanga[1], Ila Bharati[2], Jessie Fecteau[2], Thomas Kipps[2], Davorka Messmer[2], Tracy Handel[1]
[1]*Phamacology, University of California, San Diego, La Jolla, CA, USA,* [2]*Moores Cancer Center, University of California, San Diego, La Jolla, CA, USA*

Chronic Lymphoytic Leukemia (CLL) is an adult B cell leukemia with highly variable clinical prognosis. CLL is divided into two prognostic subgroups based on the expression of the tyrosine kinase ZAP-70, as high ZAP-70 (ZAP-70+) expression correlates with more aggressive disease and low/no ZAP-70 (ZAP-70-) correlates with more indolent disease. CLL cells exhibit enhanced survival properties *in vivo* yet rapidly die in cell culture. However, coculture of CLL cells with stromal associated cells called Nurse-Like Cells (NLCs) keeps the CLL cells alive in culture, suggesting that the microenvironment plays a critical role in CLL survival. One of the factors known to be secreted by NLCs that contributes to survival *in vitro* is the chemokine, CXCL12. While CXCL12 clearly enhances CLL survival, relatively little is known regarding its mechanisms of action or differences in effects on the ZAP-70 subsets. In order to elucidate the mechanisms by which CXCL12 contributes to CLL survival, we have directly probed known survival signaling pathways, e.g. Akt and ERK1/2, and used phosphoproteomics to determine novel signaling events that may be important to this process.

Our results indicate that while CXCL12 stimulates Akt and ERK1/2 activation in both CLL subgroups, the intensity and duration of activation is enhanced in the ZAP-70+ CLLs, especially for ERK1/2. Upstream signaling events of ERK1/2 also appear to be enhanced in ZAP-70+ cells. However, expression levels and turnover rates of CXCR4, the receptor for CXCL12, were not found to differ significantly between the two subgroups. Additionally, while many similar downstream targets of Akt and ERK1/2 pathways appear to be activated in both ZAP-70 subgroups, phosphoproteomics has revealed some CXCL12-stimulation targets, e.g. HSP27, that are characteristic of select patients, highlighting the underlying heterogeneity of CLL and difficulties in fully understanding its pathogenesis.

Poster No. 98

Prognostic and Response-Predicative Roles of Stromal PDGF β-receptor Expression in Human Breast Cancer

Janna Paulsson[1], Betzabe Chavez[1], Lisa Rydén[2], Tobias Sjöblom[3], Patrick Micke[3], Karin Jirström[2], Barbro Linderholm[1], Arne Östman[1]
[1]*Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden,* [2]*Department of Laboratory Medicine, Division of Pathology, Lunds Universitet, Malmö, Sweden,* [3]*Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden*

Stromal fibroblasts contribute to tumor growth and drug sensitivity. PDGF receptor signaling is important for the stromal recruitment and growth. Previous studies have suggested heterogeneity of PDGF receptor expression in tumor stroma. We therefore investigated, by immunohistochemistry, the potential prognostic and response predicative roles of stromal PDGF receptors in breast cancer.

In a population-based cohort of breast cancers we found associations between PDGF β-receptor status and clinicopathological characteristics. High stromal PDGFβ-receptor expression was significantly associated with high histopathological grade, ER negativity and high HER2 expression. High stromal PDGF β-receptor expression also correlated with significantly shorter recurrence-free and breast cancer specific survival. The prognostic significance of stromal PDGF β-receptor expression was particularly prominent in tumors from pre-menopausal women.

In an independent material, derived from a phase III study of adjuvant tamoxifen, we analyzed the response-predicative role of stromal PDGF β-receptor expression. When patients were divided according to stromal PDGF receptor expression, it was noted that the therapeutic benefit of tamoxifen was much more prominent in the group with low stromal PDGF receptor expression. These results suggest a previously unrecognized response-predicative role of stromal PDGF β-receptor in breast cancer. The mechanistic basis for this phenomenon is currently explored in co-culture experiments where the potential PDGF-dependent influence of fibroblasts on breast cancer cell sensitivity to tamoxifen is being analyzed.
In summary our studies indicated novel prognostic and response-predicative roles of stromal PDGF receptor expression, which should be explored in the continued development of PDGF receptor inhibitors and endocrine treatments.

Poster No. 99

Co-Cultured Fibroblasts Regulate Colorectal Cancer Cell Proliferation, Migration, Invasion and Cetuximab-Sensitivity in a PDGF- dependent Manner

Cristina Peña[1], Maja Bradic Lindh [1], Arne Östman[1]
[1]*Department of Pathology-Oncology, Karolinska Institutet, Stockholm, Solna, Sweden*

PDGF tyrosine kinase receptors activation has been involved in multiple aspect of cancer growth. In solid tumors PDGF receptor signaling appears to be most important for the pericytes and fibroblasts of the tumor stroma. We have developed co-culture assays to analyze the paracrine interactions between fibroblasts (PDGFR+) and colorectal cancer (CRC) cells (PDGFR-). PDGF-dependent effects of fibroblasts on the proliferation, migration, invasion and response to EGFR inhibitor (Cetuximab) of CRC cells (HT29, SW620 and LIM1215) were analyzed in different co-culture models.
PDGF stimulation of fibroblasts increased the migration and invasion of LIM1215 and HT29 CRC cells. The fibroblast-induced migration of SW620 cells, which produce PDGFs, could be blocked by PDGF receptor inhibitors targeting the co-cultured fibroblasts. Furthermore, "priming" of matrigel with fibroblasts indicated PDGF-dependent effects on the matrigel which facilitated CRC cell invasion. Finally, PDGF stimulated fibroblasts protect CRC cells from the growth- and migration-inhibitory effect of cetuximab.
Gene-expression profiling of control and PDGF stimulated fibroblasts has been performed to identify the molecular mediators of the fibroblasts-derived paracrine effects on tumor cell migration and invasion. Approximately 10 secreted proteins where found to be up-regulated in the PDGF stimulated cells. Functional studies, with antibodies or siRNA, have been initiated for a selected subset of these genes.
In summary, these studies have identified novel PDGF dependent paracrine effects on CRC cell proliferation, migration, invasion and drug sensitivity. The ongoing identification of the molecular mediators of these paracrine effects should potentially lead to novel prognostic, response-predicative and therapeutic opportunities.

Poster No. 100

Bone Marrow Derived Cells Incorporate into the Prostate During Regrowth

Veronica Placencio[1], Taylor Sherrill[3], Xiuping Yu[2], Neil Bhowmick[1,2]
[1]*Department of Cancer Biology, Vanderbilt University, Nashville, TN, USA,* [2]*Department of Urologic Surgery, Vanderbilt University, Nashville, TN, USA,* [3]*Department of Medicine, Vanderbilt University, Nashville, TN, USA*

It is necessary to understand mechanisms of androgen refractory prostate cancer development and progression. We hypothesized that enhanced chemokine signaling results in the recruitment of immune cells to the prostate microenvironment from the bone marrow. A chimeric mouse model with GFP-labeled bone marrow was used to allow us to identify bone marrow cells recruited to the prostate. We studied how bone marrow derived cells (BMDCs) contributed to an androgen refractory response, specifically prostate regrowth. In a similar mouse model we used GFP-labeled mesenchymal stem cells (MSCs) to study this specific subset of BMDCs in response to prostate regrowth. Host mice were castrated or left intact as a control. Testosterone was given to the chimeric mice. The intact and castrated control mice had a low number of BMDCs recruited to the prostate. However, three and seven days following treatment with exogenous testosterone resulted in a dramatic increase in BMDC recruitment during prostate regrowth. Immunohistochemistry staining for F4/80 suggested that some of these BMDCs were macrophage cells. GFP labeled MSC cells were also recruited to the prostate at three days following treatment with exogenous testosterone. Interestingly, even after four weeks the fully regrown prostates retained BMDCs that appeared to be incorporated in the epithelial compartment. Double immunofluorescence staining showed that a subset of BMDCs gained the expression of p63, a basal cell marker; androgen receptor and Foxa1, an endoderm marker, in the prostate. This suggested that the incorporated cells may have either differentiated or fused with resident cells. The recruitment and eventual incorporation of BMDCs in the prostate suggests a mechanism for exogenous cells in castration-resistant prostate cancer growth.

Poster No. 101

Drastic Decreased Expression of Activating Receptors on NK Cells in Human Lung Tumor Microenvironment Impairs their Cytotoxic Functions

Sophia Platonova[1], Julien Cherfils-Vicini[1], Liana Ghazarian[1], Pierre Validire[1,2], Vincent Vieillard[4], Wolf Herman Fridman[1], Catherine Sautès-Fridman[1], Diane Damotte [1,3], Isabelle Cremer[1]

[1]*INSERM U872 Team 13, Centre de Recherche des Cordeliers, Paris, France,* [2]*Pathological Anatomy Service, Intitut Mutualiste Montsouris, Paris, France,* [3]*Pathological Anatomy Service, Hôpital Hôtel Dieu, Paris, France,* [4]*INSERM U543, Paris, France*

While NK cells were originally identified by their ability to kill tumor cells in vitro, only limited information is available on NK cells present in tumor microenvironment. Our objectives were to characterize the phenotype and function of NK cells in human Non Small Cell Lung Cancers (NSCLC) patients, in tumor microenvironment, in non tumoral lung tissue, and in the blood, and to investigate the expression of NK cell receptor ligands on tumor cells.

NK cells are present both in tumoral and non tumoral lung tissues of NSCLC patients. In the tumor, they are mainly localised in the invasive margin, but outside tertiary lymphoid structures (Ti-BALT – "Tumor-induced Bronchus Associated Lymphoid Tissues") that are induced in the tumoral area. Intratumoral NK cells are not cytotoxic even after activation with IL-2, on the contrary to NK cells from blood of the same patient, despite an activated phenotype defined by NKp44 and CD69 expression. Consistent with this observation, intratumoral NK cells display a highly significant decreased expression of activating receptors such as NKG2D, NKp30, NKp80, DNAM-1 and CD16. On the contrary, NK cells from non tumoral lung tissue or blood of NSCLC patients have the same phenotype than healthy donors. Analysis of NK cell receptor ligand expression revealed that inhibitory receptors ligands such as HLA-G and HLA-E are strongly expressed by tumor cells, but not by normal tissue, whereas activating receptors ligands such as MICA/B and ULBP1, 2, 3 are rarely expressed by tumor cells.

Altogether these results demonstrate for the first time that the NK cells display an altered phenotype and function specifically in the tumor microenvironment and that tumor cells express high levels of inhibitory receptors ligands. This suggests a local induction of escape mechanisms established by tumor cells and directed towards NK cells.

Poster No. 102

Analysis of Periostin in Human Tumors: Role in Tumour Growth and Metastasis

Dodie Pouniotis[1], Wei hui Zeng[3], Alexis Desmouliere[2], Ian Darby[1]
[1]*Cancer and Tissue Repair Laboratory, School of Medical Sciences, RMIT University, Bundoora, Victoria, Australia,* [2]*Faculty of Medicine and Pharmacy, University of Limoges, Limoges, France,* [3]*Department of Dermatology, Second Hospital of Xian Jiaotong University, Xian, China*

The tumor stroma is known to interact with cancer cells to promote growth and metastasis. Preliminary data from our laboratory has identified differentially expressed proteins that are either over-expressed or under-expressed in the tumor stroma and tumoral tissue compared to surrounding 'normal' peri-tumoral tissue from the same patients with cholangiocarcinoma. A novel marker of myofibroblasts that may be involved in stimulating myofibroblast proliferation, migration and differentiation, periostin, was markedly increased in the tumour stroma of these patients. Periostin is a unique extracellular matrix protein, whose deposition is enhanced by mechanical stress and the tissue repair process. Periostin deposition in the stroma of invasive tumours has been described in the literature. Stromal cell secretion of periostin has only recently been shown to correlate with epithelial to mesenchymal transition of human pancreatic cancer cells indicating stromal cells influence on cancer development. The significance of periostin and its secretion by stromal cells in normal and neoplastic tissue has not yet been fully clarified. We assessed the expression patterns of periostin in a number of different human tumors by immunohistochemistry and showed localised expression in the tumor stroma of lung, colon, liver, renal, breast, stomach, pancreatic, thyroid, ovary, uterine, prostate and skin cancers. Interestingly, increased staining was also keen in non-neoplastic fibrotic kidney, skin and liver tissue suggesting a possible role in epithelial to mesenchymal transition in human tissue. Further investigations will be carried out to elucidate autocrine and paracrine regulation of periostin in stromal and cancerous cells using cell-based and animal-based models as well as human tissue and to further our understanding of its role in tumour growth and metastasis.

Poster No. 103

Elucidating the Role of Macrophages in Distinct Tumor Microenvironments

Stephanie Pyonteck[1,2], Bedrick Gadea[1], Hao-Wei Wang[1,2], Eric Holland[1], Johanna Joyce[1]
[1]*Cancer Biology and Genetics Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA,* [2]*Weill Graduate School of Medical Sciences, Cornell University, New York, NY, USA*

Recent research has revealed tumor-associated macrophages (TAMs) can facilitate the malignant progression of cancer, and our aim is to determine the role of TAMs in two distinct microenvironments: the brain and pancreas.

We utilize the RCAS-TVA model of gliomagenesis where somatic cell gene transfer of PDGF-B into transgenic nestin-TVA;Ink4a/ARF-/- mice induces brain tumors that recapitulate the histopathology of human glioblastoma multiforme. Using immunohistochemistry and flow cytometry we have shown that macrophages are the predominant immune cell type within gliomas and that TAM density correlates with tumor grade. Actin-GFP bone-marrow transplants have shown that glioma TAMs derive from both brain resident microglia and peripheral bone marrow-derived cells. Microglia in the contralateral hemisphere opposite the tumor are highly proliferative relative to TAMs and normal microglia. Current studies aim to deplete macrophages from gliomas to determine their role in tumor development and progression.

RIP1-Tag2 (RT2) transgenic mice express SV40-T-antigen under the control of the rat insulin promoter leading to the development of

multiple pancreatic islet tumors. To determine the role of TAMs in the pancreatic microenvironment, RT2 mice were crossed to CSF-1 null macrophage deficient mice. There is a progressive increase in macrophage density in wild-type RT2 tumors, and in line with a tumor-promoting role of TAMs, both tumor number and tumor burden are decreased in CSF-1 null RT2 mice. Histological invasion scoring has revealed a more invasive phenotype of CSF-1 null RT2 tumors relative to controls. This may be due to compensatory macrophage recruitment via a CSF-1 independent mechanism, which is under investigation.

In conclusion, while the source of TAMs may be dependent on tissue context, macrophage recruitment is a critical step in cancer development and progression in both the pancreatic and brain tumor microenvironments.

Poster No. 104

A Distinct Macrophage Population Determines Mammary Tumor Pulmonary Metastasis

Binzhi Qian[1], Jeffrey W. Pollard[1]
[1]*Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY, USA*

There is a growing appreciation of the importance of tumor-stroma interactions for tumor progression and metastasis. In the tumor stroma, macrophages are very abundant and have been shown to enhance these malignant processes. We have used an experimental metastasis assay to elucidate the significance of macrophages in promoting the two final limiting steps of metastasis: target organ seeding and persistent growth. Our data demonstrate that the pulmonary seeding and persistent growth of Polyoma virus middle T antigen induced mammary tumor cells are correlated with host colony stimulating factor 1 (the major growth factor for macrophages) gene copy number and the numbers of macrophages recruited to lung metastasis. To further determine the macrophage contributions, liposome encapsulated Clodronate was used to deplete macrophages *in vivo*; this treatment reduced the efficiency of both rate-limiting steps in the pulmonary lung metastasis assay. FACS analysis revealed a recruitment of CSF-1R+CD11b +Gr1- cells in the metastasis bearing lung. CD11b+cells were deleted in vivo with diphtheria toxin (DT) treatment in mosaic animals generated by bone marrow transplant using a transgenic mouse expressing human DTR driven by the CD11b promoter as a bone marrow donor. The deletion of CD11b+cells reduced the tumor cell seeding efficiency and growth rate in lung. Further intact lung 3D imaging study revealed that tumor-macrophage interaction is critical for tumor cell extravasation. In addition, CCL2/CCR2 signaling was found to be important for the recruitment of these macrophages and critical for tumor cell seeding. Together these data demonstrated the importance of a recruited myeloid cell population displaying a distinct phenotype in tumor cell extravasation, seeding and growth in a metastatic target organ and revealed the potential of targeting CSF-1 and CCL2 signaling in blocking metastasis.

Poster No. 105

Activity of MMP-2 and MMP-9 and their Inhibitor in Breast Cancer Tissue

Sandra Radenkovic[1], Gordana Konjevic[1,2], Katarina Karadzic[1], Momcilo Inic[1], Kristina Gopcevic[2]
[1]*Department of experimental immmunology, Institute of oncology and radiology of Serbia, Belgrade, Serbia,* [2]*Medical School University of Belgrade, Belgrade, Serbia*

Matrix-metalloproteinases (MMPs) are of essential importance for tumor cell invasion and metastasis. Two of their members, proMMP-2 and proMMP-9 are proteolytic enzymes involved in the process of tumor invasion by mediating degradation of basement membrane and remodeling of extracellular matrix. They are secreted as latent pro-enzymes (proMMP-2 and proMMP-9) which are activated by proteolytic cleavage and are inhibited by forming complexes with a class of endogenous inhibitors of MMPs, TIMPs. Imbalance between MMPs and TIMPs can lead to cancer metastasis.

We analyzed the activity of proMMP-2 and proMMP-9, as well as the activity of active MMP-2 and MMP-9 in breast cancer and surrounding tissue of 24 patients (clinical stage I and II) by gelatin zymography. In order to verify the activity of MMPs, we performed MMP inhibition test on zymography. Expression of TIMP-1 was assessed in tumor cell lysates by Western blotting using anti-TIMP-1 antibody.

The analysis of activity of ProMMP-2 and ProMMP-9 shows significantly higher activity in tumor tissue compared to surrounding healthy tissue. In our study we show that tumor tissue compared to surrounding healthy tissue of patients shows a higher activity of active forms of MMP-2 and MMP-9. Tumor tissue of patients compared to surrounding healthy tissue shows lower expression of TIMP-1, inhibitor of MMP-9 activity. We give data of enzyme and pro-enzyme higher activity of MMP-2 and MMP-9 in breast cancer tissue of patients and lower expression of TIMP-1, inhibitor of MMP-9 activity in breast cancer tissue. MMP-2 and MMP-9 activation participate in processes associated with cancer progression and understanding the processes of MMPs activation and regulation may have significant benefits in clinical interpretation. The reported higher MMP-2 and MMP-9 activity in breast cancer tissue suggests a role of MMP-2 and MMP-9 in prognostic stratification of breast cancer patients and in designing new therapeutics.

Poster No. 106

Loss of Adamts1 Protease Reduced Metastasis and Increased Apoptosis in the MMTV-PymT Mammary Tumor Model

Carmela Ricciardelli[1], Kate M. Frewin[1], Izza A. Tan[1], Elizabeth D. Williams[2], Kenneth Opeskin[3], Melanie A. Pritchard[4], Wendy V. Ingman[1], Darryl L. Russell[1]

[1]*Discipline of Obstetrics and Gynaecology, Research Centre for Reproductive Health, University of Adelaide, Adelaide, Australia,* [2]*Centre for Cancer Research, Monash Institute of Medical Research, Monash University, Clayton, Victoria, Australia,* [3]*Department Anatomical Pathology, St Vincent's Hospital, Melbourne, Victoria, Australia,* [4]*Centre for Functional Genomics and Human Disease, Monash Institute of Medical Research, Monash University, Clayton, Victoria, Australia*

Adamts1 (a disintegrin and metalloprotease with thrombospondin motifs1) is a protease known to remodel the extracellular matrix (ECM) surrounding tumors through cleavage of proteins, such as the extracellular proteoglycan versican. In breast cancers with highly elevated metastatic activity Adamts1 is found to be upregulated, and recent studies have identified Adamts1 is required for hormone mediated lymphangiogenesis in the ovary. In this study we investigated whether Adamts1 plays an essential role in mammary cancer metastasis using the MMTV-PymT mammary tumor model. Adamts1$^{-/-}$PymT mice displayed significantly reduced mammary tumor burden compared to the wildtype littermates and increased survival. Importantly the number and area of lung metastases was significantly reduced in Adamts1$^{-/-}$/PymT mice. Histological examination revealed an increased proportion of tumors with ductal carcinoma *in situ* in and a lower proportion of high grade tumors in Adamts1$^{-/-}$/PymT mice compared to Adamts1$^{+/+}$/PymT mice. The reduced tumour burden in Adamts1$^{-/-}$/PymT mice was associated with an increased apotoptic index but not associated with alterations in the proliferative index nor vascular density. Interestingly tumors from Adamts1$^{+/+}$/PymT mice had increased levels of versican compared to Adamts1$^{-/-}$/PymT mice but unaltered hyaluronan levels. Overall, this study provides strong *in vivo* evidence that Adamts1 is non-redundantly involved in breast cancer growth and metastasis. We propose that Adamts1 promotes the remodelling of peritumoral ECM facilitating the release of tumour cells from the primary tumour and their invasion into blood and lymphatic vessels for ultimate dissemination to distal sites.

Poster No. 107

A Chemokine Receptor Profile of Melanoma Brain Metastasis

Orit Sagi-Assif[1], Sivan Izraely[1], Anat Klein[1], Tsipi Meshel[1], Ido Nevo[1], Ilana Yron[1], Galia Tsarfaty[2], Dave S.B. Hoon[3], Isaac P. Witz[1]
[1]*Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel-Aviv, Israel,* [2]*Diagnostic Imaging Department, Sheba Medical Center, Tel-Hashomer, Israel,* [3]*Department of Molecular Oncology, John Wayne Cancer Institute, Saint John's Health Center, Santa Monica, CA, USA*

Brain metastasis indicates that melanoma reached its terminal stage. Since efficient therapies for brain metastasis do not exist, it is essential to identify why melanoma frequently metastasizes to the brain and identify therapeutic targets.

Chemokines, essential constituents in the immune system, attract leukocytes expressing respective receptors to insulted tissue sites. Cancer cells expressing chemokine receptors have hijacked this chemo-attraction mechanism to migrate to distant organs and form metastasis in these organs.

To identify chemokine receptors that might be involved in melanoma homing to the brain, we checked the expression of chemokine receptors in cell lines of cutaneus melanoma and melanoma brain metastasis.

Three lines of cutaneous melanoma and five lines of melanoma brain metastasis were analyzed for the expression of 19 chemokine receptors and for the expression of the cell-bound chemokine CX3CL1. Five chemokine receptors (CCR3, CCR4, CXCR3, CXCR7 and CX3CR1) and the chemokine CX3CL1 were expressed both on cultures of cutaneous melanoma and of melanoma brain metastasis.

No significant differences were measured between the expression of these chemokine receptors by the cutaneous melanomas and the melanoma brain metastasis.

Preliminary immunohistochemistry analyses performed with sections from primary cutaneous melanoma and melanoma brain metastasis confirmed the expression of these chemokine receptors in patient material.

We have at our disposal melanoma variants which grow in nude mice either as local tumors or as brain metastasis. These 2 types of variants were derived from the same patients having therefore, an identical genetic background. The chemokine receptor profile of the variants was similar to that of the local and metastatic melanoma cell cultures mentioned above.

Ongoing work focuses on the functional significance of the chemokine receptors expressed by brain-metastasizing melanoma cells.

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Poster No. 108

Prognostic Value of Angiogenic Markers in Childhood Acute Lymphoblastic Leukemia

Pascale Schneider[1,3], Odile Costa[3], Nimrod Buchbinder[1], Jean-Pierre Vannier[1,3], marc vasse[2,3]
[1]*Pediatric Hemato-Oncology, CHU Charles Nicolle, Rouen, France,* [2]*Laboratory of Hematology, CHU Charles Nicolle, Rouen, France,* [3]*Groupe de Recherche MERCI (EA 3829), Faculte de Medecine Pharmacie, Rouen, France*

The mechanisms of tumoral invasion in solid tumors are related to angiogenesis, endothelial adhesion and cell migration and similar mechanisms have been hypothesized for hemopathies, especially acute leukemia. An increased medullary angiogenesis has been observed on bone marrow biopsies of children with ALL (1). However, no correlation between angiogenesis and the prognosis of ALL has been clearly established. In our work, we focused on pro-and anti-angiogenic markers (bFGF, VEGF, endostatin) in

urine and/or plasma of 39 patients at diagnosis. Lymphoblasts mRNA expression (RT-PCR) has shown that VEGF and endostatin partially originate from lymphoblasts, whereas bFGF seems to have a stromal origin. Quantification in the supernatant of lymphoblasts confirmed these findings in half of the cases studied. Plasmatic and urinary levels of VEGF of patients were not significantly higher than in controls, but higher in relapsing patients ($p<0.006$). Patients' urinary bFGF levels were significantly higher at diagnosis than in controls (545 vs 44 pg/mmol creatinine, $p<0.005$), but interestingly bFGF levels were lower for patients with high proliferation criteria. For a small subset of patients, we cultured leukemic cells with and without fibroblasts and observed a reduction of the apoptosis rate (annexin V test), especially in patients with high urinary levels of bFGF. Among the patients with a bFGF level within the normal range, most cases showed no influence of fibroblasts on apoptosis, suggesting that a subset of leukemias with a high proliferation rate could have a growth independent of the medullary microenvironment.

(1) Perez-Atayde AR, Sallan SE, Tedrow U, Connors S, Allred E, Folkman J. Spectrum of tumor angiogenesis in the bone marrow of children with acute lymphoblastic leukemia. Am J Pathol 1997; 150:815–821.

Poster No. 109

Cellular and Molecular Interactions of Renal Carcinoma Cells with the Human Bone Marrow Microenvironment

Yvonne Schueler[1], Wilhelm K. Aicher[2], Joerg Hennenlotter[3], Gerd Klein[1]

[1]*Center for Medical Research, University of Tuebingen, Tuebingen, Germany,* [2]*Department of Orthopedic Surgery, University of Tuebingen, Tuebingen, Germany,* [3]*Department of Urology, University of Tuebingen, Tuebingen, Germany*

Bone metastasis occurs frequently in renal cell carcinoma (RCC) patients leading to excessive osteolytic lesions. There is increasing evidence that the bone marrow microenvironment plays an important role in the homing of disseminated tumor cells. However, little is known about the mechanisms leading to bone tropism. Here, we performed cell adhesion and migration assays using RCC cell lines A498 and CRL1611 and primary isolated RCCs to investigate the influence of bone marrow components on cellular functions of renal tumor cells. Cell-matrix adhesion assays revealed a strong binding of RCC cells to extracellular matrix molecules expressed in the human bone marrow including collagen type I and IV, laminin isoforms, osteopontin or tenascin-C, which were partly mediated by β1-integrins. Cell-cell adhesion assays showed a moderate binding of RCC cells to primary human osteoblasts. The attachment to stromal cell lines, however, was significantly weaker. To investigate the influence of bone marrow cells on tumor cell migration, we performed cell migration assays using conditioned media of these cells. Wound healing assays with tumor cells showed that osteoblasts, but not osteoclasts or stromal cells, secrete factors which led to faster wound closure, indicating an increased migration ability of the tumor cells. This was not affected by hydroxyurea, a cell proliferation inhibitor, indicating that these effects are due to migration. Microarrays were performed using RNA isolated from RCC cells either treated with osteoblast-conditioned or control medium. Cultivation in osteoblast-conditioned medium resulted in an up-regulation of a cancer-associated cell membrane glycoprotein which is currently analyzed in detail. These data demonstrate that RCC cells preferentially interact with osteoblasts and extracellular matrix components of the human bone marrow and show increased migration ability in response to osteoblast-derived factors suggesting a possible mechanism for facilitated homing of RCC cells into bone.

Poster No. 110

Tumor-Lymphatic Cross Talk Contributes to Tumor Progression and Invasion

Jacqueline D. Shields[1], Amine Issa[1], Iraklis C. Kourtis[1], Melody A. Swartz[1]

[1]*Institute for Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland*

Changes in the immunological equilibrium and escape from immune surveillance are critical events for the progression of a developing tumor. Likewise, tumor derived vascular endothelial growth factor C (VEGF-C) is known to stimulate lymphatics at the tumor periphery and promote metastasis to draining lymph nodes. CCL19 and CCL21 are produced by both lymphatic endothelium and reticular stroma guiding antigen presenting cells (APCs) to LN and driving co-localization of $CCR7^{+}$ APCs and naïve T cells within the lymph node. Furthermore, we recently demonstrated that tumors use autologous CCL21 secretion and lymphatic function to escape a growing tumor. To this end, we investigated how lymphatic growth factors and lymph node chemokines influence the developing tumor-lymphatic microenvironment and ensuing immune response. We engineered tumor cells to secrete different levels of CCL21 and VEGF-C. Using *in vitro* co-culture models and complementary *in vivo* studies we demonstrate that several tumor cell lines express functional VEGFR-3; hence tumor-derived VEGF-C could act autologously on tumor cells to promote their invasion through a 3D matrix, by increasing their motility and proteolytic activity. In addition to peritumoral lymphatic expansion, tumor-secreted VEGF-C also increased CCL21 production by lymphatic endothelium. Increased tumor volumes were observed in these VEGF-C-overexpressing tumors compared with control counterparts and coincided with a switch in the inflammatory compartment

towards a regulatory phenotype. A sustained loss of CCL21 at the tumor site permitted an effective tumor specific immune response to develop. These results indicate that modulation of the tumor-lymphatic microenvironment not only promotes metastasis through VEGF-C-CCL21 cross-talk strategies but is also necessary for manipulation and control of the anti-tumor immune response.

Poster No. 111

Accumulation and Role of Resident and Bone-Marrow Derived Macrophages in Glioma Pathogenesis

Malgorzata Sielska[1], Konrad Gabrusiewicz[1], Eliza Kwiatkowska[2], Dariusz Kowalczyk[2], Aleksandra Ellert-Miklaszewska[1], Joanna Kucharska[1], Bozena Kaminska[1]
[1]*Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland,* [2]*Department of Cancer Diagnostics and Immunology, Great Poland Cancer Center, Poznan, Poland*

Malignant glioblastomas are characterized by infiltration of tumour tissue with brain macrophages that may consist up to 30% of tumour mass and create specific microenvironment contributing to tumour progression. A relative proportion of brain resident and peripheral monocyte/macrophages to gliomas is poorly defined. We generated chimeric mice with the immune system reconstituted after irradiation with hematopoietic GFP-bone marrow cells. The dsRed-GL261 glioma cells were implanted to the brains of 16-weeks old C57BL/6 chimeric mice. Two weeks after implantation, tumour bearing hemispheres were isolated and the number of $CD11b^{+}CD45^{low}$ microglial cells or $CD11b^{+}CD45^{high}$ macrophages was determined by flow cytometry. The increase of the percentage of microglial cells and macrophages was observed in tumor-bearing hemispheres. We found that peripheral GFP^{+} macrophages comprise above 60% of GFP^{+} cells in the tumour. Peripheral GFP^{+} cells accumulated inside and around tumours. A co-localization of Iba-1^{+} cells (macrophages/microglia) with GFP^{+} cells has been detected by confocal microscopy. Counting of double-stained cells revealed that above 50% of Iba-1^{+} cells are peripheral macrophages. To study a functional contribution of microglia/macrophages to glioma growth, invasion and pathogenesis, we employed osteopetrotic mice (*op/op*) which possess a spontaneous mutation in the macrophage colony-stimulating factor (M-CSF/CSF-1) gene. Mice homozygous for the osteopetrosis mutation are viable but exhibit a generalized macrophage deficiency, monocytopenia, deficient microglia/macrophage responses and defective bone remodeling. The studies of growth of GFP-GL261 glioma cells in *op/op* mice will facilitate understanding of contribution of microglia/macrophages to glioma microenvironment and growth. Our studies demonstrate that in addition to accumulation of brain resident macrophages (microglia), also blood-borne macrophages migrate to the tumour and consist of a significant population of tumour-associated macrophages.

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Poster No. 112

The Effect of Human Placental Explants on Breast Cancer Cell Line MCF7; To stay or to STAT?

Shelly Tartakover Matalon[1,3], Adi Mizrahi[1,3], Gali Epstein[1,3], Amin Shneifi [2], Liat Drucker[1,3], Meir Pomeranz[4], Ami Fishman[3,4], Michael Lishner[1,2,3]
[1]*Oncogenetic Laboratory, Meir Medical Center, Kfar Saba, Israel,* [2]*Department of Internal Medicine A, Meir Medical Center, Kfar Saba, Israel,* [3]*Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel,* [4]*Department of Obstetric and Gynecology, Meir Medical Center, Kfar Saba, Israel*

Introduction: Pregnant women with breast cancer often present with an advanced disease and have decreased estrogen receptor (ER) levels. In spite of that, metastases are rarely found on the placenta which suggests that the placenta is a nonsupportive microenvironment for the cancer cells. Indeed, we have found that MCF7 cells (ER positive, breast cancer cells) when cocultured with first trimester placental explants had elevated apoptosis, G1/G0 cell cycle arrest accompanied by cell detachment and migration. The aim of the study presented here was to analyze which MCF7 signaling pathways were affected by the placenta.
Methods: MCF7-eGFP cells cultured on matrigel with or without placental explants were separated from the placenta by cell sorting and their mRNA was subjected to microarray analysis. ERa/STAT3/mTOR expression and phosphorylation levels were analyzed in these cells.
Results: Trophoblast cells differentiate into extravillous trophoblast cells (EVT) which migrate into the matrigel. The effects (apoptosis/proliferation/detachment) were mainly observed in MCF7 cells that were located near the EVT cells. Microarray results have demonstrated significant changes in genes related to cell adhesion, glycan, breast carcinoma estrogen and JAK-STAT pathways. Decreased ERa and elevated pmTOR and STAT3 proteins detected by immunobloting confirmed our findings at the transcript level. Moreover, promoter analysis of significantly affected genes in this array demonstrated an enrichment of motifs located at the transcription start site that match annotation for ISRE (interferon-stimulated response element), suggesting the activation of interferon (INF) signaling.
Conclusion: Our results suggest that the placenta attenuates MCF7 growth in its vicinity by modulating INF/STAT and inducing detachment/migration and apoptosis. Published data demonstrated that the above pathways may indeed stimulate these processes. The involvement of these signaling pathways in cell migration/apoptosis and the fate of the detached MCF7 cells will be further studied.

Poster No. 113

Identification of Secretory Stromal Gene Signature of the Ovarian Tumor Microenvironment and Implications for Ovarian Cancer Progression

Melissa Thompson[1], Lina Albitar[2], Kwong-Kwok Wong[1], Michael Birrer[3], Samuel Mok[1]
[1]*Department of Gynecologic Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA,* [2]*Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital, Boston, MA, USA,* [3]*Department of Medicine, Massachusetts General Hospital, Boston, MA, USA*

The stromal microenvironment provides structural support and myriad signaling cues, which can significantly affect cell growth and development. The tumor-associated stromal microenvironment has been shown to play a key role in many cancers by influencing tumor initiation, invasion and metastasis. Our objective was to identify specific genes that are differentially regulated in the stromal component of high-grade late-stage serous ovarian cancer that may contribute to ovarian cancer progression.
Microarray analysis of RNA isolated from 10 microdissected normal ovarian fibroblasts, and the epithelial and stromal component of 16 high-grade late stage serous ovarian cancers revealed that 40 secreted proteins are overexpressed more than 5 fold by cancer-associated fibroblasts exclusively. Quantitative RT-PCR validated the overexpression of several genes, including sFRP2, by the cancer-associated fibroblasts. Clinical data correlated stromal sFRP2 overexpression with poorer overall survival and chemoresistance in patients with high-grade late stage serous ovarian cancer, suggesting that sFRP2 promotes ovarian cancer progression.
In vitro functional studies illustrate increased ovarian cancer cell line growth in response to sFRP2.
Our results illustrate a direct and specific signaling linkage from the tumor microenvironment to tumor cells that contributes to tumor progression.

Poster No. 114

Stromal Fibroblast-Derived Periostin Promotes Cancer Progression and Serves as Diagnostic and Poor Prognostic Factors in Cholangiocarcinoma

Chanitra Thuwajit [1,7], Kusumawadee Utispan [2,7], Yoshimitsu Abiko [3], Komkrid Jarngkaew[4], Anucha Puapairoj [5,7], Siri Chau-in [6,7], Peti Thuwajit [1,7]
[1]*Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok-Noi, Bangkok, Thailand,* [2]*Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Muang, Khon Kaen, Thailand,* [3]*Department of Biochemistry and Molecular Biology, Nihon University School of Dentistry at Matsudo, Matsudo, Japan,* [4]*Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok-Noi, Bangkok, Thailand,* [5]*Department of Pathology, Faculty of Medicine, Khon Kaen University, Muang, Khon Kaen, Thailand,* [6]*Department of Surgery, Faculty of Medicine, Khon Kaen University, Muang, Khon Kaen, Thailand,* [7]*Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Muang, Khon Kaen, Thailand*

Cholangiocarcinoma (CCA) is a major health problem in Thailand. It is well recognized to contain abundant fibrous stroma with activated fibroblasts. Our group has recently isolated primary culture CCA fibroblast (Cf) from CCA tissues and revealed that Cf induced human biliary epithelial and CCA cell proliferation. However, molecular mechanism of fibroblasts in CCA remains unclear. Here, we indicated periostin (PN) secreted from cancer fibroblasts as diagnostic and prognostic factors, and had carcinogenic role in CCA. By comparing gene expression profile of Cf and non-tumorigenic liver fibroblasts, 1,466 genes were up-regulated whereas 495 genes were down-regulated in Cf. PN was verified up-regulated expression in Cf by real time PCR and western blotting. Immunohistochemistry of PN in CCA tissues (n=139) revealed that PN was solely in tumor stromal fibroblasts. More than 80% of CCA cases had low to high level of PN, but slight expression was found in benign liver tissues and hepatocellular carcinoma. The overall survival of CCA patients with high PN expression was significantly lower than those who had low level (P=0.029). Multivariate analysis indicated that high PN expression was an independent poor prognosis factor (P=0.039). Spearman correlation analysis indicated that PN mRNA level significantly correlated with PN level in tissues (P=0.045). In addition, *in vitro* study revealed that PN could induce CCA proliferation by driving cells into S+G2/M of cell cycle. Taken together, it is likely to conclude that high PN expression in CCA tissues can be used as a diagnostic factor to distinguish CCA from hepatocellular carcinoma. Fibroblast-derived PN in the tumor microenvironment may play important role in induction of cancer progression and serves as a poor prognostic factor.

Poster No. 115

Lack of the α2β1 Integrin Decreases Squamous Cell Carcinoma Metastasis in K14-HPV16 Transgenic Mice

Thuy Tran[1], Lynda O'Rear[1], Mary Zutter[1]
[1]*Department of Pathology, Vanderbilt University Medical Center, Nashville, TN, USA*

The α2β1 integrin is a heterodimeric cell surface receptor for collagen, laminin, and other extracellular matrix proteins. Expressed on the epidermal basal layer and on several inflammatory cell populations, the α2β1 integrin regulates orderly, cellular proliferation and innate and adaptive immune system function. Using the K14-HPV16 model of epithelial carcinogenesis and the α2β1 integrin-null mouse, we evaluated

the α2β1 integrin's impact on squamous cell carcinoma (SCC) development and progression. We hypothesized that the integrin plays a role in SCC pathogenesis through keratinocyte signaling within the local microenvironment or through chronic inflammation.

Our data show that loss of the α2β1 integrin in K14-HPV16 mice does not alter SCC latency, prevalence, anatomic location, or histologic grade. HPV-positive, α2β1 integrin-null animals (HPV/KO), when compared with wild-type, HPV-positive (HPV/WT) littermates, have: reduced tumor metastasis by 43%, decreased $Ki67^+$ tumor cell proliferation ($p=0.0059$), fewer tumor multiplicity, and decreased tumor, $LYVE\text{-}1^+$ lymphatic vessels ($p=0.021$). Intratumoral HPV/KO lymphatics occupy only 0.029±0.048% of the 20X field versus the 0.59 ± 0.92% seen in HPV/WT tumors ($p=0.031$). Peritumoral $LYVE\text{-}1^+$ vessel area are less in HPV/KO mice ($p=0.013$). Mast cells express the α2β1 integrin, use integrin-signaling mediated IL-6 secretion, and increase epithelial neoplastic change through inducing chronic inflammation. Mast cells are decreased ($p = 0.019$) in HPV/KO mice ears at 6-months-of-age compared to age-matched HPV/WT mice ears. Plasma IL-6 levels are decreased in HPV/KO relative to HPV/WT, tumor-bearing animals ($p=0.014$).

Our data demonstrate the α2β1 integrin plays a critical role in regulating metastasis to regional lymph nodes; decreased metastasis seen in HPV/KO mice may result from reduced lymphangiogenesis or vessel function. Future studies focus on the α2β1 integrin's role in regulating structure and function of pathologic lymphatic vessels and determining whether mast cell-lymphatic crosstalk alters lymphangiogenesis.

Poster No. 116

The Matrixmetalloproteinase-11 is Overexpressed in Tumor Blood Vessels and Supports Angiogenic Sprouting Processes and Metastasis

Gerold Untergasser[1], Johann Kern[1], Martina Zimmermann[1], Günther Gastl[1], Eberhard Gunsilius[1]
[1]*Tumorbiology and Angiogenesis Laboratory, Depart. of Internal Medicine V, Medical University Innsbruck, Innsbruck, Austria*

Angiogenesis and metastasis of tumors is strongly dependent on the expression of proteases that cleave basement membranes and extracellular matrix. Transcriptome analysis of normal and tumor blood vessels in colorectal cancer revealed an overexpression of MMP-11 in the tumor endothelium. These data could be confirmed by immunohistochemistry clearly showing immunoreactive MMP-11 in blood vessels of the tumor and fibroblasts of the reactive stroma. Adenoviral overexpression of MMP-11 did not affect proliferation of HUVECs, but significantly supported angiogenic sprouting of endothelial cell spheroids in a collagen matrix. In contrast to GFP, MMP-11 transfected cells increased cumulative sprout length and number of sprouts/spheroid.

MMP-11 overexpressing B16F10 melanoma cells were generated by the sleeping beauty transposase system and grafted into the chorioallantoic membrane (CAM) of chicken embryos. In comparison to mock-transfected cells, MMP-11 overexpressing B16F10 cells showed no increased proliferation *in vitro*, but a significant higher rate of metastasis in the chicken embryo xenograft assay. Our data support the hypothesis, that tumor endothelial cells secret MMP-11 to support angiogenic sprouting processes and metastasis of the tumor.

Poster No. 117

Matrix Metalloproteinases Impact Metastatic Growth in the Liver Microenvironments of Steatosis and Steatohepatitis

Michael VanSaun[1,2], In Kyu Lee[3], Lynn Matrisian[1], Lee Gorden[1,2]
[1]*Department of Cancer Biology, Vanderbilt University, Nashville, TN, USA,* [2]*Department of Surgery, Vanderbilt University, Nashville, TN, USA,* [3]*Department of Surgery, The Catholic University of Korea, St. Mary's Hospital, Yeongdeungpo-gu, Seoul, Korea Republic*

Non-alcoholic fatty liver disease (NAFLD), encompassing steatosis and progression to non-alcoholic steatohepatitis (NASH) are liver disorders of increasing clinical significance. We hypothesize that steatosis and steatohepatitis establish early permissive microenvironments for metastatic seeding and tumor progression in the liver. Specifically, we hypothesize that MMP12 (macrophage metalloelastase) and MMP13 (collagenase-3) are important regulators of tumor growth in the setting of NAFLD. MMP12 can process latent TNF alpha and it is important for macrophage migration and immune-mediated injury response. MMP13 can cleave fibrillar collagens and is potentially involved in collagen remodeling of fibrotic liver disease associated with NAFLD. Mice in the C57Bl/6 background were fed a 42% fat diet for three months to induce hepatic steatosis. Affymetrix microarray analysis was performed on steatotic vs. normal liver to determine candidate genes altered between these liver microenvironments. Of 715 significant changes noted in gene expression, the matrix metalloproteinases MMP12 and MMP13 were the most significantly upregulated genes in the steatotic microenvironment. MMP12 as well as MMP13 deficient mice both developed high fat diet induced hepatic steatosis. To determine whether these MMPs affected metastasis, normal and steatotic MMP deficient mice underwent splenic injection experimental metastasis assays. Preliminary data showed similar results between wildytpe and MMP13 deficient mice. However, loss of MMP12 resulted in decreased number of metastases compared to wildtype for steatotic livers. Conclusions: Modulation of host factors is known to be important in tissue/site specific susceptibility to cancer metastases. The matrix metalloproteinases 12 and 13 were upregulated in the condition of hepatic steatosis and MMP12 was found to effect the establishment of metastatic tumors in this permissive microenvironment. Improved understanding of alterations to host factors in

the setting of NAFLD and their mechanisms of action may lead to a better understanding of microenvironmental host response to metastasis and tumor progression.

Poster No. 118

Characterization of CD90-positive Cells in the Peripheral Blood of Tumor Patients

Kathleen Wagner[1], Klaus Höffken[1], Joachim H. Clement[1]
[1]*Deptartment of Haematology, Oncology, Bone Marrow Transplantation, Clinic for Internal Medicine II, University Clinic Jena, Jena, Germany*

Aims: Interactions between epithelial tumor cells and the surrounding milieu are an essential regulatory component of tumor development. Especially the contribution of the crosstalk between epithelial tumor cells and tumor-associated fibroblasts (TAFs) on tumor progression and metastasis formation is of emerging interest. Therefore, we ask whether circulating TAFs could be detected in the peripheral blood of tumor patients.
Methods: CD90 (Thy-1) is a putative marker of TAFs. A fluorescence-scanning-cytometer (scan^R) was used to detect and quantify vital CD90-positive cells in blood samples from individual tumor patients. For further analysis CD90-positive cells were separated from leukocyte fractions pooled from different tumor patients using an immunomagnetic cell separation technology (ROBOSEP®). The CD90-positive fraction was subsequently analyzed by immunofluorescence and immunohistochemistry.
Results: In cell culture experiments we established CD90 as a highly specific marker for fibroblasts. The amount of CD90 positive cells in unseparated blood samples varied from 0 up to 54,000 cells/ml and changes over time. The CD90-positive cells were enriched immunomagnetically from the leukocyte fraction pooled from tumor-patients. By immunofluorescence we approved the cell vitality and verified that the separated cells do not belong to the sub-population of CD34-positive blood stem cells. Up to now more than 300 patients with solid tumors (e.g. breast, bladder, kidney) were tested for the presence of CD90-positive cells. Further analysis showed the expression of CD29 which is involved in metastasis formation and CD105 a marker for activated fibroblasts in a subset of the CD90-positive cell fraction.
Conclusions: We could show for the first time that CD90-positive cells circulate in the peripheral blood of breast cancer patients. Ongoing studies should evaluate their suitability as diagnostic or prognostic factor.

Poster No. 119

VEGFR1 Expression by Bone Marrow-Derived Myeloid Cells Mediates Tumor Metastasis via Suppression of Anti-angiogenic Factors

Jared Wels[1], Maria Rosario Andre[1], Selena Granitto[1], Rosandra N. Kaplan[1,2], Beth Psaila[1], John Lawrence[1], Stefano Rivella[1], Shahin Rafii[1], David Lyden[1,2]
[1]*Cell and Developmental Biology, Weill Cornell Medical College, New York, NY, USA,* [2]*Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

VEGF receptor 1 (VEGFR1) expression by bone marrow-derived cell (BMDC) populations associated with primary tumors as well as the metastatic microenvironment has been reported[1,2]. This receptor has been used to describe cell populations of myeloid progenitor or monocyte/macrophage lineage with pro-angiogenic and metastatic function. However, the role of VEGFR1 activity in these contexts remains unclear. In the present study, we tested the effect of lentiviral-mediated knockdown of VEGFR1, specifically within BMDCs, on the development of spontaneous metastases. We report that downregulation of VEGFR1 expression in the bone marrow had a modest effect on primary B16 subcutaneous tumor growth and subsequent tumor cell seeding at early-metastatic sites, yet drastically reduced the occurrence of micro- and macro-metastatic foci. Microarray analysis of RAW 264.7 monocyte/macrophages transduced with VEGFR1 shRNA showed the upregulation of key anti-angiogenic factors, including CXCL4 (platelet factor-4) and pigment epithelial derived factor (PEDF). Upregulation of these factors was drastically enhanced in VEGFR1-deficient RAW cells and primary bone marrow-derived myeloid cells lacking VEGFR1 when co-cultured with B16 tumor cells. Functional analyses of VEGFR1-deficient BMDCs indicate these cells inhibit endothelial cell survival *in vitro*. Additionally, co-injection of VEGFR1-deficient myeloid cells with B16 tumor cells suppressed subcutaneous tumor growth due to apparent defects in functional vessel formation. These novel findings indicate that VEGFR1 expression controls the angiogenic activity of tumor-associating myeloid cells by suppressing the expression of potent angiostatic chemokines and that blocking this pathway can significantly inhibit tumor metastasis. Our results clearly demonstrate a functional role for VEGFR1 expression within BMDCs in promoting metastatic progression by mediating an angiogenic microenvironment.
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2 Lin, E. Y. et al. VEGF Restores Delayed Tumor Progression in Tumors Depleted of Macrophages. Mol Oncol 1, 288–302, (2007).

Poster No. 120

Blood Serum Concentration of sHLA-G and sRCAS1 in Women Treated for Gynecological Malignancies as Indicators of the Status of the Tumor Microenvironment

Pawel Blecharz[1], Pawel Mach[1], **Lukasz Wicherek[1]**
[1]*Department of Gynecology, Obstetrics and Oncology, Jagiellonian University, Krakow, Poland*

Background:
The suppression of the immune system is a crucial event in the development of malignancy, particularly in cases of cancer relapse. It

has not, however, been common practice to evaluate the suppressive influence of cancer cells on the immune system, even though the soluble forms of RCAS1 and HAL-G can be detected in the blood serum of patients suffering from gynecological malignancies, and elevated levels seem to be related to cancer progression. Certainly, the participation of both these proteins in inhibiting the cytotoxic immune response has been well documented. In our study, we took serial measurements of the levels of both proteins over the course of the applied therapy in order to determine their usefulness for revealing the relationship between the applied therapy and the size and degree of the tumor suppressive environment.

Methods:

We measured both the sRCAS1 and sHLA-G blood serum concentration levels in a group of 85 patients treated for gynecological malignancy. The group included 38 patients with ovarian cancer, 33 with endometrial cancer, and 14 with uterine cervical carcinoma. We assessed the levels of these proteins using ELISA Kits through a series of measurements taken before and after surgery.

Results:

In patients with both ovarian and endometrial carcinomas, the blood serum concentration levels of both sRCAS1 and sHLA-G were found to be statistically significantly higher before surgery when compared with the levels following surgery. In the patients treated surgically due to cervical carcinoma, the blood serum concentration level of sRCAS1 was statistically significantly higher before treatment as compared to after. No such differences, however, were observed in the sHLA-G blood serum concentration levels of the women in this group.

Conclusion:

The detected levels of the blood serum concentration of sRCAS1 and sHLA-G may prove to be useful indicators of the status of the tumor microenvironment.

Poster No. 121

The Unique Cadherin Switch in Ovarian Tumor Progression

Natalie Aizenberg[1], Shmuel Argov[2], Benjamin Piura[3], Ilana Yanai-Inbar[2], Elroei David[1], Marina Wolfson[1]
[1]*The Shraga Segal Department of Microbiology and Immunology, Ben Gurion University of the Negev, Beer-Sheva, Israel,* [2]*Department of Pathology, Soroka University Medical Center, Beer-Sheva, Israel,* [3]*Gynecologic Oncology Unit, Soroka University Medical Center, Beer-Sheva, Israel*

Tumor progression to a metastatic stage is accompanied by profound changes in tumor cell phenotype. Tumor microenvironment plays an important role in this process by regulating tumor cell gene expression by variety of soluble and cell-associated molecules. Cells in primary tumor dissemble established cell-cell junctions, become more migratory, release tissue-remodeling enzymes, and make new cell-cell contacts forming secondary tumor masses (metastases). In epithelial tumors, these changes are referred as epithelial-mesenchymal transition (EMT). Cadherins, transmembrane proteins responsible for cell-cell interactions, play a central role in EMT. Switch from E-to-N-cadherin in EMT has a profound effect on tumor cell phenotype and behavior.

Here we described the unique pattern of cadherin switch in ovarian tumors, namely, N-to-E-cadherin. Immunohistochemical staining of 80 cases of ovarian primary tumors and their metastases demonstrated that (i) primary tumors expressed either N- or E-cadherin; (ii) N-cadherin expression was dependent on differentiation state of the tumor: N-cadherin in well-differentiated ovarian tumors was replaced by E-cadherin in poorly differentiated tumors; (iii) ovarian tumor metastases expressed exclusively E-cadherin.

To further investigate the role of E-cadherin in development of metastatic phenotype, we expressed a full length E-cadherin cDNA in E-cadherin-negative SKOV3 human ovarian carcinoma cells. Several E-cadherin expressing clones were studied as an *in vitro* model of ovarian tumor metastases. E-cadherin expression resulted in more aggressive phenotype characterized by new adhesion properties, higher migration and invasion potential, increased proliferative capacity and resistance to taxol (anti-cancer drug used in ovarian cancer therapy).

We conclude that ovarian tumor progression is associated with mesenchymal-epithelial transition, namely, with N-to-E-cadherin switch. Given that expression of cadherins could be transcriptionally and epigenetically regulated by various microenvironmental signals, these results suggest the crucial importance of microenvironment in ovarian tumor progression.

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Poster No. 122

A "Go or Growth" Model Based on Cell-Cell Interactions in Brain Tumours

Mathilde Badoual[1], Christophe Deroulers[1], Basile Grammaticos[1]
[1]*Physics, Paris 7 Diderot University, Paris, France*

Glioblastomas are malignant brain tumours associated with poor prognosis, due to the capacity of glioma cells to invade normal brain tissue.During their migration, cancerous astrocytes interact with other cancerous cells (homotype interactions) as well as with normal motionless astrocytes (heterotype interactions), in particular through gap junctions. These interactions appear to strongly influence the migration of glioma cells. We have developped a cellular automaton where the strength of each type of interaction is ajustable, in order to describe the migration of glioma cells.

From this automaton, we were able to derive a macroscopic diffusion equation, where the diffusion coefficient is original compared to other classical models, as it is non linear. We show that the inhibition of homotype gap junctions leads to the increase of cell migration, whereas when both homotype and heterotype gap junctions are involved, their inhibition leads to a reduction of migration. This result is in agreement with experimental data [5]. Our model also accounts for the variability of the expression of connexin 43 (the major junctional protein in astrocytes) through

different tumours. We suggest that the various migrating behaviours observed among cells in a tumour correspond to different expressions of connexin 43 and we propose a model for the "go or grow" hypothesis, based on a differential connexin 43 expression.
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Poster No. 123

Nerve Growth Factor-Expressing Stromal Cells in the Microenvironment of Hepatic Colorectal Carcinoma Metastasis: Clinical Occurrence and Functional Implicationsin Preclinical Models

Felisa Basaldua[1], Aritz Lopategi[1], Beatriz Arteta[1], Andrés Valdivieso[2], Jorge Ortiz de Urbina[2], Fernando Vidal-Vanaclocha[2]
[1]*Department of Cell Biology and Histology, Basque Country University School of Medicine, Leioa, Bizkaia, Spain,* [2]*Hepatobiliar Tumor Surgery Sevice, Cruces Hospital, Cruces-Baracaldo, Bizkaia, Spain*

Nerve growth factor (NGF) is increased during hepatic regeneration and carcinogenesis, but its role during hepatic metastasis is unknown. A tissue-array collection of metastases from 24 patients who had undergone hepatic excision of colorectal adenocarcinoma metastases was used to investigate NGF and neurotrophin receptor expression by cancer and stromal cells. NGF immunostaining of cancer cells only occurred in 2 out of 24 patients with hepatic metastases, while around 80% of patients had metastases with NGF-expressing stromal cells. Conversely, high affinity TrkA neurotrophin receptor immunoreactivity was mainly concentrated in cancer cells, with low expression occurring in tumor stroma. However, NGF immunostaining of tumor stroma and cancer cell immunostaining with anti-ki67 antibodies did not correlate, suggesting that NGF was not associated to metastatic cell proliferation. Anti-alpha-smooth muscle actin antibodies revealed that majority of metastasis-associated NGF-expressing cells had a myofibroblast phenotype. Interestingly, NGF immunoreactivity was unequivocally localized to desmin-expressing hepatic stellate cells (HSC) —prototypic myofibroblast precursors—, and peri-metastatic hepatocytes, located at the invasion front of metastases. NGF-expressing hepatocytes had phenotypic features suggesting epithelial-to-mesenchymal transition. A similar immunostaining pattern was reproduced in the experimental hepatic colonization of C26 and 51b colorectal cells. Consistent with in situ findings, NGF increased by two-fold in the hepatic blood from metastasis-bearing mice. NGF also significantly increased in the supernatant of both HSC given tumor cell-conditioned medium(CM),and hepatocytes given tumor-activated HSC-CM, but not tumor cell-CM. Recombinant NGF dose-dependently increased chemotactic migration, but not proliferation and adhesion of neurotrophin receptor-expressing tumor cells in vitro. HSC migration-stimulating activity of VEGF and tumor-activated hepatocytes was also NGF-mediated as shown with anti-NGF antibodies. Our results demonstrate that hepatocyte- and HSC-derived myofibroblasts secrete NGF in the hepatic metastasis microenvironment of colorectal carcinoma and suggest that NGF contributes to hepatic metastasis development through the specific activation of tumor and stromal cell migration.

Poster No. 124

Transcript Profiling for Epithelial - Mesenchymal Transition (EMT) Search for EMT Signature and Validation on Clinical Samples

An De Bondt[2], **Thierry Grand-Perret[1],** Janine Arts[1], Tamara Geerts[1], Lutgart Janssen[1], An Boeckx[1], Nele Vloemans[1], Ilse Van den Wyngaert[2], Willem Talloen[3], Hinrich Göhlmann[2], Pieter J. Peeters[2]
[1]*Oncology Discovery, Ortho Biotech Research & Development, a division of Janssen Pharmaceutica NV, Beerse, Antwerpen, Belgium,* [2]*Functional Genomics and Molecular Profiling, Johnson & Johnson Pharmaceutical Research & Development, a Division of Janssen Pharmaceutica NV, Beerse, Antwerpen, Belgium,* [3]*Nonclinical Biostatistics, Johnson & Johnson Pharmaceutical Research & Development, a Division of Janssen Pharmaceutica NV, Beerse, Antwerpen, Belgium*

Background:
Patient stratification becomes increasingly important for metastatic cancer treatment. Initiation of metastasis involves invasion and increased cell motility, which has many similarities to Epithelial-Mesenchymal-Transition (EMT), including a loss of cell-cell adhesion mediated by E-cadherin down-regulation.
Aim:
The aim of this study is to identify a set of genes that could be a biomarker for metastatic risk to be used on tumor biopsies. More specifically, a gene expression signature discriminating epithelial from mesenchymal cell phenotypes.
Methods:
First we have focused on known genes related to EMT based on literature. Second, we investigated whether we could identify another unbiased set of genes, solely based on expression data of cell lines, which can discriminate epithelial from mesenchymal cells. A refined principle component analysis, based on this subset of genes, identifies the weight of each gene in this signature. Taking these weights together with their expression levels make up a so-called composite gene expression measure. This has been applied to data from clinical samples.
Results:
Identification of affected biological processes when comparing epithelial with mesenchymal cell lines confirmed that cell-cell adhesion is highly affected. Analysis on gene level revealed that a set of 24 genes could clearly discriminate epithelial from mesenchymal cell lines. The identified composite gene expression measure clearly subdivided expression data from clinical samples

in 2 groups. Moreover, the composite gene expression measure showed a correlation with the pathological grade available for the clinical samples.

Conclusion:

This 24-gene signature revealed that clinical samples consisted of two distinct subpopulations. This suggests that the composite gene measure may predict whether a patient biopsy is enriched with epithelial or with mesenchymal cells. It could also give an idea of pathological grade of the sample making this signature a potential biomarker for patient stratification allowing personalized therapy.

Poster No. 125

Loss of R-Cadherin Facilitates Mammary Tumor Progression and Metastasis

Rachel Hazan[1]
[1]*Pathology, Albert Einstein College of Medicine, Bronx, NY, USA*

The mammary epithelium is thought to be stabilized by cell-cell adhesion mediated mainly by E-cadherin. Here we show that another cadherin, Retinal (R)-cadherin, is critical for maintenance of the epithelial phenotype. R-cadherin is expressed in non-transformed mammary epithelium but absent from tumorigenic cell lines. In vivo, R-cadherin was prominently expressed in the epithelium of both ducts and lobules. In human breast cancer, R-cadherin was downregulated with tumor progression, with high expression in ductal carcinoma in situ and reduced expression in invasive duct carcinomas. By comparison, E-cadherin expression persisted in invasive breast tumors and cell lines where R-cadherin was lost. Consistent with these findings, R-cadherin knockdown in normal mammary epithelium stimulated invasiveness and disrupted formation of acini despite continued E-cadherin expression. Conversely, R-cadherin overexpression in aggressive cell lines induced glandular morphogenesis and inhibited invasiveness, tumor formation, and lung colonization. R-cadherin also suppressed the MMP1, MMP2, and Cox 2 gene expression, associated with pulmonary metastasis. The data suggest that R-cadherin is an adhesion molecule of the mammary epithelium that acts as a critical regulator of the normal phenotype. As a result, R-cadherin loss contributes to epithelial suppression and metastatic progression.

Poster No. 126

Paradoxical Effect of MUC1/G-TRUNC Expression in Breast Cancer – Metastatic Phenotype Associated with Tumor Abrogation

Galit Horn[1,2], Avital Gaziel[1,2], Daniel H. Wreschner[1], Marcelo Ehrlich[1], Nechama I. Smorodinsky[1,2]
[1]*Department of Cell Research and Immunology, Tel-Aviv University, Tel-Aviv, Israel,* [2]*The Alec and Myra Marmot Hybridoma Unit, Tel-Aviv University, Tel-Aviv, Israel*

MUC1 is a prominent marker of breast cancer cells endowed with signal transduction potential due to its cytoplasmic domain. In the cell MUC1 undergoes alternative splicing and proteolytic processing which may lead to both membrane bound and secreted forms. Recent studies have identified the cleavage of the cytoplasmic tail of MUC1, which generates a truncated membrane bound form, as an important event in its signal transduction. In order to study the signaling potential of MUC1 devoid of a cytoplasmic tail in the establishment and maintenance of the tumorigenic phenotype we have generated MUC1/G-TRUNC, a truncated genomic fragment of the human MUC1, which encodes for both a truncated transmembrane form and a secreted form. To identify and dissect the function of different structural features of this construct, we generated additional MUC1 constructs, endowed with or devoid of a cytoplasmic tail, either as genomic fragments or cDNA. All constructs were transfected into DA3, highly malignant mouse mammary tumor cells. Only cells transfected with MUC1/G-TRUNC differed morphologically and phenotypically from parental DA3. Thus, presence of both truncated and secreted forms of MUC1 leads to the potentiation of *in-vitro* measured tumorigenic parameters and epithelial to mesenchymal transition (EMT). DA3/G-TRUNC cells demonstrate ERK-dependent increased spreading on fibronectin, and PI3K-dependent enhanced proliferation. In spite of the enhanced transformation of DA3/G-TRUNC in culture, and the maintenance of their tumorigenic phenotype in immuno-compromised mice, these cells fail to grow when implanted in immuno-competent mice unlike all other DA3 based cell lines. This suggests a tumor abrogation mechanism dependent on T-cells and on the interaction with the host microenvironment. Different molecular forms of MUC1 generated through genetic or proteolytic means may serve as a phenotype-determining regulatory mechanism. The role of cellular context and tumor microenvironment concomitantly determines the readout of the activation of specific signaling pathways.

Poster No. 127

3D Collagen Type I Matrix Protects Tumor Cells Against the Antimigratory Effect of Doxorubicin

Emilie Millerot-Serrurot[1], Wojciech Witkowski[1], Marie Guilbert[1], Georges Said[1], Laurence Schneider[1], Jean-Marie Zahm[2], Roselyne Garnotel[1], **Pierre Jeannesson**[1]
[1]*University of Reims, MEDyC CNRS UMR 6237, Reims, France,* [2]*Hôpital Maison Blanche, INSERM UMRS903, Reims, France*

The cell microenvironment, especially extracellular matrix (ECM) proteins is considered to play an important role in the tumor cell response to chemotherapeutic drugs. We have previously reported that the highest non toxic dose of the antracycline drug, doxorubicin, displays a marked antimigratory effect on human fibrosarcoma HT1080 cells when cultured in a conventional way, on tissue culture plastic (Int J Oncol. 2004; 24: 1607–15), which was not observed when cells were grown on ECM proteins (Cancer Sci. 2008; 99: 1699–705). The present study was designed to investigate whether the cell microenvironment can influence the antiinvasive effect of doxorubicin when these tumors cells are grown in a 3 dimensional (3D) context which simulates a natural microenvironment. For this purpose, we define migratory

parameters by time-lapse videomicroscopy, the integrin expression, and the activation state of FAK and GTPase RhoA, two proteins involved in the formation of focal adhesion complexes and cell movement. In 3D matrix, the highest non toxic dose of doxorubicin does not affect cell migration and cell trajectories. Concerning the integrin expression, and the activation state of FAK and GTPase RhoA, protectory effect of microenvironnement was also observed. In conclusion, this in vitro study demonstrates the lack of antiinvasive effect of anthracyclines in a 3D environment which is generally considered to better mimic the phenotypic and morphological behaviour of cells in vivo. Consistent with the previously shown resistance to the cytotoxic effect in 3D context, our results shed more light on the importance of the matrix configuration on the tumor cell response to antiinvasive drugs.

Poster No. 128

PPAR-g Ligands Inhibit Acquisition of Mesenchymal Phenotype During Epithelial-mesenchymal Transition

Ajaya Kumar Reka[1], Jun Chen[1], Bindu Kurapati[1], **Venkateshwar Keshamouni[1]**
[1]*Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, University of Michigan, Ann Arbor, MI, USA*

Tumors cells acquire metastatic capabilities by undergoing epithelial-mesenchymal transition (EMT). In lung cancer cells, we demonstrated that TGF-b-induced EMT confers a migratory and invasive phenotype in-vitro and promotes metastasis in-vivo. We have also shown that activation of nuclear hormone receptor, peroxisome proliferator activated receptor (PPAR)-g with its ligands, inhibits the growth and metastasis of lung cancer cells. Many pathways have been implicated in PPAR-g mediated inhibition of tumor progression, but the mechanisms by which PPAR-g activation may inhibit metastasis are not clear. Here we tested the hypothesis that PPAR-g activation may inhibit EMT contributing to its anti-metastatic effects. Activation of PPAR-g by synthetic ligands or by a constitutively active form of PPAR-g, did not prevent TGF-β-induced E-cadherin loss or the fibroblastoid morphology. However, the induction of mesenchymal markers (vimentin, N-cadherin) and MMPs by TGF-b were significantly inhibited. Consistently, activation of PPAR-g also inhibited EMT-induced migration and invasion of A549 cells. It has been shown that Zinc finger E-box binding homeobox 1 (Zeb1) regulates EMT by repressing epithelial gene expression and inducing mesenchymal gene expression. Here we demonstrate that activation of PPAR-g inhibits TGF-b-induced Zeb1 expression but had no effect on TGF-b-induced Smad phosphorylation or expression. Furthermore, effects of PPAR-g ligands on Zeb1, vimentin and MMP expression were attenuated by siRNA mediated knockdown of PPAR-g indicating above responses are PPAR-g dependent. Together our data demonstrates that PPAR-g activation regulates EMT by selective inhibition of mesenchymal phenotype partly through inhibition of Zeb1 expression and offers PPAR-g ligands as potential therapeutics against tumor metastasis.

Poster No. 129

Up-Regulation of Protease-Activated Receptor-1 (PAR-1) by Galectin-3 via AP-1 Activation in Human Gastric Cancer

Seok-Jun Kim[1,2], Ji-Young Shin[1], Kang-Duck Lee[1], Jae-Yeol An[3], Il-Ju Choi[1], Kyung-Hee Chun[1]
[1]*Gastric cancer Branch, Division of translational & clinical research I, National Cancer Center Institute and Hospital, Goyang-si, Gyeonggi-do, Korea Republic,* [2]*Department of Biological Science, Sungkyunkwan University, Suwon-si, Gyeonggi-do, Korea Republic,* [3]*School of Medicine & Dental Institute, University of London, London, UK*

PAR-1 has been studied to play a significant role in cancer metastasis. PAR-1 is activated by thrombin and initiates the signal transduction across the membrane to activate intracellular G proteins, which regulate pathways for cell migration and adhesion. The expression of PAR-1 was also reported about the association with gastric cancer progression, however the regulation mechanism (s) of PAR-1 is still unclear. Here, we demonstrated galectin-3 regulates the expression of protease-activated receptor1 (PAR1), which promotes gastric cancer cell migration through its activation. Galectin-3, a member of the β-galactoside-binding proteins, is also involved in tumor metastasis but its roles also need to study.
When the expression of galectin-3 was knock-downed by small interfering RNA (siRNA), the decrease of PAR-1 expression was detected in MKN-28 gastric cancer cells. Not only PAR1 expression, galectin-3 siRNA treatment also reduced MMP-1 and PAR-1 target genes such as MMP-2 and MMP-9. Down-regulation of both of galectin-3 and PAR-1 by its siRNA resulted in decrease of cell migration and change of cell morphology to round shape. Over-expression of galectin-3 showed the increased PAR-1 expression and cell migration. However, its increasing induced by over-expression of galectin-3 was blocked by PAR-1 silencing, suggesting that galectin-3 promotes cell migration through PAR-1 up-regulation. To determine how galectin-3 modulates PAR-1 expression, we found out the expectation site of AP-1 binding on PAR-1 promoter and detected the interaction with galectin-3 and c-jun/fra-1. After galectin-3 silencing, c-jun and fra-1 could not bind on PAR-1 promoter by ChIP assay.
Taken together, we suggest that galectin-3 increases cell motility through up-regulation of PAR-1 expression, and galectin-3 can serve as potential target molecule in the prevention and/or therapy of gastric cancer metastasis.

Poster No. 130

RECK Restoration by Targeting Histone Deacetylase Blocks Hypoxia-Induced Migration and Invasion of Cancer Cells

Hye Won Jeon[1], Sun Hee Lee[1], **You Mie Lee[1]**
[1]*School of Life Sciences and Biotechnology, College of Natural Sciences, Kyungpook National University, Daegu, Korea Republic*

Hypoxia is a strong signal for cell migration and invasion in cancer. The reversion-inducing cysteine-rich protein with Kazal motif (RECK), a tumor suppressor, inhibits cancer cell migration and

invasion and is frequently silenced in aggressive tumor cells by histone deacetylases. However, the effect of RECK silencing in several cancer cells in a hypoxic microenvironment has not been fully identified. Here we investigated that hypoxia suppresses RECK expression and restoration of RECK by using the strategy of HDAC inhibition inhibits cancer cell migration and invasion. HDAC inhibitors including trichostatin A (TSA) completely restored RECK expression suppressed by hypoxia in the H-Ras MCF10A cell line (human breast cancer) and the HT1080 cell lines (human fibrosarcoma). TSA suppressed the activity of MMP-2 and MMP-9 induced by hypoxia and significantly inhibited the hypoxia-stimulated migration and invasion of both cancer cells. RECK overexpression significantly inhibited the hypoxia-induced migration and invasion, suggesting the inhibitory role for RECK in hypoxic conditions. We also demonstrate that silencing of HDAC1 using small interfering (si) RNA suppressed hypoxia-induced RECK downregulation. In conclusion, the inhibition of HDAC successfully restored the expression of RECK under hypoxic conditions. This resulted in the inhibition of cancer cell migration and invasion through the repression of MMP-2 and MMP-9 activity.

Poster No. 131

Probing the Role of E-cadherin in Metastasis Using Real-Time Protein Modulation and Intravital Imaging

Hon Leong[1], Shruti Nambiar[1], Balaji Iyengar[1], Ann F. Chambers[1], John Lewis[1]
[1]*Oncology, London Regional Cancer Program, London Health Sciences Centre, London, ON, Canada*

The ability of tumor cells to migrate, invade and intravasate requires the deregulation of interactions with adjacent cells and the extracellular matrix. A major challenge of cancer biology is to observe the dynamics of the proteins involved in this process in their functional and physiologic context. To address this, we developed an E-cadherin chimera fused to both GFP and a FKBP-destabilization domain (DD) that constitutively targets the protein for proteasome degradation until stabilized by SHIELD-1, a small molecule that binds reversibly to the DD. This approach allows one to dynamically modulate E-cadherin activity at the post-translational level by varying the levels of SHIELD-1. Using the highly metastatic MDA-MB-231-LN cell line, we demonstrate that in the absence of SHIELD-1, E-cadherin is observed only in punctate cytoplasmic vacuole pools that co-localize with 20S proteasome. Within 30 minutes of SHIELD-1 treatment, a shift in localization to the plasma membrane is seen with concurrent formation of cell-cell adherens junctions. SHIELD-mediated induction of E-cadherin significantly reduces cell migration and invasion compared to un-induced MDA-MB-231LN cells expressing the E-cadherin chimera and vector control MDA-MB-231LN cell line. Using an experimental *in vivo* human breast cancer metastasis model in the chick embryo, we observe retraction of cell protrusions and formation of adherens junctions in MDA-MB-231LN cells with SHIELD-1 treatment. Moreover, cells transform from a spindle-shaped morphology into a rounded morphology, resembling a mesenchymal-to-epithelial morphological transition. Using this dynamic protein modulation strategy with intravital imaging, we will be able to quantify the impact of dynamic E-cadherin modulation *in vivo* during each rate-limiting step of metastasis.

Poster No. 132

Hyperoxic Treatment induces Mesenchymal-to-Epithelial Transition in a Rat Adenocarcinoma Model

Ingrid Moen[1], Anne M. Øyan[2,3], Karl-Henning Kalland[2,3], Karl Johan Tronstad[1], Lars A. Akslen[2,4], Martha Chekenya[1], Per Øystein Sakariassen[1], Rolf K. Reed[1], Linda EB Stuhr[1]
[1]*Department of Biomedicine, University of Bergen, Bergen, Norway,* [2]*The Gade Institute, University of Bergen, Bergen, Norway,* [3]*Department of Microbiology, University of Bergen, Bergen, Norway,* [4]*Department of Pathology, University of Bergen, Bergen, Norway*

Background: Tumor hypoxia is considered to be relevant for several aspects of tumor pathophysiology, for tumor growth and progression, and epithelial to mesenchymal transition (EMT). We now report that hyperbaric oxygen (HBO) treatment induced mesenchymal to epithelial transition (MET) in a dimetyl-α-benzantracene induced mammary rat adenocarcinoma model, and the MET was associated with extensive coordinated gene expression changes and less aggressive tumors.
Methods: One group of tumor bearing rats was exposed to HBO treatment (2 bar, pO_2 = 2 bar, 4 exposures à 90 minutes), whereas the control group was housed under normal atmosphere (1 bar, pO_2 = 0.2). Treatment effects were determined by assessment of tumor growth, tumor vascularisation, tumor cell proliferation, cell death and gene expression profile.
Results: Tumor growth was significantly reduced (~16%) after repeated HBO treatment compared to day 1 levels, whereas control tumors increased almost 100% in volume. A significantly decrease in tumor cell proliferation and tumor blood vessels, together with an increase in cell death, are consistent with tumor growth reduction and tumor stroma influence after hyperoxic treatment. Gene expression profiling showed that HBO induced a MET with increased expression of cell attachment gene modules.
Conclusion: Hyperoxia induces a coordinated alteration of entire gene modules of cell junctions, attachments and MET, which leads to less aggressive DMBA-induced mammary tumors. This indicates that oxygen *per se* might be an important factor in the "switch" from EMT to MET in vivo. HBO treatment also attenuates tumor growth and changes tumor stroma by targeting the vascular system, having anti-proliferative and pro-apoptotic effect.

Poster No. 133

BMP2 Upregalates the Migration and Invasion of Gastric Cancer Cells via PI3K/Akt-Raf-NF-κB Pathways

Myoung Hee Kang[1], Han-Na Kang[1], Jung-Lim Kim[1], Yong Park[2], Jun-Suk Kim[2], Sang-Cheul Oh[2], Young A. Yoo[3]
[1]*Graduate School of Medicine, Korea University College of Medicine, Korea University, Seoul, Korea Republic,* [2]*Division of*

Oncology/Hematology, Department of Internal Medicine, Korea University College of Medicine, Korea University, Seoul, Korea Republic, [3]Brain Korea 21 Program for Biomedical Science, Korea University College of Medicine, Korea University, Seoul, Korea Republic

Bone Morphogenetic Proteins (BMPs), members of the TGF-β superfamily, has been reported to enhance migration, invasion, and metastasis of various types of cancer cell lines, but its role in the invasive phenotype of gastric cancer cells is not yet known. Here, we was investigated a role and mechanism for BMP2-Akt-Raf signaling as it relates to the metastatic potential of gastric cancer. We found that stimulation of BMP2 in gastric cancer cells enhanced cellular motility and invasiveness, but addition of BMP4 did not. Meanwhile, blockade of BMP2 pathway by Noggin (a BMP signaling inhibitor) or anti-BMP2 neutralizing antibodies inhibited BMP2-induced invasiveness of gastric cancer cells. The phosphorylation of Akt and c-Raf was also enhanced by treatment with BMP2, but not Noggin, anti-BMP2 neutralizing antibodies. To investigate the functional relationship between BMP2 and Akt signaling in regulating the metastatic progression of human gastric cancer, we performed Matrigel invasion assay using cells either transfected with kinase-dead Akt or treated with either LY294002 or PD98059. Blockade of the Akt kinase using kinase-dead Akt, LY294002, or PD98059 in the presence of BMP2 significantly inhibited the gastric cancer cell invasiveness in association with a decreased expression of phospho-Raf. In addition, In addition, phosphorylation of IκBα, IκBα degradation, and the nuclear translocation of NF-κB was also enhanced by treatment with BMP2, but not Noggin, BMP2 neutralization antibodies, LY294002, or PD98059. Furthermore, we investigated whether the serum BMP-2 level from patients had any association or tendency with progression status of gastric cancer. No significant difference in the mean serum BMP-2 levels was observed between control and EGC (Early Gastric Cancer without lymph node metastasis) groups. However, metastatic disease group had significantly higher level of serum BMP than those of control and EGC groups. Overall, BMP2 signaling pathway promotes invasiveness of gastric cancer cells through activation of PI3K/Akt-Raf-NF-κB pathway.

Poster No. 134

Highlighting the Peritumoral Areas of Human Skin Cancer Biopsies by Infrared Spectral Microimaging

David Sebiskveradze[1], Cyril Gobinet[1], Elodie Ly[1], Michel Manfait[1], Olivier Piot[1], Valeriu Vrabie[2], **Pierre Jeannesson[1]**
[1]*MéDIAN, CNRS UMR 6237 MEDyC, University of Reims Champagne-Ardenne, Reims, France,* [2]*CReSTIC, University of Reims Champagne-Ardenne, Reims, France*

Infrared micro-spectral imaging is an efficient label-free optical method to analyze biological samples and to provide spatially resolved information on the basis of their biochemical composition. Recent studies have shown its potential to detect and characterize cancerous tissues in their early stages, independently of visual morphology. Infrared micro-imaging could thus be developed as a sensitive, nondestructive and objective diagnostic tool in clinical oncology. The discrimination between tumoral and peritumoral tissues relies on the highlighting of subtle infrared spectral differences. For this, we developed an algorithm based on fuzzy classification techniques which permits to automatically identify both the tumoral areas and their normal counterparts. This approach has been directly performed on formalin-fixed paraffin-embedded tissue sections of human skin cancers (squamous cell carcinoma and melanoma), without chemical dewaxing. The constructed infrared colorcoded spectral images allow recovering the different histological structures automatically. However, more than reproducing classical histology, our algorithm can give access to interesting information on the assignment of the infrared images pixels to the tissular structures. For each pixel, fuzzy classification provides with membership values, permitting to nuance their assignment. Such data are very valuable for the pixels located at the interface between tumoral tissue and its microenvironment. Thus, heterogeneous transitional areas between tumor and environmental normal tissue were identified for the examined tissue sections. These areas cannot be identified on hematoxylin-eosin staining or by conventional classification of infrared data, such as K-means. They are characterized by a gradual increase of the membership value of the pixels, from tumor to normal tissue to reach a maximum. Then, this value sharply decreases at the edge of the normal tissue. Experiments are underway to define the molecular assignments of the spectral variations observed in these peritumoral areas.

(DS is a recipient of a doctoral fellowship from INCa).

Poster No. 135

TGF-beta Promotes NSCLC Cell Migration towards the Lymphatic Endothelium by a CCR5-mediated Mechanism

Elizabeth Salvo[1], Saray Garasa[1], Álvaro Teijeira[1], Erik Olliemüller[1], Marta Irigoyen[1], Ana Rouzaut[1,2]
[1]*Divison of Oncology, Center for Applied Medical Research (CIMA), Pamplona, Navarra, Spain,* [2]*Department of Biochemistry, University of Navarra, Pamplona, Navarra, Spain*

Transforming growth factor (TGF-beta) is a pleiotropic cytokine that plays a dual function in lung cancer, acting as suppressor at initial stages of tumor growth, but becoming oncogenic at later cancer stages.
Although recent studies have described a mechanism whereby the TGF-beta induce mammary cancer cells to disrupt the capillary walls and seed metastases to lung, the role of this cytokine in lung tumor cell intravasation in the lung lymphatic vasculature remained obscure.
In this study, we use *in vitro* models to describe that upon TGF-beta exposure, H157 NSCLC, enhance their migration towards the leukocyte chemokines MIP1-alpha with the concomitant induction of its receptor CCR5 on NSLCL cells surface. These effects were specifically blocked by DAPTA an inhibitor of CCR5 signalling

and by the TGF-beta inhibitor SB431542. Subsequently, we observed that TGF-beta treated NSCLC showed also increased adhesion and transmigration through the lymphatic vessels in the presence of MIP1-alpha gradients.
Lastly, to provide a mechanistic support for TGF-beta mediated tumor cell adhesion to this endothelium, we analyzed the integrins and integrin receptors that showed modified expression after TGF-beta exposure, observing that there was an induction of the integrins alphavbeta3 and alphavbeta5 in NSCLC cells while that of their receptor, the protein L1 did not change on lymphatic endothelial cells. After specific blockade of these integrins and confocal microscopy analysis we could definitively affirm that they intervene in NSCLC adhesion to the lymphatic endothelium.
These results provide the first *in vitro* evidence of the implication of TGF-beta induced CCR in the onset of the metastatic spread of NSCLC through the lymphatics.

Poster No. 136

Butyric Acid Rich Microenvironment Induces Epithelial to Mesenchymal Transition (emt) in Colon Cancer Cells

Jacinta Serpa[1,2], Francisco Caiado[1,2], Cheila Torre[1,2], Tânia Carvalho[1,2], Cristina Casalou[1,2], Luís Gonçalves[3], Pedro Lamosa[3], Sérgio Dias[1,2]
[1]*Angiogenesis Lab fom "Centro de Investigação em Patobiologia Molecular", Portuguese Institute of Oncology, Francisco Gentil, Lisbon, Portugal,* [2]*Intituto Gulbenkian de Ciência, Oeiras, Portugal,* [3]*Intituto de Tecnologia Quimica e Biológica, Oeiras, Portugal*

Butyric acid is a short chain fatty acid (SCFA), a final product of bacterial fermentation of dietary fibers in colon. Butyric acid controls cell proliferation and apoptosis due to its action as a histone deacetylase inhibitor; as such, butyrate and butyrate-derived drugs are commonly used in cancer therapy with varying success. Despite the high butyrate concentration in colonic lumen, some colon cells are resistant to the butyrate effect and can give rise to aggressive colon cancers. In the present report, we characterize the effects of butyrate exposure on butyrate-resistant colon cancer cells. In vivo, sub-cutaneous tumours formed by butyrate pre-treated HCT15 (resistant colon cancer cells) proliferated more and were more angiogenic than tumours induced by non-treated cells. Similarly, intravenous inoculation of butyrate pre-treated HCT15 cells resulted in the formation of pulmonary micro-metastases, while mice injected with non-treated cells did not develop metastases. In vitro, we show HCT15 cells are able to fully metabolise butyrate. Butyrate treatment regulated the expression of angiogenic factor VEGF and its receptor KDR (VEGFR-2) at the transcriptional level. Butyrate treatment also induced the expression of b-oxidation acyl-dehydrogenases (SCAD and MCAD), MMP2, MMP9, a2 and a3 integrins, and reduced E-cadherin, suggestive of epithelial to, mesenchymal transformation (EMT). These results suggest that butyrate resistant colon cancer cells exposed to butyrate-rich microenvironment undergo metabolic and phenotypic changes resulting in enhanced proliferation, angiogenesis and metastasis. These results reveal the mechanistic basis for the clonal selection of very aggressive and butyrate resistant colorectal cancers.

Poster No. 137

The Biophysical Environment Affects Tumor-Fibroblast Interactions: Interstitial Flow Drives Fibroblast-Enhanced Tumor Invasion via Autocrine TGF-β1 Gradients

Adrian Shieh[1], Melody Swartz[1]
[1]*Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland*

Fibroblasts in the tumor microenvironment promote cancer progression and invasion through various mechanisms. We previously demonstrated that fibroblasts respond to interstitial flow (Ng et al., 2005), and since flow is an important part of the tumor microenvironment, we asked how flow affects tumor-fibroblast crosstalk and cancer invasion. In a modified transwell assay with a 3-D matrix, fibroblasts significantly and synergistically enhanced melanoma cell invasion *only* with interstitial flow. This synergy depended on endogenous, but not exogenous, TGF-β1. We therefore hypothesized that highly localized gradients of TGF-β1 were driving this synergistic response, and that the fibroblasts responded to these gradients to help direct tumor cell invasion. Cell-localized gradients could be generated by interstitial flow and secreted proteases, as we previously showed (Fleury et al., 2006). Interstitial flow alone increased fibroblast migration by 3-fold; in the presence of tumor cells, flow enhanced fibroblast migration 6-fold. This migration was TGF-β1-dependent. Fibroblasts produced most of the TGF-β1, as tumor cell-fibroblast gels contained 113 pg of TGF-β1, compared to 15 pg in tumor cell only gels. To generate an autologous TGF-β1 gradient, fibroblasts would need to activate latent growth factor, possibly via matrix metalloproteinases (MMPs). Inhibiting MMP activity resulted in a 47% decrease in flow-stimulated fibroblast migration, and a 40% reduction in fibroblast / flow-mediated tumor cell migration. These results suggest that fibroblasts secrete latent TGF-β1, activate it via MMPs, and generate a gradient in the direction of interstitial flow. Further, these data support the notion that fibroblasts chemotact up autocrine TGF-β1 gradients and direct tumor cell invasion. This behavior represents a previously undescribed mechanism by which tumor cells could migrate to lymphatic vessels, towards which interstitial flow is directed, leading to lymph node and organ metastases.

Poster No. 138

Hepatic Tumor-Stroma Crosstalk Guides Epithelial to Mesenchymal Transition at the Tumor Edge

Franziska van Zijl[1], Markus Mair[2], Agnes Csiszar[3], Doris Schneller[1], Gudrun Zulehner[1], Heidemarie Huber[1], Robert Eferl[2], Hartmut Beug[3], Helmut Dolznig[4], Wolfgang Mikulits[1]
[1]*Medical University Vienna, Department of Inner Medicine I, Institute of Cancer Research, Vienna, Austria,* [2]*Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria,* [3]*Research Institute of Molecular Pathology, Vienna, Austria,* [4]*Medical University Vienna, Institute of Clinical Pathology, Vienna, Austria*

The tumor-stroma crosstalk is a dynamic process fundamental in tumor development. In hepatocellular carcinoma (HCC), the progression of malignant hepatocytes frequently depends on transforming growth factor (TGF)-beta provided by stromal cells. TGF-beta induces an epithelial to mesenchymal transition (EMT) of oncogenic Ras-transformed hepatocytes and an upregulation of platelet-derived growth factor (PDGF) signaling. To analyze the influence of the hepatic tumor-stroma crosstalk onto tumor growth and progression, we co-injected malignant hepatocytes and myofibroblasts. For this, we either used *in vitro* activated $p19^{ARF}$ myofibroblasts or *in vivo* activated myofibroblasts derived from physiologically inflamed livers of $Mdr2/p19^{ARF}$ double null mice. We demonstrate that co-transplantation of myofibroblasts with Ras-transformed hepatocytes strongly enhances tumor growth. Genetic interference with the PDGF signaling decreases tumor cell growth and maintains plasma membrane-located E-cadherin and beta-catenin at the tumor-host border, indicating a blockade of hepatocellular EMT. We further generated a collagen gel-based three dimensional HCC model *in vitro* to monitor the myofibroblast-induced invasion of micro-organoid HCC spheroids. This invasion was diminished after inhibition of TGF-beta or PDGF signaling. These data suggest that the TGF-beta/PDGF axis is crucial during hepatic tumor-stroma crosstalk, regulating both tumor growth and cancer progression.

Poster No. 139

The Role of PI3K/Akt Signaling and MMP(s) in Shh/Gli-mediated EMT and Metastatic Potential of Gastric Cancer

Young A. Yoo[1], Myoung Hee Kang [2], Han Na Kang [2], Jung Lim Kim[2], Jun Suk Kim [3], Sang Cheul Oh[3]
[1]*Brain Korea 21 Program for Biomedical Science, Korea University College of Medicine, Korea University, Seoul, Korea Republic,* [2]*Graduate School of Medicine, Korea University College of Medicine, Korea University, Seoul, Korea Republic,* [3]*Division of Oncology/Hematology, Department of Internal Medicine, Korea University College of Medicine, Korea University, Seoul, Korea Republic*

The activation of Sonic hedgehog (Shh) signaling is involved in the progression and invasion of various tumors, including gastric carcinoma. Epithelial-mesenchymal transition (EMT) and matrix metalloproteinases (MMPs) have been implicated in facilitating the invasion and metastatsis. Herein, we investigated the impact of phosphoinositide 3-kinase (PI3K)/Akt pathway and MMPs activity on the Shh/Gli-mediated EMT and invasion of gastric cancer cells. We found that stimulation of N-Shh in gastric cancer cells enhanced cellular motility and invasiveness and induced a full EMT process characterized by Snail induction and E-cadherin down-regulation. Accompanying EMT, Shh also induces increased expression and activity of MMP-9. Meanwhile, blockade of Shh/Gli signaling by Cyclopamine (a Shh signaling inhibitor), anti-Shh neutralizing antibodies, or Gli siRNA also restored these changes of EMT markers and activity of MMP-9 and inhibited N-Shh-induced invasiveness of gastric cancer cells. The phosphorylation of Akt was also enhanced by treatment with N-Shh, but not cyclopamine, anti-Shh neutralizing antibodies, or Gli siRNA. Blockade of the Akt kinase using DN-Akt or LY294002 in the presence of N-Shh significantly inhibited the Shh-induced EMT, activity of MMP-9, and invasiveness. Furthermore, knock-down of MMP-9 by its siRNA results in an decrease in invasiveness of gastric cancer cells treatment with N-Shh. Immunohistochemistry on gastric tumor biopsies showed that the levels of Gli, E-cadherin, MMP-9 and phosph-Akt expression were enhanced in cases of metastatic gastric cancer than in cases of primary gastric cancer. Moreover, the strong correlation between Gli and E-cadherin, MMP-9 or phospho-Akt expression was also observed in lymph node metastasis specimens. These data indicate that Shh/Gli signaling pathway promotes EMT and invasiveness of gastric cancer cells through activation of PI3K/Akt pathway and upregulation of MMP-9.

Poster No. 140

Relevance of CD44 to the Poor Prognosis of Basal Breast Cancers

Suzanne McFarlane[1], Ashleigh Hill[1], Susie Conlon[2], Tony O'Grady[2], Nicola Montgomery[1], Karin Jirstrom[3], Elaine Kay[2], David Waugh[1]
[1]*Centre for Cancer Research & Cell Biology, Queen's University Belfast, Belfast, Northern Ireland, UK,* [2]*Royal College of Surgeons in Ireland, Dublin, Ireland,* [3]*Centre for Molecular Pathology, Lund University, Malmo, Sweden*

CD44 is a transmembrane adhesion molecule and principal receptor for hyaluronan (HA). Expression of CD44 has been documented to have a key role in breast cancer metastasis. We conducted an immunohistochemistry (IHC) study of CD44s expression in breast cancer tissue microarrays (TMAs) and found that CD44s expression significantly associated with node positive tumours ($p=0.0209$) and distant recurrence ($p=0.0427$). Furthermore CD44 expression was associated with the basal phenotype of breast cancer ($p=0.018$). Basal breast cancers are known to have a poor prognosis and the aim of this study was to gain insight into the role of CD44 in the poor prognosis of basal breast cancers. For this we used a subclone of the basal-like breast cancer cell line MDA-MB-231 that specifically metastasises to bone. Bone homing MDA-MB-231BO cells displayed increased CD44, alpha5 and beta1-integrin expression relative to the parental cells and were more adherent to bone marrow endothelium (BMEC) and fibronectin. HA-induced CD44 signaling increased beta1-integrin expression and activation and induced phosphorylation of the cytoskeletal proteins cortactin and paxillin. HA-induced paxillin phosphorylation was attenuated by depletion of CD44

and cortactin using RNAi and following administration of neutralizing antibodies to beta1-integrin receptors, suggesting that it is a downstream target of CD44-promoted, integrin-mediated signaling. Inhibition of this signaling cascade by RNAi-mediated depletion of CD44, cortactin or paxillin or by addition of neutralizing antibodies against beta1- and alpha5beta1-integrins attenuated MDA-MB-231BO cell adhesion to BMECs and the alpha5beta1-integrin substrate, fibronectin. Furthermore IHC confirmed alpha5 and beta1-integrin expression in breast TMAs and correlated CD44 expression with alpha5 expression ($p = 0.044$). We propose this CD44 induced, integrin-mediated signaling pathway contributes to the efficient extravasation of basal breast cancer cells across endothelial barriers and their colonisation of the metastatic niche.

Poster No. 141

Identification and Description of Novel CAF-derived Stimulators of Prostate Cancer: The Chemokine CXCL14

Martin Augsten[1], Christina Hägglöf[1], Eleonor Olsson[2], Panagiotis Tsagozis[1], Sabine Vorrink[1], Åke Borg[2], Lars Egevad[1], Arne Östman[1]
[1]*Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden,* [2]*Department of Oncology, Lund University, Lund, Sweden*

The tumor stroma of solid tumors harbours many different cell types that are contributing to an intense crosstalk with the cancer cells and thereby promote tumor growth and progression. One of the major cell types of the tumor stroma are cancer-associated fibroblasts (CAFs). CAFs attract increasing attention because of their critical contributions to tumor development and metastasis. Using an integrative approach we identified several novel factors in CAFs derived from prostate cancer patient biopsies. For one of the soluble factors identified, the chemokine CXCL14, we describe a novel, tumor- promoting activity when expressed by CAFs.

Analyses of matched normal and tumor tissue revealed up-regulation of CXCL14 in cancer-associated fibroblasts of a majority of prostate cancer. Fibroblasts over-expressing CXCL14 promoted the growth of prostate cancer xenografts, accompanied by increased tumor angiogenesis and macrophage infiltration. Mechanistic studies demonstrated that autocrine CXCL14-stimulation of fibroblasts enhance migration and proliferation of fibroblasts. CXCL14- producing fibroblasts, but not recombinant CXCL14, enhanced *in vitro* proliferation of prostate cancer cells and *in vivo* angiogenesis. Furthermore, expression profiling led to the identification of several molecules that putatively mediate CXCL14- action in the fibroblasts.

These studies thus identify CXCL14 as a novel autocrine stimulator of fibroblasts, with multi-modal tumor-stimulatory activities. In more general terms, our findings emphasize the importance of CAFs in tumor growth and suggest a novel mechanism whereby cancer-associated fibroblasts achieve their pro- tumorigenic phenotype. The generic strategy used for the identification of these novel stimulators of prostate cancer growth is predicted to also be applicable in other tumor types.

Östman and Augsten, Curr Opin Genet Dev. 2009 19: 67–73.
Augsten *et al*., Proc Natl Acad Sci U S A. 2009 106: 3414–3419

Poster No. 142

Radiation Induces Invasiveness of Pancreatic Cancer via Upregulation of Heparanase

Esther Bensoussan[1], Amichay Meirovitz[1], Irit Cohen[1], Immanuel Lerner[1], Benito Casu[2], Israel Vlodavsky[3], Michael Elkin[1]
[1]*Department Of Oncology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel,* [2]*Ronzoni Institute, Milan, Italy,* [3]*Technion-Israel Institute of Technology, Haifa, Israel*

Pancreatic cancer is one of the most aggressive neoplasms with an extremely low survival rate. Because most pancreatic carcinoma patients miss the opportunity for complete surgical resection at the time of diagnosis, radiotherapy remains a major component of treatment modalities. However, pancreatic cancer often shows resistance to radiation therapy. Ionizing radiation (IR)-induced aggressiveness is emerging as one of the important mechanisms responsible for limited benefit of radiation therapy in pancreatic cancer, but the identity of downstream effectors responsible for this effect remains poorly investigated.

Here we report that IR promotes pancreatic cancer aggressiveness through up-regulation of the heparanase. Heparanase is a predominant mammalian enzyme capable of degrading heparan sulfate (HS), the main polysaccharide component of the basement membrane and other types of extracellular matrix (ECM). Cleavage of HS by heparanase leads to disassembly of ECM, enables cell invasion, releases HS–bound angiogenic and growth factors from the ECM depots, and generates bioactive HS fragments. We found that clinically relevant doses of IR augment invasive ability of pancreatic cells *in vitro* and *in vivo* via induction of heparanase. Our results indicate that effect of IR on heparanase expression is mediated by Egr1 transcription factor. Moreover, specific inhibitor of heparanase enzymatic activity abolished IR-induced invasiveness of pancreatic carcinoma cells *in vitro*, while combined treatment with IR and the heparanase inhibitor, but not IR alone, attenuated orthotopic pancreatic tumor progression *in vivo*.

The proposed up-regulation of heparanase by IR represents a new molecular pathway through which IR may promote pancreatic tumor aggressiveness, providing explanation for the limited benefit from radiation therapy in pancreatic cancer. Our research is expected to offer a new approach to improve the efficacy of radiation therapy and better define target patient population in which such approach could be particularly beneficial.

Poster No. 143

The Tumor Inhibitory Capacity of the Aged Organism Microenvironment is Limited by the Aggressiveness of the Tumor

Orit Itzhaki[1], Tatiana Kaptzan[1], Ehud Skutelsky[1], Judith Sinai[1], Ginnette Schiby[1], Monika Huszar[1], Annette Siegal[1], Judith Leibovici[1]
[1]*Department of Pathology, Sackler Faculty of Medicine, Tel- Aviv University, Tel- Aviv, Israel*

Incidence of neoplasia is known to increase with age while tumor growth and metastatic spread proceed, paradoxically, at a slower rate in aged as compared to young patients. Although not a general feature, this intriguing phenomenon is observed in many human and experimental tumors. We have shown this particular behavior in the AKR lymphoma and B16 melanoma.
Understanding the mechanisms of this interesting phenomenon is of importance, particularly in view of the possibility that these mechanisms may eventually suggest modalities for age- adjusted anti- tumoral therapy. We have previously shown that one such mechanism is increased tumor cell apoptosis in the old animals. In the present study we tried to verify whether the induction of tumor cell apoptosis in the aged depends on the malignancy of the tumor. We used variants of malignancy of the AKR lymphoma and tested the degree of apoptosis in young and old mice in several such variants. According to various cellular (Apoptag staining, DNA flow cytometry) and molecular (ladder type DNA fragmentation, Bcl-2, Fas receptor and caspase expression) characteristics of apoptotic cells, we found that tumor cell apoptosis was increased in tumors of old as compared to those of young mice in all variants. This age-dependent increased apoptosis was however inversely proportional to AKR lymphoma malignancy.
Our results may indicate that the inhibitory capacity of the old organism tumor microenvironment is limited by the aggressiveness of the tumor. We have previously found that low malignancy variants of AKR lymphoma are more prone to apoptosis than high malignancy variants. It is therefore expected that inducing tumor cell apoptosis as a therapeutic modality in the old can be more effective at early stages of tumor development than at late ones.

Poster No. 144

Pre-Adipocytes and "Reorientated" Adipocytes Contribute to the Desmoplastic Reaction in Breast Cancer: A New Link between Breast Cancer and Obesity?

Ludivine Bochet[1,2,3], Béatrice Dirat[2,3], Stéphanie Dauvillier[1], Christophe Roubeix[1,3], Philippe Valet[2,3], Catherine Muller[1,3]
[1]*Team Microenvironment, Cancer and Adipocytes (MICA), Institute of Pharmacology and Structural Biology CNRS UMR 5089, Toulouse, France,* [2]*Team AdipOlab, INSERM U858, I2MR, Toulouse, France,* [3]*Université de Toulouse, Toulouse, France*

In a variety of tumours, such as breast carcinomas, a desmoplastic response, characterized by the presence of a dense collagenous stroma comprising fibroblast-like cells, is observed and is thought to contribute to tumour progression. Peritumoral fibroblasts are composed of several subpopulations that are morphologically undistinguishable and their origins remain debated. Most of the studies have focused on the activation of fibroblasts present in the interstitium (the so-called myofibroblasts) and very little attention has been given to adipocytes, although it is obvious that in breast, early local tumour invasion results in immediate proximity of cancer cells to adipose tissue. We demonstrate here that the tumour cells modify both the mature and precursors components of the surrounding adipose tissue leading to the accumulation of an activated population with morphological features of fibroblast cells. Using an original 2D system, where an insert separates the two cell populations, we first demonstrate that mature adipocytes cocultivated with breast tumour cells for 5 to 8 days exhibit a loss of lipid content, a decrease in differentiation markers (shown by qPCR and Western blots) and underwent morphological changes into fibroblast-like cells associated to cytoskeleton reorganization. Tumour cells were also able to profoundly inhibit the adipogenesis of pre-adipocytes grown in adipogenic conditions. Interestingly, this population of adipocyte-derived fibroblasts (ADF) exhibit a profibrotic phenotype (with enhanced fibronectin and collagen I production) and enhanced migratory capacities. Ongoing experiments are performed in our laboratory to assess the presence of these ADF in human breast tumours. Our results might provide an explanation for the poor prognosis observed in localised breast cancer in obese women, since the nature of the desmoplastic reaction and the secretion pattern of the ADF might be profoundly altered in this physiopathogical condition.

Poster No. 145

The Endothelial KSHV GPCR Signaling Pathways is Active in Human Kaposi Sarcoma

Julie Dwyer[1,2], Mamta Sumbal[1,2], Armelle Le Guelte[1,2], Laetitia Douguet[1,2], Nina Fainberg[3], J. Silvio Gutkind[3], Philippe A. Grange[4], Nicolas Dupin[4], Julie Gavard[1,2]
[1]*Institut Cochin, Universite Paris Descartes, CNRS (UMR 8104), Paris, France,* [2]*INSERM, U567, Paris, France,* [3]*Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institute of Health, Bethesda, Maryland, USA,* [4]*UPRES-EA1833 Laboratorie de Recherche en Dermatologie, Centre National de Reference Syphilis, Paris, France*

Kaposi Sarcoma (KS) are opportunist tumors, associated with the herpes virus-8 infection, also named as Kaposi Sarcoma Herpes Virus. KS development is indeed highly favored by immune-depression, such as AIDS malignancies. Although KS incidence is reduced in HIV-infected patients through the use of antiretroviral tri-therapies, recent epidemiological data show that KS is the second most frequent tumor in AIDS patients in western countries. KS are multiple tumor lesions, highly angiogenic, highly

inflammatory, and involved in neoplastic cells as well as transformation of the microenvironment most likely through paracrine effects. Recently, it has been demonstrated that the expression of the viral G protein coupled receptor (vGPCR) in the endothelial compartment is sufficient alone to recapitulate formation and progression of Kaposi Sarcoma in mice; making this model and this viral protein in particular, a powerful tool to study the pathology of KSHV. However, the functional effects of vGPCR expression on endothelial homeostasis are far from being addressed. Here we show that vGPCR expression in endothelial cells induces an increase in paracellular permeability through a PI (3)Kinase/Rac pathway and involves the activation of the kinase PAK. This leads to the further phosphorylation of VE-cadherin and the subsequent remodeling of endothelial junctions. Importantly, this signaling pathway was also found active in 12 out of 14 KS samples analyzed. Our results suggest that endothelial vGPCR signaling mechanisms are functional in KS microenvironment, placing endothelial transformation as a key cellular target for therapeutic intervention.

Poster No. 146

The Role of Different Subtypes of Macrophages in Colorectal Cancer

Sofia Edin[1], Maria L. Henriksson[1], Roger Stenling[1], Jörgen Rutegård[2], Åke Öberg[2], Per-Arne Oldenborg[3], Richard Palmqvist[1]
[1]*Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden,* [2]*Department of Surgical and Perioperative Sciences, Surgery, Umeå University, Umeå, Sweden,* [3]*Department of Integrative Medical Biology, Histology and Cellular Biology, Umeå Univsersity, Umeå, Sweden*

Colorectal cancer (CRC) is the second most common cause of cancer deaths in the western world. We have previously shown a correlation between high macrophage infiltration and improved survival in CRC. Tumour associated macrophages (TAMs) play complex roles in tumourigenesis since they can both prevent and promote tumour progression. According to a suggested hypothesis classically activated M1 macrophages mainly act to prevent tumour progression and metastasis, whereas alternatively activated M2 macrophages instead have mainly tumour promoting functions.
We have applied an immunohistochemical approach to determine the degree of M1 and M2 macrophage infiltration in clinical specimens of CRC and related the results to various clinicopathological variables. A total of 434 consecutive CRC specimens collected over the period 1995 through 2003 were stained for iNOS (M1 marker) and CD163 (M2 marker). The average infiltration along the invasive tumor margin was semiquantitatively evaluated using a four-graded scale. We observed a statistical correlation between the amount of iNOS (M1) and CD163 (M2) positive cells ($p<0.001$). Furthermore, patients harbouring iNOS high tumours had a significantly better prognosis than iNOS low tumours. An inverse association between tumour stage and the amount of iNOS positive macrophages ($p<0.001$) was found. Accordingly, the prognostic significance for iNOS macrophages was lost when including tumour stage in the multivariate analysis. *In vitro* cell culture models using primary human monocytes or a monocytic cell line (MonoMac6) were used to study the functional roles of M1 and M2 macrophages in tumour cell migration and invasion In conclusion, our results support the view that TAMs are important in tumour progression and for patient outcome.

Poster No. 147

Human Interferon-α and Holocene Grain Wash-Out Affects the Growth of Neoplastic Cells *in vitro*

Bratko Filipič [1], Evgen Šooš [2], Hrvoje Mazija [3], Srečko Koren[1]
[1]*Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia,* [2]*Kloštar Ivanić, Croatia,* [3]*Department of Avian Diseases with Clinic, Veterinary Faculty, University of Zagreb, Zagreb, Croatia*

The Holocene started by withdrawal of pleistocene glacier. The holocene sants containing uniform holocene minerals occured. Grained show unusual biological/microbiological activity, like antifungal against *Peronospora sp., Phytophthora sp.*
The entity of the patent invention lies in the use of the holocene grain wash-out and different Human Interferons and combinations between them in the prevention of growth and multiplication of Human neoplastic cells *in vitro*. [1)]
Grain samples (»Svijetli« (Light), »Tamni« (Dark)) and Human Interferons: HuIFN-αN3 (Human leukocyte Interferon) (1000 I.U./ml) (reference value) and rHuIFN-α2 (1000 I.U./ml) (reference value) were used. Samples from river Drava sants, near Koprivnica, grained by »Star mix« technology giving fine grain with 60–80 μm size were used. The following wash-out (»suspensions«) were prepared: (1) 10% Monoethylene-glycole, (2) 10% PBS (Phosphate buffer saline).CaCo-2 (Colon cancer carcinom) cells were used and WISH (Human amniotic cell line) cells as control. Cells were treated either with grain wash-out (Monoethylene glycole, PBS), HuIFN-αN3, rHuIFN-α2 or with different combinations between them in ratio: 1:1, 1:2, 2:1. The 50% cell growth inhibition test was used. The meaning of the data: The higher is the dillution till well giving 50% cell growth inhibition, beter is the substance. The conclusions:(1)The holocene grain wash-out (10% suspension) show the AP (Antiproliferative) activity against CaCo-2 cells in vitro.(2)The AP actvitiy of Monoethylene glicole wash-out is higher than these obtained from PBS (3)The obtained AP activity can be enhanced up to 4x by combination with HuIFN-αN3 but not with rHuIFN-α2. (4)For the optimal enhancement of Holocene grain wash-out AP activity in vitro different natural subtypes contained in HuIFN-αN3 are needed.
1)Kesteli B., Filipič B., Šooš E. (2007): Ways of use of natural extracts of Holoce- ne minerals and Interferons on the growth of neoplastic cells.(In Croatian) IPO; Republic of Croatia, Patent No.: P20080400A

Poster No. 148

Toxicity Studies of Cancer Drugs in Engineered Cell Environments

Maria Håkanson[1], Mirren Charnley[1], Marcus Textor[1]
[1]*Laboratory for Surface Science and Technology, Department of Material Science, ETH Zurich, Zurich, Switzerland*

It is widely acknowledged that cancer progression and behavior is affected by the microenvironment [1]. Furthermore, substantial evidence exists that demonstrates the dependence of the drug response in cancer cells on cues of the surrounding environment, such as dimensionality [2] and ECM composition [3]. Such insights partially explain the discrepancies between observed drug efficiency *in vitro* and *in vivo*. This work aimed to use controlled engineered cell environments to improve the understanding of the role of external cues on drug response.
We used a microwell array, previously developed in our group [4], which enables the culture of cells in a 3D environment with control of cell cluster size down to the single cell level. It also allows the control of the biochemical interface with the cells. Initially we studied the influence of dimensionality on the response to taxol on the breast carcinoma cell line, MCF-7.
Cancer cells cultured in microwells showed an increased resistance to taxol in comparison to cells cultured on flat substrates. A similar change in drug response was observed for cells in cell-derived fibronectin matrices. These results in two 3D systems, of different complexity, demonstrate that dimensionality is an important factor for determining the responsiveness of cells to drugs. In addition, the results showed that the microwell array can be used as an in vivo mimic, and is therefore a promising tool for the screening of anti-cancer drugs.
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Poster No. 149

FAP-positive Fibroblasts Express FGF1 and Increases Migration and Invasion of Colon Cancer Cells

Maria L. Henriksson[1], Sofia Edin[1], Anna M. Dahlin[1], Per-Arne Oldenborg[2], Åke Öberg[3], Bethany Van Guelpen[1], Jörgen Rutegård[3], Roger Stenling[1], Richard Palmqvist[1]
[1]*Department of Medical Biosciences/Pathology, Umeå Universtiy, Umeå, Sweden,* [2]*Department of Integrative Medical Biology, Section for Histology and Cell Biology, Umeå Universtiy, Umeå, Sweden,* [3]*Department of Surgical and Perioperative Sciences, Surgery, Umeå Universtiy, Umeå, Sweden*

Background: Colorectal cancer is one of the leading causes of cancer deaths in western countries, with death generally resulting from metastatic disease. In recent years, the importance of the tumor microenvironment, including tumor-associated fibroblasts, has paid increasing attention.
Aim: To analyze the effect of Fibroblast activation protein (FAP)-expressing fibroblasts on colon cancer cell migration and invasion in experimental cell studies. We also investigated the expression pattern of FAP in tumor-associated fibroblasts during transformation from benign to malign colorectal tumors.
Methods and results: In immunohistochemical analyses, FAP was expressed in fibroblasts in all carcinoma samples examined (n=61), whereas all normal colon (n=12), hyperplastic polyps (n=16) or adenoma (n=55) samples were negative for FAP. In *in vitro* studies, conditioned medium from HCT-116 colon cancer cells, but not LT97 adenoma cells, induced FAP expression in colon fibroblasts. FAP-expressing fibroblasts increased the migration of colon cancer cells in Boyden chamber experiments, and when co-cultured with FAP-expressing fibroblasts in a three-dimensional organotypic cell culture model, the invasive behavior of the cancer cells was increased. In contrast, FAP-expressing fibroblasts did not increase the invasiveness of the adenoma cell line. Conditioned medium from FAP-expressing fibroblasts increased the invasion in colon cancer cells, indicating an involvement of mechanisms other than the protease activity of membrane-bound FAP. Further cell culture analysis showed that FGF1-expression is increased in FAP-expressing fibroblasts.
Conclusions: We demonstrate a novel function for FAP-expressing fibroblasts. Our findings also suggest that FAP may be a potential diagnostic marker for early invasion in colorectal cancer.

Poster No. 150

CCL1 is a Novel Therapeutic Target for the Modulation of Treg Function with Implications for Cancer Immunotherapy

Dominique B. Hoelzinger[1], Shannon E. Smith[1], Noweeda Mirza[1], Ana Lucia Dominguez[1], Soraya Zorro-Manrique[1], Joseph Lustgarten[1]
[1]*Departmen of Immunology, Mayo College of Medicine, Scottsdale, AZ, USA*

The genetic instability of tumors ensures a changing landscape of mutated or over-expressed tumor associated antigens (TAAs). The presence of tumor-specific lymphocytes in tumors is evidence that TAAs are targets for T-cell immunity. In spite of this, established tumors rarely generate endogenous immunity leading to successful tumor eradication. A key reason for poor TAA immunity is that the tumor microenvironment becomes progressively more immunosuppressive as the tumor develops, inhibiting anti-tumor immune activity. The

immunosuppressive milieu within tumors is largely brought about by the presence of T-regulatory cells (Tregs), which maintain self-tolerance by directly inhibiting T-cells, NK cells and dendritic cells. Depletion of Tregs enhances antitumor immune responses, however, it also affects the number of T-effector cells. Previous studies indicate that intratumoral injections of CpG-ODN strongly reduces the levels of Tregs within the tumor, and that the decrease in Tregs is mainly mediated by IL-6. Since IL6 promotes growth of some human cancers, alternate pathways to inactivate Tregs were sought through microarray analysis, resulting in gene candidates that can be exploited to modulate the function of Tregs. Chemokine (C-C motif) ligand 1 (CCL1) was expressed by Tregs and its neutralization both inhibited Treg conversion and suppressive function without affecting the function of T-effector cells. The combination of CpG-ODN and anti-CCL1 treatments induce complete rejection of tumors in BALB-neuT tolerant mice. Tumor rejection was coincident with changes in the lymphocyte composition in the tumor microenvironment. Tumors of CpG+anti-CCL1 treated mice have decreased in Treg numbers and a concomitant accumulation of tumorcidal cells within the tumor. We propose that neutralization of CCL1 can be used as an adjuvant to anti-tumor immunotherapy, as a means of reversing Treg dependent immunosuppression within the tumor.

Poster No. 151

Novel Role of Tumor-Derived ExtracellularHsp90 as an Essential Mediator of Prostate Cancer Cell Migration and Stromal Cell Activation: Evidence for Autocrine and Paracrine Functions

Venkatesababa Samanna[1], Udhayakumar Gopal[1], **Jennifer Isaacs[1]**
[1]*Department of Cell and Molecular Pharmacology, Hollings Cancer Center, Medical Uninversity of South Carolina (MUSC), Charleston, SC, USA*

Prostate cancer (PCa) is one of the most common and lethal diseases among men. Although early cancer is often curative, subsequent metastatic spread of tumor cells renders the disease untreatable. Treatment failure is also due to a poor understanding of the contribution of the tumor microenvironment to disease progression. We find that a number of PCa cells secrete heat shock protein 90 (Hsp90). This extracellular Hsp90 (eHsp90) acts in a manner distinct from the intracellular chaperone and has been implicated in regulating cell motility in other models. Interestingly, we find that eHsp90 expression correlates with cancer aggressiveness. Consistently, the more aggressive and metastatic PCa cells secreted several fold more eHsp90 relative to their weakly tumorigenic matched counterparts. Interference with this pathway by antibody or drug-mediated neutralization of native eHsp90 dramatically impaired tumor cell migration, thereby implicating eHsp90 in a constitutive pathway culminating in cell migration. Concomitant with inhibition of eHsp90, the activation of downstream mediators such as FAK, Src, and ERK were attenuated. The multifunctional receptor LRP1 (LDL-receptor Related Protein-1) has been proposed as the receptor for eHsp90. We find that silencing of LRP1 similarly reduced PCa signaling and migration, implicating an eHsp90-LRP1 signaling axis in PCa development. Addition of Hsp90 to prostate stromal cells, which lack Hsp90 secretion, potently stimulated ERK activation and cell motility, implicating paracrine effects. ERK activation was inhibited by pretreatment with an inhibitor of MMP activity, suggesting that eHsp90 modulates ERK signaling and MMP activity to modulate cell migration. We propose that PCa aggressiveness may be due in part to increased secretion of eHsp90, which then activates the stroma to further support tumor growth.

Poster No. 152

IL-6 Promotes Pancreatic Cancer Progression by Intractions of Fibroblasts

Hidenobu Kamohara[1], Takatoshi Ishiko[1], Hiroshi Takamori[1], Hideo Baba[1]
[1]*Department of Gastroenterological Surgery, Kumamoto University, Graduate School of Medical Sciences, Kumamoto, Kumamoto, Japan*

Introduction:
IL-6 has pleiotropic function and are produced by various immunnocompetent cells, as well as cancer cells. Some studies have been demonstrated IL-6 play an important role in evading host immune surveillance in tumor microenvironment, but interactions of fibroblasts has not been fully understood. Therefore, the aim of this study is to reveal role of fibroblasts in pancreatic tumor microenvironment. Especially, we evaluate induction and biologicial function of IL-6 in pancreatic carcinoma cells after the stimulation with various cytokines and fibroblast associated factors.
Methods and Results:
All 7 tested cell lines expressed gp130 and IL-6Ralpha mRNA, 2 cell lines (Hs7667 and Capan1) expressed IL-6 mRNA in serum free condition by RT-PCR and Northern blotting. Hs766T cells were stimulated with or without cytokines. Northern blotting revealed TNFalpha and IL-1beta upregulated IL-6 mRNA, but not IL-6, IL-8 and LIF. IL-6 did not affect cell proliferation by WTS assay, but promoted cell motility and chemoinvasion significantly. To identify IL-6 expression by interaction between pancreatic carcinoma cells and fibroblasts, we used two established fibroblastic cell lines (MRC-9 and WI-38)isolated from human embryonal lung tissues. Serum free conditioned medium (CM) were collected after incubation for indicated periods. Hs766T produced CM (Hs766T-CM)induced IL-6 and IL-8 mRNA in MRC-9 and WI-38 cells. MRC-9 CM and WI-38-CM did not affect in Hs-766 T cells. Co-culture between Hs766T and MRC-9 cells induced IL-8 mRNA drastically.

Conclusion:
Communication of pancreatic carcinoma cells with fibroblasts affect IL-6 expression and that could contribute to pancreatic cancer progression. Regulation of IL-6 expression in tumor microenvironment would be important for pancreatic cancer therapy.

Poster No. 153

The Anti-Angiogenic Activity of Bortezomib is Blocked by GRP-78 Secreted by Tumor Cells

Johann Kern[1], Gerold Untergasser[1], Christoph Zenzmaier[2], Guenther Gastl[1], Eberhard Gunsilius[1], Michael Steurer[1,3]
[1]*Tumor Biology & Angiogenesis Laboratory, Department of Internal Medicine V Innsbruck, Medical University of Innsbruck, Innsbruck, Tirol, Austria,* [2]*Institute for Biomedical Aging Research, Austrian Academie of Sciences, Innsbruck, Tirol, Austria,* [3]*Laboratory for Molecular Genetics, Department of Internal Medicine V, Medical University of Innsbruck, Innsbruck, Tirol, Austria*

Anti-angiogenic effects of the proteasome inhibitor bortezomib were analyzed *in vivo* using tumor xenografts *in the* chicken chorioallantoic membrane (CAM) assay. Bortezomib's inhibitory effects on CAM vascularization were abrogated in the presence of distinct tumor xenografts suggesting a soluble inhibitory factor secreted by tumor cells. Using size-exclusion and ion-exchange chromatography as well as mass spectroscopy. GRP-78, a chaperone protein of the unfolded protein response, normally expressed and retained in the endoplasmatic reticulum was identified as being responsible for bortezomib inhibition. In fact, a variety of bortezomib-resistant solid tumor cell lines (PC-3, HRT-18), but not bortezomib-sensitive myeloma cell lines (U266, OPM-2) were found to secrete high amounts of GRP-78. In fact, recombinant GRP-78 confered bortezomib resistance to endothelial cells and knock down of GRP-78 in PC-3 cells resulted in loss of bortezomib resistance. Preliminary data using immunoprecipitation and proteasome activity assays suggest that the mechanism of action of GRP-78 induced bortezomib resistance is not due to direct interference with the compound or the proteasome but rather further downstream due to inhibition of apoptotic signalling. In conclusion, distinct solid tumor cells secrete GRP-78 thereby gaining resistance to bortezomib. These findings describe a hitherto unknown mechanism of resistance to proteasome inhibitors and may offer a novel strategy to increase the susceptibility of solid tumor cells to bortezomib.

Poster No. 154

The Effect of Platycodin D on Breast Cancer-Induced Bone Destruction

Sun Kyoung Lee[1,2], Kwang-Kyun Park[1,2], Yeong-Shik Kim[3], Young-Wan Ha[3], Won-Yoon Chung[1,2]
[1]*Department of Oral Biology, Oral Cancer Research Institute, Oral Science Research Institute, Brain Korea 21 Project, College of Dentistry Yonsei University, Seoul, Korea Republic,* [2]*Department of Applied Life Science, The Graduate School, Yonsei University, Seoul, Korea Republic,* [3]*Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul, Korea Republic*

Breast cancer is the most common cancer affecting women in the United States and other countries. In individuals with breast cancer, the frequency of bone metastasis is much higher than other organ metastases. Breast cancer cells secrete osteolytic factors, such as parathyroid hormone-related protein (PTHrP), interleukin (IL)-1β, -6 and -11. These factors stimulate stromal/osteoblastic cells to over-express receptor activator of nuclear factor-kappa B ligand (RANKL), which is required to induce osteoclast formation/activation. Over-expression of RANKL results in increased osteoclast formation and bone resorption. The subsequent bone resorption induces the release of various growth factors from the bone matrix, such as transforming growth factor (TGF)-β, insulin-like growth factor (IGF)-Iand -II. The released growth factors stimulate the proliferation of cancer cells. The interaction between tumor cells and bone cells, called to 'vicious cycle', is crucial for the initiation and promotion of skeletal metastasis. We found that platycodin D (PD), a major constituent of triterpene saponins found in the root of *Platycodon grandiflorum*, inhibited the viability of human breast cancer MDA-MB-231 cells, in a dose-dependent manner. However, PD did not influence the secretion of osteolytic factors in MDA-MB-231 cells and RANKL/OPG ratio in osteoblasts treated with conditioned media of MDA-MB-231 cells. PD suppressed RANKL-induced osteoclast formation/activation through down-regulation of c-Fos and nuclear factor of activated T cells 1 (NFATc1) in mouse bone marrow-derived macrophage (BMM) cells. PD also induced apoptosis in osteoclasts. Consistent with the in vitro effect, PD showed the inhibitory effect on tumor growth and tumor-induced bone destruction in vivo. Taken together, our results indicate that PD may block breast cancer-induced osteolysis by decreasing the viability of cancer cells and inhibiting RANKL-induced osteoclast formation.

Poster No. 155

Pten in Stromal Fibroblasts Suppresses Mammary Epithelial Tumors

Anthony J. Trimboli[1,2], Carmen Z. Cantemir-Stone[3], Fu Li[1,3], Julie A. Wallace[3], Anand Merchant[3], Nicholas Creasap[1,2], John C. Thompson[1,2], Enrico Caserta[1,2], Hui Wang[1,2], Jean-Leon Chong[1,2], Shan Naidu[1,2,4], Guo Wei[1,3], Sudarshana M. Sharma[3], Julie A. Stephens[5], Soledad A. Fernandez[5], Metin N. Gurcan[6], Michael B. Weinstein[1,2], Sanford H. Barsky[7], Lisa Yee[8], Thomas J. Rosol[4], Paul C. Stromberg[4], Michael M. Robinson[9], Francois Pepin[10,11], Michael Hallett[10,11], Morag Park[10,12], Michael C. Ostrowski[3,13], **Gustavo Leone[1,2,13]**
[1]*Department of Molecular Genetics, College of Biological Sciences, The Ohio State University, Columbus, Ohio, USA,* [2]*Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Columbus, Ohio, USA,* [3]*Department of Molecular and Cellular Biochemistry, College of Medicine, The Ohio State University, Columbus, Ohio, USA,*

[4]*Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA*, [5]*Center for Biostatistics, Office of Health Sciences, The Ohio State University, Columbus, Ohio, USA*, [6]*Department of Biomedical Informatics, The Ohio State University, Columbus, Ohio, USA*, [7]*Department of Pathology, The Ohio State University, Columbus, Ohio, USA*, [8]*Department of Surgery, School of Medicine, The Ohio State University, Columbus, Ohio, USA*, [9]*Center for Molecular and Human Genetics, Columbus Children's Research Institute, Columbus, Ohio, USA*, [10]*Department of Biochemistry, Rosalind and Morris Goodman Cancer Center, McGill University, Quebec, Canada*, [11]*McGill Center for Bioinformatics, McGill University, Quebec, Canada*, [12]*Department of Oncology, McGill University, Quebec, Canada*, [13]*Tumor Microenvironment Program, Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, USA*

The tumor stroma is believed to contribute to some of the most malignant characteristics of epithelial tumors. However, signaling between stromal and tumor cells is complex and remains poorly understood. Here we show that the genetic inactivation of *Pten* in stromal fibroblasts of mouse mammary glands accelerated the initiation, progression and malignant transformation of mammary epithelial tumors. This was associated with the massive remodeling of the extra-cellular matrix (ECM), innate immune cell infiltration and increased angiogenesis. Loss of *Pten* in stromal fibroblasts led to increased expression, phosphorylation (T^{72}) and recruitment of Ets2 to target promoters known to be involved in these processes. Remarkably, *Ets2* inactivation in *Pten* stroma-deleted tumors ameliorated disruption of the tumor microenvironment and was sufficient to decrease tumor growth and progression. Global gene expression profiling of mammary stromal cells identified a Pten-specific signature that was highly represented in the tumor stroma of breast cancer patients. These findings identify the Pten-Ets2 axis as a critical stroma-specific signaling pathway that suppresses mammary epithelial tumors.

Poster No. 156

Recombinant Human Erythropoietin Promotes Proliferation of Cervical Cancer Cell Lines in vitro and in vivo

Tania Lopez-Perez[1], Vilma Maldonado-Lagunas[2], Leticia Rocha-Zavaleta[1]
[1]*Department of Molecular Biology and Biotechnology, Biomedical Research Institute, National University of Mexico, Mexico City, Mexico,* [2]*Department of Biomedical Research in Cancer, National Cancer Institute, Mexico City, Mexico*

Human erythropoietin (EPO) is a hormone produced by the kidney that circulates into the bloodstream. EPO binds to its specific receptor (EpoR) on the surface of erythroid progenitors inducing their proliferation, survival and differentiation into mature erythocytes. Functional EpoR expression, together with EPO production, has also been documented in nonhematopoietic sites including some tumors. Since recombinant human erythropoietin (rHuEPO) is widely used in cancer patients to correct anemia several studies have evaluated its role in tumors. It has been suggested that EpoR may contribute to the development of these tumors. We focused on the study of the effect of rHuEPO in cervical cancer cell lines. Expression of EpoR was detected in cell lines HeLa, SiHa and C33 by flow cytometry. rHuEPO significantly increased proliferation of all cell lines. Pre-incubation with a neutralizing anti-EPO antibody, or with Lovastatin abated rHuEpo-induced proliferation. We also detected that rHuEPO promotes the growing of HeLa tumors in athymic female mice. Interestingly we observed that rHuEPO actived several members of the JAK/STAT pathway. Our data suggest that rHuEPO plays a critical role in proliferation of cervical cancer.

Poster No. 157

Bone Marrow Stromal Cell Gene Expression Profiles Associated with Increased Migration of Breast Cancer Cells in an In-vitro Co-culture System

Konstantin Koro[1], Stephen Parkin[1], Cay Egan[1], **Anthony Magliocco**[1]
[1]*Department of Oncology, University of Calgary, Calgary, AB, Canada*

Introduction:
The development of bone metastasis from breast cancer is a common and fatal complication of the disease. Understanding the biological mechanisms underpinning this process will be vital to the development of effective treatment modalities. The development of bone metastasis involves a complex series of events including bone homing, migration and invasion. We have developed a innovative co-culture system composed of breast cancer cells grown in association with bone stromal cells (BSCs) derived from orthopedic bone reamings from cancer free patients. This system enables in-vitro study of the interactions of breast cells and benign bone stromal cells. We have shown that primary bone derived stromal cell cultures are superior to HS68 fibroblast cultures in stimulating migration of MCF-7 and MDA-MB-231 breast cancer cells in transwell migration assays. We performed gene expression microarray analysis to detect the differences in gene expression patterns between BSCs and HS68 cells.
Materials and Methods:
Total RNA was isolated from cultures of HS68 and BSCs. Affymetrix HU133 Plus 2 GeneChip® arrays were used to analyze gene exprssion. Six isolates of BSCs were compared with three isolates of HS68 cells.
Results:
There were 471 differentially expressed genes using stringent criteria. Bioinformatics analysis indicated these genes were significantly more likely to cluster into developmental process pathways P=1.4E–10. Several messages coding for secreted molecules were also identified including Hepatocyte growth factor.
Conclusions:
The bone derived stromal co-culture system coupled with gene expression profile analysis is a powerful method to study the microenvironmental interactions leading to breast metastasis to bone.

Poster No. 158

Mural Cell Connexin 43 is Required for Inhibition of Endothelial Proliferation and is Inactivated by Tumor Cells

Mayur Choudhary[1], Wenhong Chen[1], Keith Barlow[1], Christine McMahan[1], **Linda Metheny-Barlow[1]**
[1]*Department of Radiation Oncology, Wake Forest University Health Sciences, Winston-Salem, NC, USA*

The tight contact between mural cells (vascular smooth muscle cells and pericytes) and the underlying endothelium stabilizes a mature blood vessel and renders the endothelium quiescent. In tumors, contact between mural cells and endothelial cells is decreased and abnormal, which allows tumor vessels to be leaky and proliferative. However, the mechanism by which tumors prevent proper association between mural cells and the endothelium is unknown. Since gap junction communication between mural cells and endothelial cells plays an important role in vessel communication and mural cell differentiation, we sought to determine the effects of tumors on the gap junction protein Connexin 43 (Cx43) on vascular cells. Here we demonstrate that short term treatment of mural cells with media conditioned by breast tumor cells stimulates a rapid and sustained inactivating phosphorylation of Cx43 at the protein kinase C (PKC) site Ser368, and that Cx43 is phosphorylated at this site on the vasculature of xenograft tumors. We found that longer term (24 hours) treatment of mural cells with media conditioned by breast or brain tumor cells leads to downregulation of Cx43 protein levels in mural cells, while media conditioned by actively proliferating monocytes lacks this activity. The decrease in Cx43 protein results both from decreased mRNA expression and proteasomal degradation of the protein. We have further demonstrated that functional Cx43 is required for mural cell-induced endothelial quiescence, as control siRNA transfected mural cells can reduce proliferation of co-cultured endothelial cells, while mural cells in which Cx43 has been knocked down by siRNA lack this activity. Our data are consistent with the notion that tumor cells can induce angiogenesis by disrupting or preventing junctional contacts between endothelial cells and mural cells, thus releasing endothelial cells from mural cell-induced quiescence. Vascular Cx43 may therefore represent a novel target for anti-angiogenic or vascular normalization strategies.
Supported in part by NIH CA138727.

Poster No. 159

Investigating a Role for CCN3 in the Promotion of Breast Cancer Metastasis to Bone

Veronique Ouellet[1,2], Jenna Fong[3], Svetlana Komorova[2,3,4], Bernard Perbal[5], Danh Tran-Tanh[6], Eitan Amir[7], Mark Clemons[7], Peter Siegel[1,2,8]
[1]*Goodman Cancer Centre, McGill University, Montreal, Quebec, Canada,* [2]*Department of Medecine, McGill University, Montreal, Quebec, Canada,* [3]*Department of Dentistry, McGill University, Montreal, Quebec, Canada,* [4]*Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada,* [5]*Research and Development, L'Oreal, Clark, New Jersey, USA,* [6]*Department of Pathology, The Princess Margaret Hospital, Toronto, Ontario, Canada,* [7]*Department of Orthopaedic Surgery, The Princess Margaret Hospital, Toronto, Ontario, Canada,* [8]*Department of Biochemistry, McGill University, Montreal, Quebec, Canada*

Breast cancer is the most frequent and the second most lethal cancer affecting women in Canada. The skeleton is a common site for breast cancer metastasis; however, the reasons for this are not fully understood. We have used mouse models to isolate 4 T1 breast cancer cell populations that aggressively metastasize to bone and have compared them to cells that are weakly bone metastatic. Through gene expression profiling, we have identified ccn3 (nov), which is expressed at higher levels in the aggressively bone metastatic cells versus those that weakly metastasize to bone. We have verified that our bone metastatic breast cancer cells overexpress ccn3 mRNA and that elevated levels of CCN3 protein are detected in the conditioned media of the bone metastatic 4 T1 sub-populations.
To determine the relevance of CCN3 expression in human breast cancer, we have interrogated ccn3 expression in publically available gene expression datasets and have observed a correlation between ccn3 expression and the luminal sub-type. These results are interesting in light of the fact that breast cancers that metastasize to the bone are most likely to be of the luminal subtype. Finally, we have performed immunohistochemical staining of CCN3 in bone metastases derived from patients with breast cancer and have found that CCN3 is expressed in every lesion (20/20). Together, these data implicate CCN3 as an interesting target associated with breast cancer bone metastasis.
Given the osteolytic nature of the bone metastases that develop in our 4 T1 breast cancer model, we wished to test the hypothesis that CCN3 plays a causal role in promoting the formation of osteolytic lesions through the inhibition of osteoblast differentiation. Using primary cultures of mouse bone marrow cells, we confirmed that a recombinant CCN3 protein impaired osteoblast differentiation. We are now investigating potential signaling pathways through which CCN3 may function to impair osteoblast differentiation and tip the balance towards enhanced osteoclastogenesis.

Poster No. 160

Differences in the Nemosis Response of Normal and Cancer-associated Fibroblasts from Patients with Oral Squamous Cell Carcinoma

Kati Räsänen[1], Ismo Virtanen[2], Reidar Grenman[3], Antti Vaheri[1]
[1]*Department of Virology, Haartman Institute, University of Helsinki, Finland,* [2]*Anatomy, Institute of Biomedicine, University of Helsinki, Finland,* [3]*Head and Neck Surgery, Turku University Central Hospital, Turku, Finland*

Tumor microenvironment plays a major role in cancer progression and activated fibroblasts, among them cancer-associated fibro-

blasts (CAFs), are key components of the tumor stroma. Nemosis is a novel type of fibroblast activation induced by cell-cell clustering. Formation of a fibroblast spheroid causes overexpression of genes involved in inflammation and tumor progression, resembling the expression pattern found in CAFs.

We used paired normal skin fibroblasts and cancer-associated fibroblasts and primary and recurrent oral squamous cell carcinoma (SCCs) cells. Nemosis response, as observed by induction of COX-2 and VEGF, HGF/SF and FGF7 and CAF markers a-SMA, FSP1 and FAP differed between these fibroblast populations. One of the normal fibroblast strains, FB-43, upregulated COX-2 in nemosis, but FB-74 cells did not. In contrast, CAF-74 spheroids expressed COX-2 but CAF-43 cells did not. Alpha-SMA protein was expressed in both CAF strains and in FB-74 cells, but not in FB-43 fibroblasts; its mRNA levels were downregulated in nemosis. FSP1 mRNA was downregulated in normal fibroblasts, but not in CAFs, whereas FAP was upregulated in all fibroblasts. Growth factor mRNA levels were upregulated to variable degree. CAFs increased the colony formation of primary tumor UT-SCC cell lines, but normal fibroblasts inhibited the anchorage-independent growth of recurrent UT-SCC cells.

These results clearly demonstrate that fibroblasts obtained from different individuals vary in gene expression and this is reflected in their capability to respond to nemosis. Nemosis, an *in vitro* model of fibroblast activation, may have its *in vivo* counterpart in cancer-associated fibroblasts and is a valuable tool in studying the variations between fibroblasts obtained from different individuals. Work on nemosis may also reveal new therapeutic means to modulate unwanted inflammation and tumor progression.

Poster No. 161

The Telomeric Protein TRF2 Controls a Cell-Extrinsic Anti-Cancer Barrier via Activation of Natural Killer Cells

Thomas Simonet[1], Adeline Augereau[1], Sébastien Pinte[1], Serge Bauwens[1], Béatrice Horard[1], Renée Grataroli[1], Antonella Stoppacciaro[2], Annamaria Biroccio[3], Carlo Leonetti[3], Cécile Cognet[4], Eric Vivier[4], Eric Gilson[1]

[1]*Laboratory of Molecular Biology of the Cell, CNRS UMR5239, Ecole Normale Supérieure de Lyon, Lyon, France,* [2]*Experimental Medicine and Pathology Department II, Faculty S. Andrea, Rome, Italy,* [3]*Regina Elena Cancer Institute, Rome, Italy,* [4]*Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, Marseille, France*

Several recent data showed that deprotected telomeres can suppress oncogenesis by engaging senescence or apoptosis, providing an explanation for the up-regulation of telomerase observed in the vast majority of human cancers. Interestingly, an increased dosage of TRF2, a key factor to preserve telomere protection and acting independently of the telomerase pathway, is also observed in various human malignancies and contributes to carcinogenesis in mice. However, very little is known on the role of TRF2 in cancer. We demonstrate here that a reduced activity or expression of TRF2 in human tumor cells can impair their ability to form xenografts in immunocompromised mice without engaging a cell intrinsic program of proliferation arrest. Strikingly, this antitumor effect does not correlate with overt telomere deprotection, DNA damage response and senescence. These data suggest that cell extrinsic mechanisms limit tumor formation upon TRF2 inhibition. We further demonstrate that the anti-tumor properties of TRF2 inhibition rely on activation of natural killer (NK) cells. These findings suggest that the overexpression of TRF2 observed in different types of human cancer contributes to bypass innate immunosurveillance. Consequently, TRF2 emerges as a multifunctional oncogenic protein and a promising therapeutic target.

Poster No. 162

Therapy of Minimal Residual Tumour Disease: β-galactosylceramide Inhibits Growth of Recurrent HPV16-associated Neoplasms after Surgery and Chemotherapy

Jana Šímová[1], Marie Indrová[1], Jana Bieblová[1], Romana Mikyšková[1], Jan Bubeník[1], Milan Reiniš[1]

[1]*Department of Tumour Immunology, Institute of Molecular Genetics AS CR, Prague, Czech Republic*

Natural killer T (NKT) cells are potent modulators of anti-tumour immunity. Their protective effects can be achieved upon their activation by glycolipid ligands presented in the context of the CD1d molecule. These CD1d-binding glycolipid antigens have been described as potent therapeutic agents against tumours, infections, as well as autoimmune diseases. On the other hand, their repeated administration can result in NKT cell anergy and serious adverse effects. Immunoregulatory and therapeutic effects of glycolipid ligands depend on their structure and modes of administration. Therefore more studies are needed for optimization of the particular therapeutic settings. This study was focused on tumour-inhibitory effects of 12 carbon acyl chain β-galactosylceramide (C12 β-D-GalactosylCeramide) on the growth of HPV16-associated neoplasms transplanted in the syngeneic mice. Treatment of tumour bearing mice with β-galactosylceramide 3–14 days after transplantation of tumour cells significantly inhibited growth of the MHC class I-positive (TC-1), as well as MHC class I-deficient (TC-1/A9) HPV16-asssociated tumours. Moreover, administration of β-galactosylceramide after surgical removal of TC-1 tumours inhibited growth of tumour recurrences. Similar results were obtained in the treatment of the tumours after chemotherapy. Beta-galactosylceramide treatment turned out to be also synergistic with immunotherapy based on administration of IL-12-producing cellular vaccines. These results suggest that β-galactosylceramide, whose antitumour effects have not been studied in detail, can be effective for treatment of minimal residual tumour disease as well as an adjuvant for cancer immunotherapy.

Poster No. 163

TNF-α Fosters Mammary Tumorigenesis Contributing to Efficient Tumor Vascularization and to Pro-Tumoral Phenotype of Tumor Associated Macrophages

Claudia Chiodoni[1], Sabina Sangaletti[1], Claudio Tripodo[2], Chiara Ratti[1], Rossana Porcasi[2], Rosalba Salcedo[3], Giorgio Trinchieri[3], Mario Paolo Colombo[1]
[1]*Department of Experimental Oncology, Immunotherapy and Gene Therapy Unit, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy,* [2]*Dipartimento di Patologia Umana, Università degli Studi di Palermo, Palermo, Italy,* [3]*Center for Cancer Research, Cancer and Inflammation Program, National Cancer Institute, Frederick, Maryland, USA*

Solid tumors comprise tumor cells and surrounding stromal cells, mostly of hematopoietic origin. Cancer cells and infiltrating leukocytes communicate through a complex network of pro-inflammatory molecules; among them critical are the transcription factor NF-kB and the inflammatory mediator TNF-α, which, through a multifaceted interaction, eventually promote cancer development and progression, at least in some tumor types. We have investigated the role of TNF-α in HER-2/neuT (NeuT) transgenic mouse model of mammary carcinogenesis spontaneously developing carcinomas during life time. Bone-marrow transplantation (BMT) experiments from TNF-α KO mice into NeuT recipients significantly delay the onset and reduce the number of affected mammary glands, indicating that the relevant source of TNF-α fostering tumor promotion is of BM origin. BMT experiments performed at different time points during tumor progression (8, 15, 20 weeks of age) indicate that TNF-α is critical in early steps of mammary tumorigenesis but still active also at later time points when carcinomas in situ and invasive carcinomas are already present. Analysis of tumor organization and vasculature points out significant differences in the two types of chimera: wild type-transplanted mice show a well-differentiated nest-like growth pattern, branching fibrovascular stromal meshwork with structured vessels, and limited foci of epithelial necrosis, whereas tumors from TNF-α-KO-transplanted mice display a disorganized structure with gross stromal axes and defective vascularization; extended necrosis, involving also the stroma and perivascular areas, is present. Immunohistochemistry for leukocyte infiltration indicates that the lack of TNF-α alters the number and localization of some leukocyte subtypes, in particular CD206+ cells that are highly represented throughout the stroma of tumors from wild type chimeras, and scarce in tumors from TNF-α deficient chimeric animals. From this work we expect to uncover the role of TNF-α in the various phases of mammary transformation and progression and to identify the best time window to neutralize its activity using specific monoclonal antibody.

Poster No. 164

Tumor Infiltrating Lymphocytes in CpG Island Methylator Phenotype (CIMP) Subgroups of Colorectal Cancer in Relation to Prognosis

Anna M. Dahlin[1], Bethany Van Guelpen[1], Maria L. Henriksson[1], Maria Jacobsson[1], Vincy Eklöf[1], Roger Stenling[1], Jörgen Rutegård[2], Åke Öberg[2], Richard Palmqvist[1]
[1]*Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden,* [2]*Department of Surgical and Perioperative Sciences, Surgery, Umeå University, Umeå, Sweden*

Background: Even though colorectal cancer patient prognosis depends to a large extent on tumor stage, complementary markers are needed. It is well-known that a high degree of infiltrating lymphocytes in and around the tumor improve patient prognosis. Recently the CpG Island methylator phenotype (CIMP), characterized by a high degree of hypermethylation, has been associated with disease outcome. Furthermore, patients with tumors displaying microsatellite instability (MSI) have a better prognosis compared to microsatellite stable (MSS) tumor patients. A high degree of infiltrating lymphocytes is a common feature of MSI tumors, whereas the level of inflammatory response is not well established in CIMP-high tumors.
Aim: To characterize the level of lymphocytic infiltration in CIMP-negative, CIMP-low, and CIMP-high tumors and relate findings to patient prognosis.
Methods: CIMP-status was determined in 499 colorectal cancer patients with quantitative real-time methylation-specific PCR (MethyLight). Immunohistochemistry (anti-CD3) was used to quantify t-lymphocytes infiltrating the tumor (TIL) and tumor stroma (in tumor front and centre).
Results: A high level of infiltrating lymphocytes was associated with a better prognosis independent of tumor stage and in all subgroups of colorectal cancer based on CIMP- and MSI-status. In CIMP-low tumors, a high degree of lymphocytes in the tumor centre was associated with an excellent prognosis (5-year cancer specific survival 91.3%). 5-year cancer specific survival in MSI tumors with a high degree of lymphocytes in the tumor front was 91.1%, while the prognosis of patients with MSI tumors with lower degrees of lymphocytic infiltration was similar to MSS tumors (60.0 and 60.2%, respectively).
Conclusion: The survival advantage of a higher level of infiltrating lymphocytes is more distinct in certain subgroups of colorectal

cancers based on CIMP- and MSI-status. These findings may facilitate a refined assessment of patient prognosis.

Poster No. 165

Space-Time Organization of T Lymphocytes in Human Tumor-induced Tertiary Lymphoid Structures

Luc de Chaisemartin[1,2,3], Jérémy Goc[1,2,3], Fella Tamzalit[1,2,3], Diane Damotte[1,2,4,6], Pierre Validire[1,2,5], Ludivine Laurans[1,2], Wolf-Herman Fridman[1,2,4], Catherine Sautès-Fridman[1,2,4], Marie-Caroline Dieu-Nosjean[1,2]

[1]*Team 13 Immunological Micro-environment and tumors, INSERM UMRS 872, Paris, France,* [2]*Centre de Recherche des Cordeliers, Paris, France,* [3]*Université Pierre et Marie Curie, Paris, France,* [4]*Université René Descartes, Paris, France,* [5]*Anatomo-pathologie, Institut Mutualiste Montsouris, Paris, France,* [6]*Anatomo-pathologie, Hôpital Hotel-Dieu, Paris, France*

The importance of the immune infiltrate in the prognosis of human cancers is now clearly established. In a retrospective study on non-small cell lung cancer (NSCLC), we have previously shown that high density of tumor-induced tertiary lymphoid structures is associated with patients' long-term survival. We thus wanted to explore the structural and cellular organization of these structures that we called Ti-BALT (Tumor-induced Bronchus-Associated Lymphoid Tissue) and their relevance in local immune responses against cancer. We used fresh human lung tumor specimens from surgical resection of NSCLC, as well as slides from paraffin-embedded and frozen biopsies. The expression of relevant molecules was assessed on fresh tumor-infiltrating lymphocytes by multicolor flow cytometry, and on biopsy slides by enzymatic and fluorescent immunohistochemistry. By Laser-microdissection, specific RNA from frozen slides was isolated for RT-PCR analysis. We showed that the Ti-BALT T cells are predominantly CD4+ central memory T cells and CD8+ naive T cells. These cells all express the CD62L marker and are in close contact with PNAd+High Endothelial Venules. We investigated the role of the chemokine system in lymphocyte migration by assessing chemokine receptor (CKR) presence on T cells subsets and found significant differences of CKR expression according to the subset. To confirm the potential role of the CKR expressed, local chemokines expression was measured on micro-dissected Ti-BALT.

We characterized T lymphocytes populations found in tumor-induced tertiary lymphoid structures and found several ligand-receptor interactions potentially implied in their migration to these structures. Knowing how these structures are organized will help to understand their prognostic value in lung cancer.

Poster No. 166

Expression and Function of the Chemokine CX3CL1 and its Receptor CX3CR1 in Human Colorectal Cancer

Marco Erreni[1,3], Giuseppe Celesti[2], Paolo Bianchi[2], Luigi Laghi[2], Fabio Grizzi[2], Graziella Solinas[1,3], Samantha Pesce[1], Alberto Malesci[2], Alberto Mantovani[1,3], Paola Allavena[1]

[1]*Istituto Clinico Humanitas, IRCCS, Rozzano, Milano, Italy,* [2]*Department of Gastroenterology, Istituto Clinico Humanitas, IRCCS, Rozzano, Milano, Italy,* [3]*Dipartimento di Medicina Traslazionale, Università degli Studi di Milano, Milano, Italy*

Experimental and epidemiological studies indicate a strong link between persistent inflammation and tumor progression, and human colorectal cancer (CRC) represents a paradigm in this connection. Major players of the cancer-related inflammation are chemokines and their receptors. Fractalkine (CX3CL1) is a peculiar chemokine, existing both as a soluble and a membrane-anchored protein. Its unique receptor, CX3CR1, is expressed on monocytes, NK, and T cells. In this study we provide evidence that CX3CL1 is expressed in human colorectal carcinoma and may modulate tumor malignant behaviour. CX3CL1 mRNA expression, evaluated in 30 CRC samples was strongly up-regulated in tumor tissues in comparison to normal colonic mucosa. CX3CL1 protein expression has been evaluated by immunohistochemistry in 172 CRC samples, classified by tumor stage, confirming a strong positivity by tumor cells. On the same series of samples, the expression of CD3 and CD68 is being investigated by immunohistochemistry and the density of tumor-infiltrating T lymphocytes and macrophages will be associated with the expression score of CX3CL1, as well as with clinical outcome of patients. Intriguingly, the receptor CX3CR1 was found expressed also by tumor cells, with a heterogeneous pattern of positivity. To better characterize the significance of the CX3CL1/CX3CR1 interaction in CRC, a multi-cellular tumor spheroids (MTS) *in vitro* assay was performed, with CRC cell lines characterized by the expression of Fractalkine and its receptor. Preliminary results indicate that both CX3CL1 and CX3CR1 are expressed by all the MTS forming cells, and that CX3CL1 is predominantly expressed by cells at the periphery of the spheroids. These data indicate a role of CX3CL1 and CX3CR1 within cancer cell interaction and in the cancer cells-immune cells cross-talk.

Poster No. 167

Pancreatic Stellate Cells – Sentinels for Tissue Damage?

Christine Feig[1], David Tuveson[1]
[1]*Tumour Modelling and Experimental Medicine (Pancreatic Cancer), Cambridge Research Institute/Cancer Research UK, Cambridge, UK*

Pancreatic cancer is the 6th leading cause of cancer deaths in the European Union. The most common malignancy is pancreatic ductal adenocarcinoma (PDA), which is almost uniformly lethal. Epidemiological and molecular studies exhibit a robust link between chronic inflammation and pancreatic cancer. Tissue injury due to premature activation of digestive enzymes is a well-described cause of hereditary chronic pancreatitis. These patients have a 100-fold increased risk of developing PDA.
Hallmarks of PDA and chronic pancreatitis are the replacement of pancreatic parenchyma with fibrotic tissue and the accumulation of immune cells with suppressive phenotypes (myeloid derived suppressor cells and regulatory T cells (Treg)). The fibrotic stroma is thought to originate from pancreatic stellate cells (PSC), a rare cell type in the healthy pancreas that, when activated, takes on a myofibroblastic phenotype. However, their physiological function and whether they are involved in creating the immunosuppressive microenvironment is unknown. Pancreatic stellate cells are (in conjunction with hepatic stellate cells) the major storage for vitamin A. Retinoic acid is an essential component for peripheral Treg priming. Immunoregulatory function has been ascribed to hepatic stellate cells. We hypothesize that PSC are tissue sentinels with antigen-presenting function responding to tissue injury by inducing an immunosuppressive response. We show that PSC express Toll-like receptors (TLR) and upon activation upregulate co-stimulatory molecules and MHCII in a mouse model of acute pancreatitis. PSC may thus be able to sense danger associated molecular patterns (DAMP) and respond by priming Treg. Therefore, the default program for non-infectious activation of PSC may be to curb excessive immune responses preventing an autoimmune attack. However, this protective program suitable for resolving acute tissues distress may be devastating in circumstances of repetitive irritation such as during chronic inflammation and pancreatic cancer precluding an immune response against the developing tumour.

Poster No. 168

Presence and Characterization of Th17 Cells in the Tumoral Microenvironment of Primary Intraocular B-cell Lymphoma

Claire Galand[1], Valérie Touitou[1], Cécile Daussy[1], Sabrina Donnou[1], Bahram Bodaghi[1], Wolf Herman Fridman[1], Catherine Sautès Fridman[1], Sylvain Fisson[1]
[1]*Department of Immune Microenvironment and Tumors (team 13), Centre de Recherche des Cordeliers, INSERM UMRS 872, Paris, France*

Despite the important role of Th17 cells in the pathogenesis of many autoimmune diseases, their presence and role in cancer remain unclear. In this work, we investigated the presence of these cells and their related cytokines in a new syngeneic model of primary intraocular B-cell lymphoma (PIOL) which is a subtype of non Hodgkin lymphomas. This model was chosen because there is no resident lymphocyte in a normal eye, so it is easier to characterize the different lymphocyte subsets recruited by the tumor. The lymphomatous B-cell line A20-IIA1.6 ($H2^d$) was injected in the posterior chamber of immunocompetent BALB/c mice ($H2^d$) and flow cytometric analysis were performed to study the tumor growth and the immune infiltrate. Concomitantly to the presence of prepolarized Th1 lymphocytes and CD4+Foxp3+ cells, Th17 cells were found and characterized by the intracellular expression of IL-17 and IL-21, but no IFNg. At the molecular level, RT-PCR analysis demonstrated the ocular expression of the messengers for IL-17, IL-21 and IL-23. Interestingly, IL-17 protein level measured by cytometric beads array showed an inverted correlation with the tumor burden. These data demonstrate that a local infiltration of IL-17 and IL-21 secreting cells occurs in a tumoral context, and it seems that Th17-related cytokines counteract the tumor development. Thus, use of Th17 cells or their related cytokines should be considered as a new therapeutic approach for non Hodgkin B-cell lymphomas and particularly with an ocular localization.

Poster No. 169

AS101 Attenuates the Severity of DSS- Induced Murine Colitis: Association with IL-17 Inhibition

Gilad Halpert[1], Yona Kalechman[1], Lea Rath-Wolfson[2], Benjamin Sredni[1]
[1]*Safdié Institute for AIDS and Immunology Research The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel,* [2]*Department of Pathology, Rabin Medical Center.Golda Campus, Petah Tikva, Israel*

Ulcerative colitis (UC) and Crohn's disease (CD) are the major chronic inflammatory bowel diseases (IBD) affecting the gastrointestinal tract (GI). UC primarily affects the mucosal lining of the colon, whereas CD affects the whole GI. Defective mucosal barrier triggers invasion of commensal enteric bacteria into the gut layers that result in aggressive immune responses. Feeding mice for several days with Dextran Sodium Sulfate (DSS) polymers in the drinking water induces acute colitis characterized by bloody diarrhea, ulceration, body weight loss and infiltration with granulocytes/mononuclear cells, reflecting human's symptoms. The present study was designed to explore the ability of the anti-inflammatory immunomodulator, ammonium tichloro [1,2-ethanediolato-O,O'] tellurate (AS101) to attenuate the severity of DSS-induced murine colitis. C57BL/6 mice received 3.5% w/v DSS in the drinking water for 7 days followed by 5 days of regular autoclaved water. Daily treatment with AS101 starting either concomitantly with DSS or 2 days later, significantly reduced occult and visible blood score vs. the DSS+PBS group. Further-

more, both treatment modes with AS101 significantly ameliorated the stool consistency score and prevented the decrease in body weight. Colon length, being much reduced in diseased mice was normalized in AS101-treated mice. Histopathology examination of the distal colon revealed destruction of the crypt structure in PBS-treated mice. Furthermore massive mononuclear cell infiltration into the mucosa and submucosa were found. In comparison, the colons of AS101-treated mice exhibited normal appearance. Treatment with AS101, either before or after disease onset, significantly reduced the inflammatory cytokine IL-17 in the colon while only AS101 given concomitantly with DSS also reduced colonic INF-γ.
These results collectively propose that inhibition of colon IL-17, and not that of INF-γ, plays an important role in attenuating murine colitis by AS101 and suggest that treatment with AS101 may be an effective therapeutic approach for controlling human IBD.

Poster No. 170

A Chimeric NKG2D/CD28/CD3 Transmembrane Type I Receptor Expressed in Human T Cells for Targeting Ewings Sarcoma

Manfred Lehner[1], Gabriel Götz[1], Florian Full[2], Armin Ensser[2], Niels Schaft[3], Jan Dörrie[3], Hinrich Abken[4], Adelheid Cerwenka[5], Peter Ambros[6], Heinrich Kovar[6], Wolfgang Holter[1]
[1]*Kinderklinik, Universitätsklinik, Erlangen, Germany,* [2]*Institut für klinische und molekulare Virologie, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany,* [3]*Dermatologie, Universitätsklinik, Erlangen, Germany,* [4]*Interne Medizin, Universität Köln, Köln, Germany,* [5]*Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany,* [6]*Children's Cancer Research Institute (CCRI), St. Anna Kinderspital, Wien, Austria*

The activating NK cell receptor NKG2D recognizes a number of evolutionary conserved ligands, which are expressed on many transformed but not on most normal cells. We analyzed the expression of NKG2D ligands in Ewing's sarcoma (EWS) and found expression in the majority of the tested cell lines, providing opportunities for NKG2D based immunotherapy of EWS. We report the construction of a chimeric NKG2D immunoreceptor by linking the extracellular ligand domain of NKG2D in reverse orientation to an IgG1-Fc/CD28/CD3zeta transmembrane signaling platform creating a chimeric type I transmembrane immunoreceptor. Primary human T cells transformed with this chNKG2D molecule expressed by either a lentiviral vector or electroporated mRNA recognize and efficiently lyse murine B cells expressing ULBP2 or MICA. Also, ligand specific stimulation of the lentivirally transduced T cells resulted in efficient long term expansion and enhanced expression density of the chNKG2D receptor. Coculture of EWS cell lines with either lentivirally transduced or mRNA transfected activated human T cells resulted in chNKG2D specific cytokine secretion and revealed high susceptibility of EWS to CD8+ and CD4+ T cell mediated cytotoxicity. These data provide the basis for further exploring the potential of a chNKG2D based immunotherapy of EWS.

Poster No. 171

IL-17 Production by γδ T Cells in Tumor Microenvironment is Involved in Shaping the Anti-Tumor Response

Yuting Ma[1], Pablo Pereira[2], Laetitia Aymeric[1], Laurent Boucontet[2], Laurence Zitvogel[1]
[1]*INSERM U805, Institut Gustave Roussy, Villejuif, France,* [2]*Unité du Développement des Lymphocytes, Institut Pasteur, Paris, France*

Our previous work showed that successful anticancer chemotherapy is dependent on CTL and IFN-γ while specific CTL priming triggered by dying tumor cells is dependent on IL-1β. Here, we demonstrated that after chemotherapy and radiotherapy, IFN-γ-producing CTL infiltrated much more intensively into tumor bed of tumor regressors compared with that of tumor progressors and untreated control. Meanwhile, tumor infiltrating γδ cells potently produced IL-17 but not IFN-γ and they were the major source of IL-17 in tumor beds of treated mice, especially in regressing tumor bed. Furthermore, the IL-17 producing γδ TILs have dominant preferential usage of Vγ4 and Vγ6. Interestingly, IFN-γ production by CD8+ TILs is closely correlated with IL-17 production by γδ TILs. Neutralizing IL-17 resulted in failure of chemotherapy in MCA205 tumor model. As we know, γδ T cells from naïve LN potently produce IL-17 upon PMA/IO stimulation. We also discovered that these γδ T cells could vigorously produce IL-17 in response to IL-1β or/and IL-23 without TCR ligation ex vivo. Moreover, IL-1β and IL-23 are much more enriched in treated tumor bed. Our data suggested that γδ T cells play a pivotal role in the success of chemotherapy by shaping and modulating host immune response to cancer through producing IL-17.

Poster No. 172

Systemic Candida Albicans Infection Promotes Inflammation-Dependent Hepatic Metastasis via Mannoprotein-Dependent Endothelial Activation

Joana Marquez[1], Beatriz Arteta[1], Aritz Lopategi[1], Juan Rodriguez[1], Andoni Ramirez[2], Fernando Hernando[2], Natalia Gallot[3], Lorea Mendoza[3], Fernando Vidal-Vanaclocha[1]
[1]*Department of Cell Biology and Histology, Basque Country University School of Medicine, Leioa, Bizkaia, Spain,* [2]*Department of Microbiology and Immunology, Basque Country University School of Sciences and Technology, Leioa, Bizkaia, Spain,* [3]*Pharmakine SL, Bizkaia Technology Park, Derio, Bizkaia, Spain*

Candida albicans is an opportunistic fungal pathogen and a major cause of morbidity in cancer patients whose immune system is compromised. Candida albicans infection involves host production of inflammatory cytokines such as interleukin (IL)-18 and tumor necrosis factor (TNF)-alpha, whose augmentations have already been correlated with metastatic occurrence of most common cancer types. However, whether the concurrent infection of this

fungal pathogen during cancer cell dissemination affects metastasis occurrence is unclear. In this study, a well-established murine model of TNFalpha/IL-18-dependent hepatic melanoma metastasis was used to study whether Candida albicans isolated from patients with systemic candidiasis can alter the ability of murine B16 melanoma (B16M) cells to colonize the liver. We demonstrated that Candida albicans increased the metastatic efficiency of B16M cells in the liver, irrespective of fungus injection route. Prometastatic effects were abrogated with antifungal ketoconazol treatment, and occurred when hepatic colonization of cancer cells took place 12 hours after Candida albicans injection. Pre-infection status also enabled a low-metastatic dose of B16M cells to metastasize in the liver at levels indistinguishable from normal mice receiving a highly-metastatic cancer cell dose. Candida albicans also accelerated the growth of established micrometastases, when mice received the fungus 4 days after cancer cell injection. Circulating candida albicans adhered to hepatic sinusoidal endothelium (HSE). They also induced TNFalpha production from HSE in vitro, which in turn enhanced endothelial cell adherence for cancer cells. Similar results were obtained when HSE cells were incubated with mannoprotein extracts from the same Candida albicans strains instead of live Candida albicans, suggesting that Candida albicans produced the remote activation of HSE via soluble mannoproteins. The HSE response to soluble mannoproteins occurred via specific mannose receptors, and the microenvironment generated by activated HSE may facilitate the ability of cancer cells to metastasize into Candida albicans-susceptible sites acting as premetastatic niches.

Poster No. 173

Tumor Infiltrating Lymphocyte Migration trough HEV Like Vessels

Ludovic Martinet[1], Ignacio Garrido[1], Philippe Rochaix[2], Jean-Philippe Girard[1]
[1]*Cancer biology department, Institut de Pharmacologie et de Biologie Structurale- CNRS UMR 5089, Toulouse, France,* [2]*anatomopathology department, Institut Claudius Régaud, Toulouse, France*

The degree of cytotoxic T lymphocyte infiltration is highly correlated with the clinical outcome of cancer patients. Tumor antigen specific T lymphocyte migration from the circulation into tumor tissues is tightly controlled by endothelial cells expression of multiple receptors such as integrins and vascular selectins. Analysis of tumor endothelium / leukocyte interaction could allow the development of novel approaches to improve the number of tumor infiltrating lymphocytes and immune therapy. Our group has more than 15 years expertise in the molecular characterisation of High endothelial venules (HEVs), specialized post-capillary venules found in lymphoid tissues that mediate high levels of naïve T lymphocyte recruitment from the blood. HEV-like vessels that are similar to HEVs from lymphoid tissues, also appear in chronically inflamed tissue and have been proposed to participate in the amplification and maintenance of chronic inflammation in auto-immune diseases. In collaboration with the Institute Claudius Regaud, we recently identified in human tumor tissues from melanoma, ovary and breast carcinoma patients, venules with HEV-characteristics. Like their lymph node counterparts, tumor HEVs display a cuboidal shape and express functional PNads (Peripheral node adressins) allowing the recruitment of $CD62L^+$ lymphocytes. In a mouse tumor model, induction of HEV-like vessels has been shown to allow naive lymphocyte recruitment, priming, and eradication of tumor cells. Therefore, although detrimental in chronic inflammatory diseases, presence of HEV-like vessels could be beneficial in human cancer. Indeed, we observed that within human tumors, HEV-like vessels were present in areas of effector memory $CD8^+$ T lymphocytes infiltrates in close contact with mature dendritic cells. A better understanding of the molecular mechanisms controlling HEV phenotype and functions may have important applications in cancer therapy for enhancing lymphocyte recruitment into tumors.

Poster No. 174

Inflammatory and Proliferative Effects on Prostate Epithelium by *Propionibacterium acnes* Infection

Jan Olsson[1,2], Sabina Davidsson[2], Johanna Drott[1,2], Oleg Alexeyev[3], Stina Rudolfsson[3], Bo Söderquist[4], Ove Andrén[5], Anders Bergh[3], Fredrik Elgh[1,2]
[1]*Department of Clinical Microbiology, Umeå University, Umeå, Sweden,* [2]*School of Health and Medical Sciences, Örebro University, Örebro, Sweden,* [3]*Department of Biomedical Sciences/Pathology, Umeå University, Umeå, Sweden,* [4]*Department of Clinical Microbiology, University Hospital Örebro, Örebro, Sweden,* [5]*Department of Urology, University Hospital Örebro, Örebro, Sweden*

Bacterial infections of the prostate are increasingly recognized as a potential risk factor for development of proliferative diseases as benign prostate hyperplasia and cancer. A phase of infection-induced inflammation is believed to precede the malignant state. We and others have identified the Gram-positive facultative anaerobic bacterium *Propionibacterium acnes* as a frequent inhabitant of prostate tissue. Currently, we are investigating *P.acnes* prevalence in prostatectomy tissue, genetic variance of isolates from prostate contra other loci, and the inflammatory and proliferative effects of the bacterial infection.
Here we present result obtained from experimental infections of cultivated prostate epithelial cells and rat prostate.
The bacterial infection was shown to induce a strong TLR2 mediated inflammatory response as seen as up-regulation and secretion of IL-6, IL-8, GM-CSF, TNF-α, G-CSF, and CCL2. In a rat prostate infection model, the *P.acnes* infection induced strong inflammation, as seen as recruitment of lymphocytes. 4 weeks post infection, foci of intense inflammation and remaining bacteria could still be visualized. The

tissue in close proximity to the infested areas exhibited increased proliferative activity, scored as brdU incorporation.
We are presently collecting *P. acnes* from prostatectomy samples, urethra and perineal skin from 100 patients, and can preliminary score the frequency of infection, both in cancerous and benign prostate tissues to 60%. Given the high prevalence in human prostates, we suggest that bacterial infections, and especially *Propionibacterium acnes*, contribute to prostate inflammation and thus contribute to a proliferation stimulating environment that facilitate the transition of prostate epithelium into higher rate of proliferation and thus disorders as hyperplasia and cancer.

Poster No. 175

Tumor Infiltrating Lymphocytes in Pancreatic Cancer

Sabita Rakshit[2], Matthias Hebrok[1], **Marina Pasca di Magliano[2]**
[1]*Diabetes Center, University of California, San Francisco, CA, USA,* [2]*Department of Surgery, University of Michigan, Ann Arbor, MI, USA*

Background: Pancreatic cancer, one of the most deadly human malignancies, is characterized by an extensive stroma, which includes fibroblasts, inflammatory cells and vascular components. Among the inflammatory cells, the components of the adaptive immune system, T- and B-lymphocytes, are abundantly represented. The contribution of the adaptive immune system in cancer is controversial, with evidence supporting its role as a protective mechanism against tumor growth, and some contradicting evidence indicating that lymphocytes contribute to maintaining a chronic inflammatory environment that favors tumor progression. In pancreatic cancer, clinical studies have shown that the quantity and class of lymphocytes located within a tumor correlate with patient survival. Studies in the Kras mouse model of pancreatic cancer that closely recapitulates the human disease have shown that regulatory T cells represent the majority of the infiltrating lymphocytes during pancreatic tumorigenesis, leading to the assumption that anti-tumor immune activity is absent in pancreatic cancer.
Objective: *Functional studies addressing the role of the adaptive immune system in pancreatic cancer are currently missing.* We set out to investigate how lymphocytes affect pancreatic tumorigenesis.
Results: We generated Kras mice that are Rag deficient (Kras; Rag-), and thus lack all T- and B- lymphocytes and compared them with Rag positive littermates (Kras;Rag+).
Surprisingly, Kras;Rag- mice showed earlier onset and higher number of pancreatic cancer precursor lesions compared to Kras; Rag+control animals. Our data indicates that absence of lymphocytes has a tumor-promoting effect in pancreatic cancer. We are currently investigating whether an immuno-surveillance mechanism is present in the early stages of pancreatic cancer, or whether a different mechanism is responsible for the faster tumor progression in Kras;Rag- mice.

Poster No. 176

Analysis of Infiltrating Cytotoxic, Th1, Th2, Treg and Th17 Cells in Patients with Colorectal Cancer: Impact on Clinical Outcome

Marie Tosolini[1], Bernhard Mlecnik[1], Amos Kirilovsky[1], Gabriela Bindea[1,2], Anne Berger[3], Tchao Meatchi[4], Zlatko Trajanoski[2], Wolf-Herman Fridman[1,5], Franck Pagès[1,5], Jérôme Galon[1]
[1]*INSERM U872 Integrative Cancer immunology (Team 15), Cordeliers Research Center,, Paris, France,* [2]*Graz University of Technology, Institute for Genomics and Bioinformatics, Graz, Austria,* [3]*Assistance Publique-Hôpitaux de Paris, AP-HP, Department of General and Digestive Surgery, Georges Pompidou European Hospital, Paris, France,* [4]*Assistance Publique-Hôpitaux de Paris, AP-HP, Department of Pathology, Georges Pompidou European Hospital, Paris, France,* [5]*Assistance Publique-Hôpitaux de Paris, AP-HP, Department of Immunology, Georges Pompidou European Hospital, Paris, France*

Objectives: The type, density, and location of immune cells in colorectal cancer have a prognostic factor superior and independent to the criteria related to the anatomic extent of the tumor (Galon et al., Science 2006). The aim of the study was to analyze the balance between the cytotoxic and helper T-cells in colorectal cancer and the impact on disease-free survival.
Methods: The tumor microenvironment was investigated in 107 frozen colorectal tumor samples by analyzing the expression of immune-related genes by Low-Density-Array on real-time PCR Taqman 7900 HT. Infiltrating cytotoxic T cells, Treg, Th1, Th17 cells of colorectal cancer patients were quantified by immunohistochemical analyses of tissue microarrays containing tissue cores from the center and from the invasive margin of the tumor. For pairwise comparisons of parametric and non-parametric data and for survival analysis, the Student's t-test,Wilcoxon rank-sum test and Logrank test were used, respectively.
Results: We first investigated the expression of cytotoxic, Th1, Th2, Treg and Th17 genes. Hierarchical clustering of a correlation matrix revealed functional clusters of genes associated with Th17 (RORC, IL17A), Th2 (IL4, IL5, IL13), Th1 (Tbet, IRF1, IL12Rb2, STAT4), and cytotoxicity (GNLY, GZMB, PERF1). High-IL17A mRNA expression level was most frequent at early stages of tumor progression. Patients with high expression of the Th17 cluster had a poor prognosis whereas patients with high expression of the Th1 cluster had prolonged disease-free survival. In contrast no prediction of the prognosis was associated with the Th2 clusters. The combined analysis of cytotoxic/Th1 and Th17 clusters gave a better discrimination for relapse. In situ analysis of IL17+ cells and CD8+ cells using tissue-microarray confirmed with these results.
Conclusion: Functional clusters associated with Th1 and Th17 cells have opposite effect on patients survival and bring complementary information.

Poster No. 177

The Effect of hCaMKIINa on TLR4-Triggered Cytokine Production of Colon Cancer Cells

Chunmei Wang[1], Nan Li[1], Xingguang Liu[1], Qinghua Zhang[1], Xuetao Cao[1]
[1]*National Key Laboratory of Medical Immunology and Institute of Immunology, Second Military Medical University, Shanghai, China*

Increasing evidences suggest that chronic inflammation contributes to cancer development and progression. One of the underlying mechanisms is proposed that tumor cell-derived inflammatory and immunosuppressive cytokines contribute to tumor immune escape and resistance to immunotherapy. Toll-like receptors (TLRs) have been implicated in tumor progression and metastasis. Our previous study showed that calcium/calmodulin-dependent protein kinase II (CaMKII) promoted TLR-triggered proinflammatory cytokine in macrophages. hCaMKIINa, a novel CaMKII inhibitory protein identified by us, suppressed the growth of colon cancer cell by inducing cell cycle arrest *in vitro* and *in vivo*. Thus we wonder whether hCaMKIINa-mediated CaMKII inhibition affects TLR4-triggered cytokine production of colon cancer cells for immune escape. In this study, we demonstrate that TLR4 is expressed on human colon cancer cell lines. TLR4 ligation promotes production of immunosuppressive cytokines IL-8 and VEGF. Overexpression of hCaMKIINa inhibits TLR4-triggered production of IL-8 and VEGF; H282R, constitutive activated CaMKII, significantly promotes TLR4-triggered IL-8 and VEGF secretion. In addition, we also observe that hCaMKIINa inhibits LPS-mediated activation of p-ERK1/2 and LPS-mediated TLR4 expression in SW620 cells. Furthermore, hCaMKIINa-mediated inhibition of ERK1/2 is necessary for suppression of TLR4-triggered IL-8 and VEGF secretion. These results suggest that hCaMKIINa-mediated CaMKII inhibition might play important roles in the suppression TLR4-triggered metastasis and immune escape of human colon cancer cells by inhibiting immunosuppressive cytokine production.

Poster No. 178

Tumor-Derived Adenosine Enhances Generation of Adaptive Regulatory T (Tr1) Cells

Theresa L. Whiteside[1], Magis Mandapathil[1,2], Stephan Lang[2], Edwin K. Jackson[3], Elieser Gorelik[1]
[1]*Pathology, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA,* [2]*Otorhinolaryngology, University of Duisburg-Essen, Essen, Germany,* [3]*Pharmacology, University of Pittsburgh, Pittsburgh, PA, USA*

Inducible $CD4^+CD25^-IL\text{-}10^+TGF\text{-}\beta^+$ regulatory T cells (Tr1) are generated upon encountering cognate antigens. In cancer patients, the Tr1 frequency is increased; in tumor and blood. However, the mechanisms used by these cells to mediate suppression are not yet defined. The ectonucleotidases, CD39 and CD73, convert ATP into adenosine which binds to the A2a receptors on effector T cells, inhibiting their functions. We reported that these ectonucleotidases are expressed in human nTreg and tumor cells. Here, we evaluated the effects of tumor-derived adenosine on the Tr1 generation and Tr1-mediated immune suppression. Tr1 were generated in co-cultures containing sorted $CD4^+CD25^{neg}$ T cells, autologous dendritic cells, low doses of IL-2, IL-10 and IL-15 (10 IU/mL each) and irradiated $CD73^+$ MDA tumor cells or $CD73^{neg}$ MCF-7 tumor cells. Proliferating Tr1 were tested for expression of the nucleotidases by multiparameter flow cytometry and their suppressor function assessed in assays with CFSE-labeled autologous $CD4^+CD25^{neg}$ responder cells (RC). ATP hydrolysis was measured in luciferase-based ATP detection assays. Adenosine in cell supernatants was analyzed by mass spectrometry. Tr1 generated in the co-cultures expressed CD39 and CD73. The $CD73^+$ tumors induced differentiation of the highest numbers of ectonucleotidase+Tr1 ($p<0.01$) relative to $CD73^{neg}$ tumors. The Tr1 generated with CD73+ tumors mediated the highest suppression of RC proliferation ($p<0.01$), hydrolyzed exogenous ATP at the highest rate ($p<0.05$) and produced high amounts of adenosine ($p<0.05$). ARL67156, an inhibitor of CD39, and ZM241385, A2A receptor antagonist, blocked Tr1-mediated suppression ($p<0.01$–0.02). Tumor-derived adenosine favors the generation of immunosuppressive CD39+ and CD73+ Tr1 cells, which have higher enzymatic activities relative to Tr1 cells generated in the $CD73^{neg}$ tumor environment. The data suggest that adenosine plays a major role in the induction of Tr1 cells, which also utilize adenosine to mediate suppression in the tumor microenvironment.

Poster No. 179

Discovery of Unique Molecular Imaging Probes for avb3-integrin from a Combinatorial Peptide Library Using a Novel 'Beads on a Bead' Approach

Choi-Fong Cho[1], Giulio Amadei[2], Leonard Luyt[2], John Lewis[1]
[1]*Medical Biophysics, University of Western Ontario, London, ON, Canada,* [2]*Chemistry, University of Western Ontario, London, ON, Canada*

Peptide-targeted nanoparticles offer an attractive multivalent platform for *in vivo* molecular imaging of the tumor microenvironment. While random combinatorial peptide libraries have shown considerable promise for the identification of peptide ligands, conventional screening strategies are labor intensive and time consuming. Here, we describe the identification of novel peptide ligands specific to avb3 integrin using a novel "beads on a bead" screening approach that significantly accelerates the identification and isolation of positive peptide hits from combinatorial peptide libraries. As a proof of principle, we took advantage of the tendency of 2 μm magnetic beads coated with the protein target (avb3 integrin) to associate differentially with the much larger 90 μm Tentagel beads coated with RGD (high affinity), KGD (low affinity) or AGD (no affinity) peptides. Positive bead hits were isolated from the negative library beads using a neodymium magnet, and specificity was validated by incubating

with avb3-expressing MDA435 (positive control) and avb3-knockdown MDA435 (negative control) tumor cells. The hit peptides were cleaved and sequenced "on bead" using a novel MALDI-TOF/MS technique developed in-house. We demonstrate here that the protein-coated magnetic beads associated with the library beads in an affinity-dependent fashion, and that the accuracy of this method is greater than 98%. A random combinatorial peptide library was screened for avb3 integrin-binding peptides, and a number of novel high-affinity peptides were identified that did not contain the RGD motif. Therefore, we expect that they may be useful to develop molecular imaging agents that do not interfere with avb3 integrin function.

Poster No. 180

Radiolabeled Cdk4/6 Inhibitors for Molecular Imaging of Tumors

Franziska Graf[1], Lena Koehler[1], Birgit Mosch[1], Jens Pietzsch[1]
[1]*Institute of Radiopharmacy, Forschungszentrum Dresden-Rossendorf, Dresden, Germany*

Overexpression of cell-cycle regulating cyclin-dependent kinases 4 and 6 (Cdk4/6) and deregulation of Cdk4/6-pRb-E2F pathway are common aspects in human tumors. The aim of our study was the evaluation of pyrido[2,3—*d*]pyrimidin-7-one derivatives (CKIA and CKIE) concerning their efficacy and suitability as small molecule Cdk4/6 inhibitors and, after iodine-124 ([^{124}I]CKIA) or fluorine-18 ([^{18}F]CKIE) radiolabeling, as radiotracers for Cdk4/6 imaging in tumors by positron emission tomography (PET).
CKIA and CKIE were analyzed concerning their biological properties (effects on cell growth, cell cycle distribution, Cdk4/6 mediated pRb-Ser^{780} phosphorylation, mRNA expression of pRb affected genes E2F-1 and PCNA) and radiopharmacological properties (cellular radiotracer uptake and PET studies) using human tumor cell lines HT-29, a colorectal adenocarcinoma cell line, FaDu, a head and neck squamous cell carcinoma cell line, and THP-1, an acute monocytic leukemia cell line, as well as phorbol ester TPA-activated THP-1 cells, as model of tumor-associated macrophages.
CKIA and CKIE were identified as potent inhibitors of Cdk4/6-pRb-E2F pathway due to decreased Cdk4/6 specific phosphorylation at pRb—Ser^{780} and downregulation of E2F-1 and PCNA mRNA expression in HT-29, FaDu and THP-1 tumor cells. This resulted in arrest of these tumor cell lines in G1 phase of the cell cycle and growth inhibition. Otherwise, in non-proliferating TPA-activated THP-1 macrophages no change of cell-cycle distribution after treatment with CKIA and CKIE was observed. Furthermore, TPA-activated THP-1 macrophages showed lower Cdk4 mRNA and protein levels, than other tumor cell lines. *In vitro* radiotracer uptake studies using [^{124}I]CKIA and [^{18}F]CKIE demonstrated tumor cell uptake, which could be blocked with both nonradioactive CKIA and CKIE. However, THP-1 macrophages showed similar radiotracer uptake like other tumor cells. Preliminary small animal PET studies in mouse tumor xenograft models further analyzed the hypothesis that radiolabeled Cdk4/6 inhibitors are suitable tracers for molecular imaging of tumors.

Poster No. 181

Characterisation of a Small, Synthetic Imaging Agent for Dying and Dead Tumour Cells

Tania Massamiri[1], **Danielle Park[2],** Amol Karwa[1], Beth Warner[1], Jan MacDonald[1], Christine Hemenway[1], Lori Chinen[1], Philip Hogg[2], Mary Dyszlewski[1]
[1]*Covidien, Imaging Solution, St. Louis, USA,* [2]*Cancer Research Centre, University of New South Wales, Sydney, Australia*

The central core of solid tumors are characterised by a high number of apoptotic and dead cells. This is due to two factors. First, tumor cells proliferate uncontrollably, and those cells ≥200 μm from a blood vessel die because of lack of oxygen. Second, the relative paucity of macrophages to dying tumor cells results in slow clearance and thus prolonged residency of apoptotic cells in the tumor core. When the tumor is subjected to chemotherapeutics, anti-hormonal agents or radiotherapy, tumor apoptosis increases. The degree of apoptosis correlates with the sensitivity of the tumor to the given treatment. Observing tumor cell apoptosis could therefore assist clinicians in evaluating treatment efficacy.
GSAO (4-(N-(S-glutathionylacetyl)amino)phenylarsonous acid) is a synthetic tripeptide trivalent arsenical that rapidly concentrates in dying and dead cells. Upon fluorescent, infrared or radioactive labelling, GSAO serves as a novel and effective imager of cell death, both *in vitro* and *in vivo*. Radiolabelled ^{111}In-DTPA-GSAO and its derivative PENAO bind specifically to dead and dying cells in a wide variety of immortalized tumor cell lines treated with various cytotoxic agents. Inhibition of apoptotic cell death by Z-VAD-FMK decreased binding of ^{111}In-DTPA-GSAO. Analysis of fluorescently labelled GSAO by flow cytometry revealed that GSAO accumulates in the late stages of apoptosis following loss of plasma membrane integrity. GSAO is retained in the cell via binding to cytoplasmic proteins, and this is mediated by cross linking of closely spaced di-thiols.
In vivo imaging of ^{111}In-DTPA-GSAO in mice bearing Lewis Lung Carcinoma and Colon Carcinoma (CT-26.WT) tumors reveal binding to dead and dying cells in both treated and untreated tumors. ^{111}In-DTPA-GSAO uptake was quantitatively greater in treated versus untreated tumors and was also greater than ^{99m}Tc-Annexin V in the same animal. Kidney and liver accumulation of ^{111}In-DTPA-GSAO was several fold less than ^{99m}Tc-Annexin V.

Poster No. 182

Proteomic Study on Human Cholangiocarcinoma

Ian Darby[1], Karine Vuillier-Devillers[2], Émilie Pinault[2], Sébastien Lepreux[3], Charles Balabaud[3], Paulette Bioulac-Sage[3], Alexis Desmouliere[4]
[1]*Cancer and Tissue Repair Laboratory, School of Medical Sciences, RMIT University, Bundoora, Victoria, Australia,* [2]*Plateau Protéomique, Faculté des Sciences et Techniques, Université de Limoges, Limoges, France,* [3]*Service d'Anatomie Pathologique, CHU Bordeaux, Hôpital Pellegrin, Bordeaux, France,* [4]*Faculté de Médecine et de Pharmacie, Université de Limoges, Limoges, France*

Cholangiocarcinoma is an adenocarcinoma of the liver which has increased in incidence over the last thirty years in many countries to reach similar levels to other liver cancers. Diagnosis of this disease is usually late and prognosis is poor, therefore it is of great importance to identify novel markers and potential early indicators of this disease as well as molecules that may be potential therapeutic targets. We have used a proteomic approach to identify differentially expressed proteins in peripheral cholangiocarcinoma cases and compared expression with paired peri-tumoral histologically normal liver tissue from the same patients. 2-D electrophoresis using DIGE labelling of the proteins with cyanine(Cy)3 and Cy5 was used to identify differentially expressed proteins. Overall, of the approximately 2400 protein spots visualised in each gel, 172 protein spots showed significant differences in expression level between tumoral and peri-tumoral tissue with $p<0.01$. Of these, 100 spots corresponding to 147 different proteins were identified by mass spectroscopy: 76 proteins were over-expressed whereas 71 proteins were under-expressed in tumoral samples compared to peri-tumoral samples. Several of the identified proteins have potential roles in control of cancer or stromal cell proliferation and survival and of control of angiogenesis. Among the over-expressed proteins were pigment epithelium derived factor (PEDF), 14,3,3 protein, periostin and a-smooth muscle actin. Immunohistochemical studies were carried out on samples from the same patient population and confirmed increased expression of 14-3-3 proteins in adenocarcinoma cells while a-smooth muscle actin and periostin were shown to be overexpressed in the tumor stroma. Double labeling showed that these latter two proteins were colocalised in stromal myofibroblasts.

Poster No. 183

Expression of Oestrogen Related Proteins in Prostate Tumor Microenvironment is Correlated to Progression of Hormone-Refractory Disease

Gaelle Fromont[1,2], Mokrane Yacoub[1], Olivier Celhay[1], Olivier Cussenot[2]
[1]*CHU - Universite de Poitiers, Poitiers, France,* [2]*CeRePP, ER2UMPC, Paris, France*

Despite increasing evidence that estrogen signalling plays a major role in both the development and progression of prostate cancer, very few studies have focused on estrogen related genes in hormone refractory prostate cancer (HRPC). The aim of the study was to compare on both tumoral and stromal cells the expression of genes related to androgen and estrogen metabolism in paired samples of prostate cancers collected before androgen deprivation therapy (ADT) and after hormonal relapse.
The study included 55 patients treated only by ADT for prostate cancer, and for whom tissues were available before treatment induction and after recurrence. Gene expressions were analysed using immunohistochemistry performed on tissue microarray, using antibodies directed against: androgen receptor (AR), phosphorylated AR (pAR), estrogen receptor alpha (ERA), estrogen receptor beta (ERB), 5 alpha reductase 1 and 2, aromatase, BCAR1 (involved in antiestrogen resistance in breast cancer), and the proliferation marker Ki67. Expressions were compared using Friedman and Wilcoxon paired tests. Predictive expressions of overall survival and the time to hormonal relapse were analysed using Log-rank and Cox tests.
When compared to hormone sensitive samples, tissues collected after hormonal relapse were characterized by increased expression of Ki67, AR, pAR ($p<0.001$), and BCAR ($p=0.03$), and by lower staining for 5AR2 ($p=0.002$), ERB ($p=0.016$), and aromatase ($p<0.001$). Shorter time to hormonal relapse was associated with high expressions of aromatase and BCAR on diagnostic biopsies, together with low stromal staining for ERA. Overall survival was significantly shorter when tissues collected after relapse displayed both high proliferation index and low ERA expression in stromal cells.
These results demonstrated a dysregulation of proteins involved not only in androgen pathways but also in estrogen synthesis and signalling during the development of HRPC. The survival advantage of ERA staining in HRPC underlines the importance of steroid signalling via the microenvironment in prostate cancer.

Poster No. 184

Is there a Relationship between the Expression of CD147 (EMMPRIN), CD44, Multidrug Resistance (MDR) and Monocarboxylate (MCT) Transporters, and Prostate Cancer (CaP) Progression?

Jingli Hao[1,2], Michele C. Madigan[3], Hongmin Chen [2], Paul J. Cozzi[1,4], Warick J. Delprado[5], Yong Li[1,2]
[1]*Faculty of Medicine, University of New South Wales, Kensington, New South Wales, Australia,* [2]*Cancer Care Centre, St George Hospital, Kogarah, New South Wales, Australia,* [3]*School of Optometry & Vision Science, University of New South Wales, Kensington, New South Wales, Australia,* [4]*Department of Surgery, St George Hospital, Kogarah, New South Wales, Australia,* [5]*Pathology, Douglass Hanly Moir, North Ryde, New South Wales, Australia*

Aim: Multidrug resistance (MDR) and metastasis are the main causes of treatment failure in prostate cancer (CaP) patients. CD147 activates proteinases and angiogenic factors in the tumour microenvironment, stimulates hyaluronan production and interacts with CD44 to regulate MDR and MCT transporters. CD44 is a key receptor for hyaluronan, critical for cell signalling and drug resistance. We investigated the expression of CD147, CD44, and transporter (MDR1) and MCT proteins in CaP progression.
Methods: CD147, CD44s and v3-10, MDR1, MCT1 and MCT4 expression was studied in human metastatic CaP cell lines (PC-3 M-luc(MDR), PC-3 M-luc, Du145, LN3, DuCaP) and primary CaP tumours, lymph node metastases and normal prostate, using immunoperoxidase, immunofluorescence and microscopy. Cell line dose-response and sensitivity (IC50) to docetaxel was measured with MTT, and correlated with CD147, CD44, MDR1, and MCT expression.

Results: PC-3 M-luc (MDR), PC-3 M-luc and Du145 cells expressed high level CD147, CD44, MDR1 and MCT. In contrast, DuCaP cells showed no CD147 or CD44, but weak MCT immunostaining. LN3 cells expressed strong CD147 and MCT, weak CD44v and MDR1, and no CD44s. Docetaxel sensitivity was positively related to CD44, CD147, MDR1 and MCT expression. Strong heterogeneous CD147, CD44, MDR1, MCT expression was found in high grade primary tumours (Gleason score >7). Heterogeneous co-localization of CD147 with CD44, MDR1 and MCT was found in PC-3 and Du145 cells, and in high grade tumours.
Conclusions: Metastatic CaP cell lines and primary CaP displayed overxpression of CD147, CD44, MDR1, MCT proteins. Interactions between these proteins could contribute to the development of CaP drug resistance and metastasis. Selective targeting of CD147 and CD44 to block their activity (alone or combined) may limit tumour metastasis, and increase drug sensitivity by modifying expression of MDR and MCT proteins.

Poster No. 185

Metallic Ion Composition Discriminates between Normal Esophagus, Dysplasia, and Carcinoma

Daniel Lindner[1], Derek Raghavan[1], Michael Kalafatis[3], Charis Eng[2], Gary Falk[4]
[1]*Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA,* [2]*Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH, USA,* [3]*Department of Chemistry, Cleveland State University, Cleveland, OH, USA,* [4]*Digestive Disease Institute, Cleveland Clinic, Cleveland, OH, USA*

Subtractive hybridization, and more recently, whole genome expression arrays have advanced our understanding of differential gene expression in neoplastic compared to normal tissues, leading to identification of several important oncogenes as well as tumor suppressor genes. We hypothesized that such changes in gene expression would not only result in differential protein expression profiles, but would also ultimately result in detectable differences in the ionic composition of normal, dysplastic, and neoplastic tissues. In a blinded fashion, we utilized atomic absorption (AA) to analyze the metallic ion composition (iron, zinc, copper, chromium, magnesium, and manganese) in normal human esophagus, low grade dysplasia, intestinal metaplasia (Barrett's esophagus), high grade dysplasia, and carcinoma. Normal esophageal epithelium consistently displayed the lowest concentration of all metallic ions analyzed. Samples representing esophageal carcinoma contained elevated concentrations of all six ions ($p< 0.025$). Copper and manganese levels were consistently able to discriminate between normal esophagus and all categories of dysplasia ($p<0.004$ and $p<0.045$, respectively) including low grade dysplasia. Thus, in cases where the histology of a biopsy is indeterminate, metallic ion composition may serve to identify epithelial dysplasia at an early stage. Results from these studies are being analyzed in light of whole genome expression arrays to identify candidate genes responsible for mediating changes in ionic profiles and their relationship to the carcinogenic process.

Poster No. 186

Overexpression of NM23A in Head and Neck Squamous Cell Carcinoma after Radiation

HaengRan Park[1], SuKi Kang[2], NamHoon Cho[1,2]
[1]*Brain Korea 21 Project for Medical Science, Yonsei Universitiy College of Medicine, Seoul, Korea Republic,* [2]*Department of Pathology, Yonsei Universitiy College of Medicine, Seoul, Korea Republic*

The main problem of radiotherapy is that some cancer cells acquire radioresistance after radiation. Remodeled tumor microenvironment(TME) is an inevitable consequence following irradiation, however, its cardinal gene expression remains unknown. We aimed to find out screen and validate surrogate genes of TME alteration related to radiation resistance(RR) to improve the poor prognosis of head and neck squamous cell carcinoma(HNSCC), which demands radiotherapy.
Head and neck cancer cell lines (SCC15, SCC25 and QLL1) with acquisition of RR until 60 Gy of cumulative dosages were established. Combined results of cDNA array and proteomics demonstrated differential expression profiles to compare with corresponding control group, non-irradiated HNSCC cell lines. Protein levels were verified retrospectively in tissue samples with locoregional failure after radiotherapy, and compared with other cell lines using western blot, immunofluorescence (IF).
On combined cDNA array and proteomics, NM23A was significantly overexpressed in RR cell lines. NM23A was also strongly expressed in tissue samples with RR. NM23A was predominantly accentuated along the tumor margin. IF revealed high expression of NM23A and partly translocation of protein into nucleus in SCC25, QLL1. This nuclear shuttling was also noted in other cell lines, including HeLa, CaKi-1, PC-3, but downregualted in sk-ov-3, and T-24. E-cadherin, HGF precursor, MMP(metrix metallo proteinase), EIF(eukaryotic translation initiation factor), EBP1 (erbB3 binding protein) and casein kinase 1 were significantly upregulated in radiation resistant cell lines.
NM23A was one of the surrogate markers to be related to RR and partly translocated into nucleus when upregulated.

Poster No. 187

From Microenvironment to Macroenvironment in Epigenetic Cancer Biology

Lyudmyla Shvachko[1]
[1]*Department of Molecular Genetics, Institute of Molecular Biology & Genetics, Kyiv, Ukraine*

Aberrant gene function and altered patterns of gene expression are key features of cancer. Growing evidence shows that the acquired

epigenetic abnormalities participate with genetic alterations to cause this dysregulation. Patterns of DNA methylation are profoundly altered in neoplasia and simultaneously include genome-wide losses of, and regional gains in, DNA methylation We purpose in understanding how epigenetic alterations participate in the earliest stages of neoplasia, and discuss the strategies to control cancer.

The explosion in our investigations of epigenetic cancer biology how alterated chromatin organization modulated DNA hypomethylation background has highlighted the importance of epigenetic mechanisms in the initiation and progression of human cancer. In this connection on the base of date obtained have been resumed that extrachromosomal satellite DNA organization is the pivotal microenvironmental feature in the initiation of epigenetic cancer biology that triggeres the heterochromatic chromocenters formation and the consequently heteropicnosis as the pivotal macroenvironment in the progression of epigenetic cancer cell biology. Taken together we conclude that epigenetic genome-wide DNA methylation is the strategy from the crucial extrachromosomal constitutive heterochromatin development to control cancer.

Poster No. 188

Matrix Metalloprotease 9 as a Prognostic Marker in Childhood Acute Lymphoblastic Leukemia

Pascale Schneider[2,3], Odile Costa[3], Elisabeth Legrand[3], Jean-Pierre Vannier[2,3], **Marc Vasse[1,3]**

[1]*Department of Biology-Hematology, CHU Charles Nicolle, Rouen, France,* [2]*Pediatric Hematology and Oncology, CHU Charles Nicolle, Rouen, France,* [3]*Groupe de Recherche MERCI (EA 3829), Faculte de Medecine Pharmacie, Rouen, France*

The matrix metalloproteases (MMPs) are endopeptidases involved in the degradation of the extracellular matrix (1). Correlations between MMP expression and increased metastatic potential of various solid tumours have been documented (2). Childhood acute lymphoblastic leukaemia (ALL) is characterized by its capacity to infiltrate different organs which can be the cause of relapses. We analyzed the expression of MMP-2, -9, -14 and TIMP-1 and -2 in a prospective study on 86 children with newly diagnosed ALL (73 B- and 13 T-lineage) and 9 children at relapse with B-ALL. Cellular expression (membrane bound and intracytoplasmic content) of MMPs and TIMPs was analysed by flow cytometry, and secreted MMPs were analysed by zymography and quantified by ELISA. Although weakly expressed on the cell surface, MMP-2 and MMP-9 were present in the cytoplasm of all ALL cases, with an average of 40% positive cells. MMP-14 expression was higher on B-ALL cells at relapse, as compared to B-ALL at diagnosis ($p<0.05$). In B-ALL, the percentage of lymphoblasts containing intracytoplasmic MMP-9 was significantly higher in patients with peripheral infiltration than in patients without ($p<0.05$), suggesting a possible role for gelatinases in peripheral organ infiltration. Nine children died and their lymphoblasts secreted higher levels of MMP-9 than children who recovered ($p<0.05$). ROC curve and Kaplan-Meier curve analysis show that a high secretion of MMP-9 (> 2450 pg/ml/10^6 cells) is associated with a lower overall survival rate, suggesting that the secretion of MMP-9 is an independent prognostic factor in childhood B-ALL.

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Poster No. 189

New Targets in Tumor Angiogenesis and Bone Metastasis

Andrei Bakin[1], Alfiya Safina[1], Huw Davies[2], Spandan Chennamadhavuni[2]

[1]*Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY, USA,* [2]*Department of Chemistry, Emory University, Atlanta, GA, USA*

Our research is focused on the development of effective therapeutics preventing cancer progression and metastasis. In tumor microenvironment, TGF-β cytokines promote tumor invasion, angiogenesis and bone metastasis. However, TGF-β is also a potent tumor-suppressor that inhibits cell growth and induces cell death. This dual role of TGF-β in cancer is an impediment in the development of anti-TGF-β therapies. The present study describes a molecular pathway underlying pro-oncogenic TGF-β activities in carcinoma cells.

The study investigated molecular pathways contributing to the metastatic potential of breast, prostate and lung carcinoma cell lines. Expression profiles and functional assays revealed that TAK1 kinase is required for TGF-β induction of MMP9, VEGF and COX2 in the metastatic cell lines. Disruption of TAK1 signaling reduces the metastatic potential of breast cancer MDA-MB-231 cells, affecting tumor invasiveness and angiogenesis. The biochemical assays showed that disruption of TAK1 reduces NFkB activity but not ERK nor p38MAPK signaling. Thus, TAK1 plays a central role in the cross-talk of TGF-β and inflammatory NFkB pathways.

Cancer-induced bone lesions present a significant complication for patients with breast cancer. To investigate if TAK contributes to osteolytic bone lesions, we used the intra-cardiac injection model with MDA-MB-231 cells. Control and dominant-negative-TAK1 cells were injected in the left ventricle of SCID mice. The X-ray and immuno-histochemistry assays revealed bone lesions in nearly 80% of mice in the control group but none in the dn-TAK group, indicating a critical role of TAK1 in bone metastases.

Our discovery provides a novel therapeutic target in cancer progression and metastasis. The current research is directed toward the development of TAK1 kinase inhibitors and to the identification of the molecular components of the TGF-β-TAK-NFkB axis.

Poster No. 190

Antibody-Mediated Inhibition of Cathepsin S blocks Tumour Invasion, Metastasis and Angiogenesis

Roberta Burden[1,2], Julie Gormley[2], Thomas Jaquin[2], Donna Small[1,2], Derek Quinn[1], Shauna Hegarty[4], Claire Ward[2], Brian Walker[1], James Johnston[2,3], Shane Olwill[2], Christopher Scott[1,2]
[1]*School of Pharmacy, Queen's University Belfast, Northern Ireland, UK,* [2]*Fusion Antibodies Ltd., Belfast, Northern Ireland, UK,* [3]*Centre for Infection and Immunity, Queen's University Belfast, Northern Ireland, UK,* [4]*Institute of Pathology, Queen's University Belfast, Northern Ireland, UK*

Antibody-based therapeutics represent a major class of drugs which have contributed greatly to an improvement in treatment for patients suffering from many forms of cancer. The major characteristics which make antibodies attractive as therapeutics are their increased specificity, long half life and reduced toxicity. Traditionally antibodies have been developed against targets such as membrane receptors or ligands where they evoke an agonistic or antagonistic response. More recently some groups, including ours, have explored their application in targeting biomarkers present in the tumour microenvironment, which may originate from more than one tumour associated cell type.
Cathepsin S (CatS) is a lysosomal cysteine protease which has been implicated in tumour cell invasion and angiogenesis in a range of different tumour types. CatS is normally restricted to the lysosomes of professional antigen presenting cells, however in tumourigenesis, the protease is secreted into the tumour microenvironment where it is involved in extracellular matrix remodelling. We have developed an antibody which specifically targets and inhibits CatS and have demonstrated efficacy in a range of in vitro and in vivo tumourigenesis models. The CatS inhibitory antibody significantly impaired invasion of a range of tumour cell lines by the Boyden Matrigel invasion assay and also disrupts capillary-tubule formation in the *in vitro* HUVEC and *ex vivo* rat aortic ring angiogenesis assays. Live-cell proteolysis assays have demonstrated that the perturbation of tumour invasion occurs as a result of the inhibitory antibody blocking CatS mediated collagen degradation. Furthermore, administration of the CatS antibody resulted in the inhibition of tumour growth, metastasis and neovascularisation in various xenograft tumour models.
In conclusion, this data highlights the potential of specifically targeting CatS within the tumour microenvironment and indicates that the CatS inhibitory antibody is an exciting experimental therapeutic which has great clinical potential.

Poster No. 191

Modulation of IL-10 and GM-CSF Production in Gliomas Leads to Decrease Tumor Growth

Konrad Gabrusiewicz[1], Aleksandra Ellert-Miklaszewska[1], Malgorzata Sielska[1], Bozena Kaminska[1]
[1]*Department of Cell Biology, The Nencki Institute of Experimental Biology, Warsaw, Poland*

Microglia (brain macrophages) are prominent in the stromal compartment of malignant gliomas. Recent evidence suggest that tumor-infiltrating cells release a variety of growth factors, cytokines/chemokines and enzymes that support tumor growth, angiogenesis, and invasion. Current understanding of molecular mechanisms of glioma pathology permits to identify microglia-glioma interactions as a novel therapeutic target. We demonstrated that cyclosporin A (CsA) affects growth/survival of cultured glioblastoma cells, interferes with glioma-microglia interactions and impairs tumorigenicity. In the present study we investigated efficacy and mechanisms mediating antitumor effects of CsA *in vivo*, with particular attention to drug influence on density and morphology of brain macrophages and level of pro/anti-inflammatory cytokines.
EGFP-GL261 glioma cells were injected into the striatum of C57BL/6 mice and tumor-bearing mice received CsA (2 or 10 mg/kg/i.p.) every 2 days starting from the 2nd or the 8th day after implantation. CsA-treated mice had significantly smaller tumors than control mice. When the treatment was postponed to 8th day, only the higher dose of CsA was effective causing 66 % tumor volume reduction. Glioma implantation caused a massive accumulation of brain macrophages within tumor. CsA-treated mice showed a diminished number of tumor-infiltrating, amoeboid brain macrophages (Iba1-positive cells). TUNEL staining revealed DNA fragmentation within infiltrating macrophages and glioma cells after CsA treatment. Production of ten pro/anti-inflammatory cytokines was determined using FlowCytomix immunoassay in total extracts from tumor-bearing hemisphere. Elevated IL-10 and GM-CSF levels were found in tumor-bearing hemisphere in comparison to naive controls. CsA treatment reduced significantly IL-10 and GM-CSF levels in brains of tumor-bearing mice. Altogether, our findings demonstrate that targeting of cytokine production, brain macrophage infiltration and their interactions with glioma cells is effective strategy to reduce glioma growth and invasion.

Poster No. 192

Microtubule Dynamics is Involved in the Control of Angiogenesis by VEGF through EB1 Localization at their Plus Ends

Géraldine Gauthier[1], **Stéphane Honore[1],** Pascal Verdier-Pinard[1], David Calligaris[1], Alessandra Pagano[1], Diane Braguer[1]
[1]*INSERM 911, Centre de Recherche en Oncologie Biologique et Oncopharmacologie, Université de la Méditerranée, Marseille, France*

Vascular Endothelial Growth Factor (VEGF) is a crucial regulator of neo-angiogenesis in cancer, promoting endothelial cell proliferation and migration. Microtubules, through their dynamic instability, control cellular processes such as division and migration that sustain tumor growth and dissemination. We have previously shown that microtubule-targeting agents (MTA) produce their anti-migratory/anti-angiogenic effects on endothelial cells through an increase in microtubule dynamics, a decrease of EB1 comets at microtubule plus ends and lower microtubule stabilization at adhesion sites (1–3). It is likely that external cues

from the tumor microenvironment are integrated at the level of microtubules to regulate these processes. To test this hypothesis, we analyzed the effect of VEGF on microtubule and EB1 dynamics in primary human endothelial cells. Autocrine VEGF inhibition using a VEGF trap strongly increased in interphase microtubule dynamic instability (+ 43%). Consistently, exogenously added VEGF (10 ng/ml) suppressed microtubule dynamic instability (− 29%). Interestingly, the suppression of microtubule dynamics occurred through their plus end stabilisation at paxillin-containing focal adhesions. Moreover, VEGF increased EB1 comet length at microtubule plus end by 32 %, without any change in its expression level. Differential post-translational modifications of EB1 were detected by 2D electrophoresis and western blotting. Their characterizations are under investigation by mass spectrometry. In conclusion, our results show (i) that microtubules integrate signals from the tumor microenvironment, (ii) that VEGF and MTA have opposite effect on microtubule and EB1 dynamics supporting the clinical benefit of the therapeutic combination of VEGF inhibitors and MTA, and (iii) suggest a potential role of EB1 protein in angiogenesis.

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Poster No. 193

3D Models to Track Endothelial Progenitors to a Tumor Site Application to In Vivo Imaging of Cell Migration

Krzysztof Szade[1,2], Witold Nowak[1,2], Catherine Grillon[1], Nathalie Lamerant-Fayel[1], Alan Guichard[1], David Gosset[1], Alicja Jozkowicz[2], Jozef Dulak[2], **Claudine Kieda[1]**

[1]*Centre de Biophysique Moléculaire, UPR 4301, CNRS, Orléans, France,* [2]*Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Kraków, Poland*

Tumor angiogenesis is crucial to support tumor cells growth and allow them to form metastasis [1]. Endothelial progenitor cells (EPC) are key players that influence tumor neovascularisation being directly incorporated into the tumor vessels [2]. Subsequently, we use progenitors of endothelium as vehicles for killer genes to be expressed preferentially in tumors [3]. This needs to determine the chemokines network that guides the progenitor and stem cells toward tumor.

Here, we study mice model of melonama (B16F10 cells) and primitive endothelial precursors isolated from mice embryo (MAgEC – Murine Aorta-gonad-mesonephros Endothelial Cells). To investigate the potential of B16F10 cells to stimulate MAgECs migration we applied two *in-vitro* methods with usage of fluorescence and pseudo confocal video microscopy, applied to dynamic phenomena using shear stress conditions and time lapse measurements on long term experiments. The first method was based on transwell inserts and visualization of MAgEC invasion through Matrigel. In the second one, 3D tumor spheroids were formed and migration of MAgEC through collagen gel towards spheroids was investigated. This allows to study the chemokine activity as we showed that CCL21 augments MAgEC sensitivity and migration potential. Such "education" may be important in cell based therapy against tumor.

In vivo studies indicated the applicability of such 3D model. By imaging using the near infra red cell tracking combined to bioluminescence we showed the active migration and localisation of the endothelial precursor cells in the sites where the tumor cells metastasize. This was confirmed by applying several methods including MRI (Magnetic Resonance Imaging), near-infrared fluorescence imaging and flow cytometry to detect and quantify the efficacy of the EPC seeking into tumor sites.

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Poster No. 194

Immunotherapeutic Strategy against EBV Latency II Malignancies

Olivier Morales[1], Stéphane Depil[1,2], Céline Miroux[1], Violaine Francois[1], Françoise Dufosse[3], Claude Auriault[4], Yvan De Launoit[1], Véronique Pancre[1], Nadira Delhem[1]

[1]*CNRS, UMR 8161, Institut de Biologie de Lille, Lille, France,* [2]*Service des Maladies du sang, CHRU, Lille, France,* [3]*Laboratoire d'Immunologie-HLA-Transplantation, CHRU, Lille, France,* [4]*CNRS, UMR 6097, IPMC, Nice, France*

The Epstein-Barr virus (EBV) is associated with several malignant diseases which can be distinguished by their patterns of viral latent gene expression. The latency II (lat.II) program is limited to the expression of the non-immunodominant antigens EBNA-1, LMP-1 and LMP-2, and is particularly associated with Hodgkin's disease, nasopharyngeal carcinomas and peripheral T/NK-cell lymphomas. Knowing that $CD4^+$ T lymphocytes may play a crucial role in controlling these EBV malignancies, we favoured an immunotherapeutic approach, based on the stimulation of a specific $CD4^+$ T cell response.

We used the TEPITOPE software to predict promiscuous MHC class II epitopes derived from the latency II antigens EBNA-1, LMP-1 and LMP-2. The predicted peptides were then submitted to peptide-binding assay on HLA II purified molecules, which allowed the selection of 6 peptides (EBNA-1: 3, LMP-1: 1, LMP-2: 2) with a highly promiscuous capability of binding. The peptide cocktail was highly immunogenic in Aβ°-DR1 transgenic mice, leading to a specific cellular and humoral Th1 response. Every peptides used in the cocktail or individually were also recognized by human $CD4^+$ memory T cells from healthy donors expressing various HLA II genotypes and from patients with Hodgkin's lymphoma (HL). We have then generated peptide-specific $CD4^+$ cell lines, and assessed their cytotoxic potential to lyse lymphoblastoid cell lines (LCLs,

Lat.III), or other EBV expressing cell lines such as T cell line (NC5, Lat.II) and monocyte cell lines (TE1, Lat.II). Finally, any changes in CD4+CD25+ regulatory T cell activity were observed in response to the peptide cocktail; avoiding the risk of aggravation of the pre-existing immuno-suppressive microenvironment, already known in EBV^+ associated malignancies.
Thus, this promiscuous peptide cocktail could be used in immunotherapeutic approaches against EBV latency II malignancies. It could be used as peptide-based vaccine or cellular therapy, with the hope of controlling the residual disease after classical treatment or to decrease the risks of relapse.

Poster No. 195

In vivo Targeting and Killing of Mouse Prostate Cancer Tissue with Vesicular Stomatitis Virus (VSV)

Maryam Moussavi[1], Ladan Fazli[2], Howard Tearle[2], Michael E. Cox[2], John Bell[3], Christopher Ong[2], William Jia[4], Paul Rennie[2,5]
[1]*Experimental Medicine, Vancouver Prostate Centre, Vancouver, BC, Canada,* [2]*The Vancouver Prostate Centre, Vancouver General Hospital, Vancouver, BC, Canada,* [3]*Centre for Cancer Therapeutics, Ottawa Health Research Institute, Ottawa, ON, Canada,* [4]*Department of Surgery and Brain Research Centre, University of British Columbia, Vancouver, BC, Canada,* [5]*Department of Urological Science, University of British Columbia, Vancouver, BC, Canada*

Prostate cancer is the most commonly diagnosed non-skin carcinoma and one of the leading causes of cancer-related mortality of men in western society. Presently there are no therapies available for advance and metastatic prostate cancer. Oncolytic viral therapy may be used as a new and alternate therapy to current treatments and provides an opportunity to efficiently direct cell death to primary and metastatic cancer cells while sparing normal cells. Vesicular Stomatitis Virus (VSV) is an oncolytic virus which is able to replicate in cells with a defective interferon (INF) response. Here, we examined the effect of a mutated VSV (AV3 strain), which expresses luciferase and has an enhanced INF-sensitivity, on the viability of prostate tumours that develop in prostate-specific PTEN null transgenic mice. Prostates of PTEN knockout and control mice were injected with $5x10^8$ pfu/ml of VSV(AV3) and monitored for luminescence over a 96 h time period using the IVIS-Xenogen machine to track the viral distribution. Both real time qPCR and plaque analysis indicated viral presence and replication in prostate tissues of PTEN null transgenic mice while little to no replication is seen in control mice. TUNEL analysis of paraffin embedded tissues demonstrated that VSV(AV3) is capable of selectively infecting and killing malignant prostate cells while sparing normal cells, specifically at the 48 h time point. This cancer-specific cell death was not due to infiltration of neutrophil into the prostate tumours of PTEN null mice as previously reported in an orthotropic mouse model. However, an increase in macrophage and B-lymphocyte infiltration into the prostates of PTEN null mice is seen when compared to control mice. In summary, our data demonstrates that VSV may be used as a potential oncolytic viral therapy to target prostate cancer.

Poster No. 196

Cell-cell Interactions Defines the Metastatic Microenvironment of Tumor Cells

Lubor Borsig[1], Heinz Läubli[1]
[1]*Institute of Physiology, University of Zürich, Zürich, Switzerland*

Recent evidence strongly suggests that cancer progression is dependent on the microenvironment consisting of stromal and inflammatory cells. Interactions of tumor cells with endothelium in a microvasculature of distant organs determine the outcome of metastasis. Previously, we could show that L-selectin deficiency reduced the recruitment of myeloid cells, and attenuated metastasis. Here we provide evidence for the molecular mechanism involved in the tumor cell-mediated activation of endothelial cells leading to formation of a metastatic niche. Selectin-mediated cell-cell interactions of tumor cells with platelets and leukocytes induce endothelial activation associated with a production of inflammatory chemokines. Enhanced expression of the key chemoattractant for monocytic cells is associated with metastatic progression. Inhibition of monocyte recruitment strongly reduced survival of tumor cell and metastasis. Our findings demonstrate that the selectin-dependent endothelial expression of chemokines contributes to the formation of a permissive metastatic microenvironment.

Poster No. 197

Anti-Tumor Activity of an Apoptosis-Targeting Peptide-Conjugated Heparin Derivative in Breast Cancer Xenografts

Sang-Moon Bae[1], Hyeri Shin[1], Jong-Ho Kim[1], Byung-Heon Lee[1], In-San Kim[1], Youngro Byun[2], **Rang-Woon Park[1]**
[1]*Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu, Korea Republic,* [2]*College of Pharmacy, Seoul National University, Seoul, Korea Republic*

HT10, a taurocholic acid-conjugated low molecular weight heparin derivative is a novel angiogenesis inhibitor. We aimed for designing a new angiogenesis inhibitor with tumor homing capability by introducing the active targeting moiety to previously developed HT10. The end-amine low molecular heparin was conjugated to Apopep-1, the apoptosis-targeting peptide, mediated by succinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate and then HT-Apopep was completed by adding taurocholic acid. The intravenous administration of HT-Apopep in MDA-MB231 human breast cancer-bearing mice

for 14 days resulted in significantly reduced tumor size compared to vehicle-treated control. The antitumor effect of HT-Apopep was dose-dependent and superior to unmodified HT10 and moreover, to bevacizumab, a humanized anti-VEGF monoclonal neutralizing antibody. Immunohistochemical analysis of tumor tissues demonstrated that HT-Apopep decreased the number of CD34-positive erythrocyte-filled blood vessels and Ki67-positive proliferating cells in tumor. These results suggest that combining the angiogenesis inhibitor with active targeting moiety improves antitumor efficacy and HT-Apopep is a promising candidate for cancer therapeutics with tumor homing antiangiogenic activity.

Poster No. 198

Different Microenvironment Alters Biological Response of a Murine Hepatocellular Carcinoma to Ionizing Radiation

Soo-Yeon Park[1], Ik Jae Lee[1], Wonwoo Kim, Jinsil Seong
[1]*Department of Radiation Oncology, Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea Republic*

Background: Tumor microenvironment alters biological response of tumors, which might lead to alteration of tumor response to radiation. Understanding the influence by different microenvironment seems to be the basic step in developing novel antitumor strategies. In this study, we investigated how biological response of a tumor differs by different microenvironment.
Materials and Methods: A syngeneic murine tumor model was established for hepatocarcinoma, HCa-I, which shows high radioresistance (50% tumor cure probability with higher than 80 Gy) and early metastasis to the lung. Tumor cells ($1X10^5$) were injected to male C3H/HeJ mice liver (orthotopic) or thigh muscle (heterotopic). The mice were observed for the tumor growth and metastasis. Tumor tissues were analyzed for CD31 and VEGF by immunochemical staining. VEGF was also analyzed in mice serum for response to radiation of 10 Gy.
Results: Tumor growth rate was faster in orthotopic than heterotopic in early time and became similar at 15 days. Number of metastatic lung nodules was much higher in orthotopic than in heterotopic (number of nodules per mouse; 136 vs 1). Endothelial cell marker, CD31, was increased in orthotopic than in heterotopic tumors by 6 fold and 7.4 fold in 9 and 15 days, respectively. Expression of VEGF was also increased in orthotopic than in heterotopic tumors by 2.3 fold and 2 fold in 9 and 15 days, respectively. The analysis of serum VEGF response showed a biphasic pattern; at 1 day after radiation it decreased in both orthotopic and heterotopic tumors. However, at day 3 after radiation, serum VEGF decreased (2.6 fold) in orthotopic tumor in contrast to increase (1.3 fold) in heterotopic tumors.
Conclusions: The present study showed different biological response of tumors by different microenvironment in tumor growth, metastasis, and related biological markers. It might be applicable to preclinical studies in developing novel therapeutic strategy.

Poster No. 199

Antagonism of Chemokine Receptor CXCR3 Inhibits Osteosarcoma Metastasis to Lungs

Emmanuelle Pradelli[1], Babou Karimdjee-Soilihi[1,2], Jean-François Michiels[3], Jean-Ehrland Ricci[4], Marie-Ange Millet[1], Fanny Vandenbos[3], Timothy J. Sullivan[5], Tassie Collins[5], Michael G. Johnson[5], Julio C. Medina[5], Annie Schmid-Alliana[1], Heidy Schmid-Antomarchi[1]
[1]*Institut National de la Santé et de la Recherche Médicale, Unité 576, Nice, France,* [2]*Centre Hospitalier Universitaire Archet II, Service de Chirurgie Générale et Cancérologie Digestive, Nice, France,* [3]*Centre Hospitalier Universitaire Pasteur, Laboratoire Central d'Anatomie Pathologique, Nice, France,* [4]*Institut National de la Santé et de la Recherche Médicale, Equipe Avenir Unité 895, Nice, France,* [5]*Amgen, Research and Development, South San Francisco, CA, USA*

Metastasis continues to be the leading cause of mortality for patients with cancer. Several years ago it became clear that chemokines and their receptors could control the tumor progress. CXCR3 has now been identified in many cancers including osteosarcoma and CXCR3 ligands were expressed by lungs which are the primary sites to which this tumor metastasize. This study tested the hypothesis that disruption of the CXCR3/CXCR3 ligands complexes could lead to a decrease in lungs metastasis. The experimental design involved the use of the CXCR3 antagonist, AMG487, and two murine models of osteosarcoma lung metastases. Following tail vein injection of osteosarcoma cells, mice that were systematically treated with AMG487 according to preventive or curative protocols had a significant reduction in metastatic disease. Treatment of osteosarcoma cells in vitro with AMG487 led to decreased migration, decreased matrix metalloproteinase activity, decreased proliferation/survival and increased caspase-independent death. Taken together, our results support the hypothesis that CXCR3 and their ligands intervene in the initial dissemination of the osteosarcoma cells to the lungs and stimulate the growth and expansion of the metastatic foci in later stages. Moreover, these studies indicate that targeting CXCR3 may specifically inhibit tumor metastasis without adversely affecting antitumoral host response.

Poster No. 200

Systems Biology: A Therapeutic Target for Tumor Therapy

Albrecht Reichle[1], Thomas Vogt[1]
[1]*Department of Hematology and Oncology, University Hospital Regensburg, Regensburg, Germany*

Tumor-related activities that seem to be operationally induced by the division of function, such as inflammation, neoangiogenesis, Warburg effect, immune response, extracellular matrix remodeling, cell proliferation rate, apoptosis, coagulation

effects, present itself from a systems perspective as an enhancement of complexity. We hypothesized, that tumor systems-directed therapies might have the capability to use aggregated action effects, as adjustable sizes to therapeutically modulate the tumor systems' stability, homeostasis, and robustness.

We performed a retrospective analysis of recently published data on 266 patients with advanced and heavily pre-treated (10% to 63%) vascular sarcoma, melanoma, renal clear cell, cholangiocellular, and hepatocellular carcinoma, hormone-refractory prostate cancer, gastric cancer, and multivisceral Langerhans' cell histiocytosis enrolled in ten multi-center phase II trials (11 centers). Each patient received a multi-targeted systems-directed therapy that consisted of metronomic low-dose chemotherapy, a COX-2 inhibitor, combined with one or two transcription modulators, pioglitazone +/− dexamethason or IFN-alpha. These treatment schedules may attenuate the metastatic potential, tumor-associated inflammation, may exert site-specific activities, and induce long-term disease stabilization followed by prolonged objective response (3% to 48%) despite poor monoactivity of the respective drugs. Progression-free survival data are comparable with those of reductionist-designed standard first-line therapies. The differential response patterns indicate the therapies' systems biological activity. Understanding systems biology as adjustable size may break through the barrier of complex tumor-stroma-interactions in a therapeutically relevant way: Comparatively high efficacy at moderate toxicity. Structured systems-directed therapies in metastatic cancer may get a source for detecting tumor-associated complex aggregated action effects as adjustable sizes available for targeted biomodulatory therapies.

Poster No. 201

The Distribution of Markers of Drug Effect Following Chemotherapy and Hypoxia-Activated Pro-Drug Treatment

Jasdeep K. Saggar[1], Ian F Tannock[1]
[1]*Division of Applied Molecular Oncology and Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada*

Previous work from our laboratory has used quantitative immunohistochemistry (IHC) to show limited distribution from tumour blood vessels of the auto-fluorescent drugs doxorubicin and mitoxantrone. Analysis of the distribution of other anticancer drugs is more difficult because most are not fluorescent, and they are not recognized directly by available antibodies. Here we investigate the use of IHC to determine the distribution of markers of drug effect, and compare that to the distribution of the fluorescent drugs mitoxantrone and AQ4N/AQ4. AQ4N is an inactive pro-drug that is selectively bioreduced in hypoxic environments to the cytotoxic metabolite, AQ4; it is structurally related to mitoxantrone, and like mitoxantrone binds with high affinity to DNA, and inhibits topoisomerase II. We have shown that AQ4N/AQ4 accumulates selectively in hypoxic regions of tumours (Tredan et al, Cancer Res 2009;69:940–7) Here, we use quantitative IHC to analyse the spatial distribution of the following molecular markers of drug effect in relation to blood vessels (recognized by an antibody to CD31) and regions of hypoxia (recognized by an antibody to EF5) of tumours treated with mitoxantrone alone, AQ4N alone, or these drugs in combination: cleaved caspase 3 (a marker of apoptosis), gamma-H2AX (a marker of DNA damage) and Ki67 (a marker of cell proliferation). Preliminary data show that compared to controls, mitoxantrone treatment causes perivascular apoptosis, while AQ4N-treated tumours have greater levels of apoptosis farther away from blood vessels. Similarly, gammaH2AX staining is increased in drug-treated tumours compared to untreated tumours, and AQ4N-treated tumours show greater gammaH2AX activation farther away from blood vessels. Quantitative statistical analysis of the distributions of markers of drug effect in relation to tumour blood vessels and to regions of tumour hypoxia is in progress, and will be compared to the fluorescence distributions of mitoxantrone and AQ4N/AQ4.

Poster No. 202

CXCR7 Antagonists Prevent and Inhibit Colon Carcinoma Metastases to Lungs

Babou Karimdjee-Soilihi[1,2], Emmanuelle Pradelli[1], Marie-Ange Millet[1], Sadaf Pakdaman[1], Violette Martini[1], Talal Al Saati[3], Nicolas Venissac[4], Daniel Benchimol[2], Jacques Abello[5], Jean-Christophe Saurin[5], Daniel Birnbaum[6], Mark ET Penfold[7], Bretton C. Summers[7], Thomas J. Schall[7], **Annie Schmid-Alliana[1],** Heidy Schmid-Antomarchi[1]
[1]*Institut National de la Santé et de la Recherche Médicale, Unité 576, Nice, France,* [2]*Centre Hospitalier Universitaire Archet I, Service de Chirurgie Générale et Cancérologie Digestive, Nice, France,* [3]*Institut Fédératif de Recherche 50, Plateau Technique de Pathologie Expérimentale, Toulouse, France,* [4]*Centre Hospitalier Universitaire Pasteur, Service de Chirurgie Thoracique, Nice, France,* [5]*Institut National de la Santé et de la Recherche Médicale, Unité 865, Lyon, France,* [6]*Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche 599 Institut Paoli Calmette, Marseille, France,* [7]*ChemoCentryx, Research and Development Department, Mountain View, CA, USA*

Preventing and eradicating metastases in target organs requires to better understand the mechanisms involved in the homing and/or development of metastases. There is mounting evidence that chemokines-receptors play a critical role in determining the metastatic progression of tumors. Our study consisted in investigating the role played by CXCR7 in metastatic colon cancer, receptors that we found significantly over-expressed in biopsies of CRC patients compared to healthy colon. To address this question in vivo, we have developed two protocols of treatment based on the systemic antagonism of CXCR7 with ChemoCentryx compounds. On the one hand, a curative treatment of tumor-bearing mice with CXCR7 antagonists was performed to evaluate their therapeutic potential to eradicate pre-established colon cancer metastases. On the other hand, a preventive treatment with these

compounds were given to the mice prior to tumor inoculation in order to assess their ability to prevent the metastatic spread of colon cancer cells to lung and liver. Our approach based on the administration of pharmacologic antagonists within animal cancer models using either murine or human cancer cells enabled us to show that CXCR7 are a key factor in the dissemination and the progression of colon cancer metastases into the lungs. Our in vitro studies performed on cancer cells suggest that the anti-tumor effects of pharmacologic blockers could reside in the inhibition of the migratory and growth/survival ability of the cancer cells induced by the corresponding chemokines (CXCL11 and CXCL12). Interestingly, however, we show that both preventive and curative CXCR7 antagonisms fail to reduce the extent of liver metastasis, thus suggesting that such receptors do not appear to play a major role in the metastatic process within this target organ.

Poster No. 203

Organ-Specific Inhibition of Metastatic Colon Carcinoma by CXCR3 Antagonism

Béatrice Cambien[1], Babou Karimdjee-Soilihi[1,2], Peggy Richard-Fiardo[1], Hanen Bziouech[1], Robert Barthel[1], Marie-Ange Millet[1], Violette Martini[1], Daniel Birnbaum[3], Jean-Yves Scoazec[4], Jacques Abello[4], Talal Al Saati[5], Michael G. Johnson[6], Timothy J. Sullivan[6], Julio C. Medina[6], Tassie Collins[6], Annie Schmid-Alliana[1], **Heidy Schmid-Antomarchi[1]**
[1]*Institut National de la Santé et de la Recherche Médicale, Unité 576, Nice, France,* [2]*Centre Hospitalier Universitaire Archet I, Service de Chirurgie Générale et Cancérologie Digestive, Nice, France,* [3]*Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche 599, Institut Paoli Calmette, Marseille, France,* [4]*Institut National de la Santé et de la Recherche Médicale, Unité 865, Lyon, France,* [5]*Institut Fédératif de Recherche 50, Plateau Technique d'Histopathologie Expérimentale, Toulouse, France,* [6]*Amgen, Research and Development Department, South San Francisco, USA*

Liver and lung metastases are the predominant cause of colorectal cancer (CRC) related mortality. Recent research has indicated that CXCR3/chemokines interactions that orchestrate hematopoetic cell movement are implicated in the metastatic process of malignant tumors, including that of CRC cells to lymph nodes. To date, however, the contribution of CXCR3 to liver and lung metastasis in CRC has not been addressed. To determine whether CXCR3 receptors regulate malignancy-related properties of CRC cells, we have used CXCR3-expressing CRC cell lines of human (HT29 cells) and murine (C26 cells) origins that enable the development of liver and lung metastases when injected into immunodeficient and immunocompetent mice, respectively, and assessed the effect of CXCR3 blockade using AMG487, a small molecular weight antagonist. In vitro, activation of CXCR3 on human and mouse CRC cells by its cognate ligands induced migratory and growth responses, both activities being abrogated by AMG487. In vivo, systemic CXCR3 antagonism by preventive or curative treatments with AMG487 markedly inhibited the implantation and the growth of human and mouse CRC cells within lung without affecting that in the liver. Also, we measured increased levels of CXCR3 and ligands expression within lung nodules compared to liver tumors. Altogether, our findings indicate that activation of CXCR3 receptors by its cognate ligands facilitates the implantation and the progression of CRC cells within lung tissues and that inhibition of this axis decreases pulmonary metastasis of CRC in two murine tumor models.

Poster No. 204

Molecular Targeting and Imaging of Tumor Endothelia

Rita Serda[1], Aaron Mack[1], Ciro Chiappini[4], Mauro Ferrari[1,2,3]
[1]*Department of Biomedical Engineering, University Texas Health Science Center at Houston, Houson, TX, USA,* [2]*Department of Experimental Therapeutics, UT MD Anderson Cancer Reseach Center, Houston, Texas, USA,* [3]*Department of Bioengineering, Rice University, Houston, Texas, USA,* [4]*Department of Biomedical Engineering, University of Texas at Austin, Austin, TX, USA*

Adhesion of therapeutic and diagnostic delivery vehicles to the endothelial lining is influenced by endothelial activation, which leads to tissue-specific differences in endothelium and corresponding differences in the expression of specific cell surface molecules. Elevated expression of E-Selectin, Vascular Cell Adhesion Molecule-1 (VCAM-1), and Inter-Cellular Adhesion Molecular-1 (ICAM-1) on tumor-associated endothelia are targets for blood borne drug delivery vehicles. The realization that blood-borne delivery systems must overcome a multiplicity of sequential biological barriers has led to the fabrication of a multistage delivery system (MDS) designed to optimally negotiate vascular transport, localizing preferential at pathological endothelia, and delivering both therapeutic and diagnostic cargo. The MDS is comprised of stage one nanoporous silicon particles that function as carriers of second stage nanoparticles. We have successfully fabricated an MDS with targeting and imaging capabilities by loading iron oxide nanoparticles into the porous silicon matrix and capping the pores with a polymer coat. The polymer also provides free amines for attachment of targeting ligands. Tissue samples from mice that were intravenously administered the MDS support the *in vivo* stability of the multi-particle system by demonstrating co-localization of silicon and iron oxide particles. Mice with breast cancer xenografts show dark contrast in the tumor by magnetic resonance imaging following injection with the MDS, supporting accumulation of iron oxide nanoparticles in the tumor. Transmission and scanning electron microscopy have been performed to view the luminal surface of the tumor endothelium following administration of the MDS.

Poster No. 205

A Soy Isoflavone Diet Inhibits Growth of Human Prostate Xenograft Tumors and Enhances Radiotherapy in Mice

Kathleen Shiverick[1], Theresa Medrano[1], Wengang Cao[2], Juan Mira[1], Yamil Selman[1], Lori Rice[3], Charles Rosser[2]
[1]*Department of Pharmacology & Therapeutics, University of Florida, Gainesville, FL, USA,* [2]*Department of Urology, University of Florida, Gainesville, FL, USA,* [3]*Department of Radiation Oncology, University of Florida, Gainesville, FL, USA*

Studies report that soy isoflavones inhibit growth in a number of carcinoma cell lines and may enhance radiotherapy. We investigated the interaction of a soy isoflavone diet (ISF) and radiation (XRT) on PC-3 human prostate xenograft tumors in mice. The PC-3 cell line is androgen-insensitive, does not express p53 or PTEN tumor suppressor genes, and overexpresses Akt, a major prosurvival pathway. Methods: Male nude mice on a soy-free control diet were injected with PC-3 prostate cancer cells into the hind flank. On day 5, half the mice were placed on a diet containing 0.5% soy isoflavone concentrate (ISF). On day 9, half the mice from each diet group were randomly irradiated to 2 Gy (XRT). Tumor sizes were monitored biweekly. Resected tumors were fixed in formalin and paraffin-embedded. Immunohistochemical staining was performed using antibodies against Akt, phosphorylated-Akt (phosAkt), TUNEL, VEGF, CD34, PCNA and vimentin. Results: The ISF and XRT treatments, respectively, each significantly inhibited xenograft tumor growth, with combination ISF+XRT (2 Gy) having additive inhibitiory effects. ISF and XRT, individually, produced inhibition of proliferation (PCNA), induction of apoptosis (TUNEL), and decreased angiogenesis (VEGF, CD34). In contrast, ISF decreased phosAkt in tumor cells, whereas XRT upregulated phosAkt, possibly as a prosurvival response to low dose radiation. In addition, XRT alone increased staining for vimentin in tumor cancer cells, a mesenchymal marker, and tumors were more invasive. The combination of ISF+XRT, however, suppressed phosAkt, as well as the transition to vimentin staining. Thus, soy alters the tumor microenvironment to sensitize to radiation killing, as well as suppress mesenchymal activation by XRT. Conclusions: Evidence shows that dietary soy isoflavones (ISF) inhibit xenograft tumor growth in mice, and also act as an adjuvant agent to sensitize to radiotherapy through distinct mechanisms within the tumor microenvironment. (Support from NIH and the Maren Foundation)

Poster No. 206

ACE-041, a Soluble ALK1-Fc Fusion Protein, is a Novel Anti-Angiogenic Compound with Anti-Tumor Activity

Nicolas Solban[1], Aaron Mulivor[1], Dianne Mitchell[1], Eileen Pobre[1], Ravi Kumar[1], Amelia Pearsall[1], Kathryn Underwood[1], Jeffrey Ucran[1], Matthew Sherman[1], Jasbir Seehra[1], Scott Pearsall[1]
[1]*Acceleron Pharma, Cambridge, MA, USA*

Activin receptor-like kinase-1 (ALK1) is a TGF-beta type I receptor found on remodeling blood vessels. *ALK1* mutations are associated with the hemorrhagic disease Hereditary Hemorrhagic Telangiectasia indicating its role in the regulation of angiogenesis. We developed a soluble ALK1 receptor, ACE-041, by fusing the extracellular domain of ALK1 to the Fc region of IgG1, to examine the potential of ALK1 inhibition as a novel anti-angiogenic therapy. ACE-041 binds circulating ligands and prevents their signaling through ALK1. RAP-041, the murine analog, was also developed for testing in rodents. Bioactivity was evaluated in cell based assays and the effect of ACE-041 on neovascularization was evaluated *in vitro* using a cord formation assay. The addition of ACE-041 reduced ALK1 signaling through both SMAD 1/5/8 phosphorylation and Id-1 expression, confirming that ACE-041 abrogates ALK1 signaling. *In vitro* stimulation of endothelial cells induces their rearrangement into vessel-like structures (cords). The addition of ACE-041 significantly inhibited their rearrangement (45%), suggesting an important role of ALK1 in neovascularization. Antiangiogenic activity of RAP-041 was demonstrated *in vivo* in a modified Basement Membrane Extract plug assay, in a chick chorioallantoic membrane assay (CAM) and in an epiphyseal hypertrophy assay. RAP-041 showed anti-tumor activity in several tumor models including a modified CAM assay and an orthotopic breast cancer model. RAP-041 treatment decreased tumor volume (up to 80%); tumor weight (up to 81%) with immunohistochemical staining demonstrating a reduction in blood vessels (decreased CD31). Taken together, these results suggest an important role of ALK1 in blood vessel formation and demonstrate the anti-angiogenic properties of ACE-041. In conclusion, ACE-041, a soluble ALK1-Fc fusion protein, is a novel anti-angiogenic compound being developed for use as a cancer therapy.

Poster No. 207

VEGF Distribution Response to Anti-VEGF Dosage Regimens: A Computational Model

Marianne Stefanini[1], Florence Wu[1], Feilim Mac Gabhann[1,2], Aleksander Popel[1]
[1]*Department of Biomedical Engineering, Johns Hopkins University, School of Medicine, Baltimore, MD, USA,* [2]*Institute for Computational Medicine, Johns Hopkins University, Baltimore, MD, USA*

Anti-VEGF therapy has shown promising results in cancer treatment but its *in vivo* mechanism of action is, to this date, poorly understood. Bevacizumab shows a synergistic effect when administrated with chemotherapy but has failed as a single-agent and, even more intriguingly, the intravenous injection of the VEGF monoclonal antibody has been reported to increase serum VEGF [1–4]. We have built an *in silico* model that comprises three compartments: blood, healthy and tumor tissues. This whole-body model includes molecular interactions involving VEGF, inter-compartmental transport (microvascular permeability and lym-

phatic removal) and clearance from the plasma. We show that the introduction of an anti-VEGF agent disrupts the VEGF distributions in tissues and blood. We predict that the increase in serum VEGF can be explained by the extravasation of the anti-VEGF agent, followed by a net flow of VEGF complexed with the anti-VEGF agent from the tissue to the blood. Such findings can lead to a better understanding of the pharmacokinetics of anti-VEGF therapy, will aid in the optimization of drug dosage regimens, and the molecular design of therapeutic agent carriers.

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Poster No. 208

Peripheral Proopiomelanocortin Expression Inhibited the Lung Metastasis of B16-F10 Melanoma by Attenuating Invasiveness and Epithelial-Mesenchymal Transition

Han-en Tsai[1,2], Guei-Sheung Liu[2,4], Ming-Hong Tai[1,2,3]
[1]*Department of Biomedical Science, National Sun Sat-sen University, Kaohsiung, Taiwan,* [2]*Department of Medical Education & Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan,* [3]*Department of Biological Sciences, National Sun Sat-sen University, Kaohsiung, Taiwan,* [4]*Bernard O'Brien Institute of Microsurgery, Melbourne University, Melbourne, Victoria, Australia*

Malignant melanoma is a highly lethal that metastasis of melanoma cells to distant organs represents the primary cause for cancer mortality, underscoring the demand of novel therapeutic alternatives. Proopiomelanocortin (POMC) is the precursor of various anti-inflammatory peptides including α-melanocyte-stimulating hormone (α-MSH). We have recently demonstrated the potential of systemic POMC expression via adenovirus gene transfer suppresses the growth of primary B16-F10 melanoma and prolongs the survival of tumor-bearing mice. In this study, we investigated whether POMC gene transfer also held promise for management of metastatic melanoma. In cell cultures, POMC gene delivery potently inhibited the motility and invasiveness of B16-F10 melanoma cells. Such inhibition was correlated with the reduced Rho activity and downregulation of Rho-ROCK signaling proteins including RhoA, RhoB, ROCK-I and ROCK-II. Besides, POMC gene transfer also disrupted the epithelial-mesenchymal transition (EMT) of melanoma cells through E-cadherin up-regulation and α-SMA down-regulation. To evaluate the anti-metastatic efficacy *in vivo*, C57BL/6 mice were intravenously administrated with luciferase-engineered B16-F10 cells at day 1, treated with adenovirus vectors at day 2, and monitored for development of lung metastasis at day 14 by counting lung foci and bioluminescence. It was found that POMC-treated mice exhibited significant reduction in lung metastasis. Therefore, the present study demonstrated for the first time the anti-metastatic potential of peripheral POMC expression for control of metastatic melanoma via perturbing EMT and Rho/ROCK pathways.

Poster No. 209

Denileukin Diftitox Selectively Depletes Regulatory T Cells and Inhibits Tumor Growth in Syngeneic Tumor Models

Mary Vermeulen[1], Lana Parent[1], Nanding Zhao[1], Diana Liu[1], Sally Ishizaka[1], Matthew Mackey[1], Natalie Twine[1], Judith Oestreicher[1], Bruce Littlefield[1]
[1]*Eisai Research Institute, Andover, MA, USA*

Denileukin diftitox (DD; ONTAK® - Eisai Inc.), a recombinant fusion protein that combines IL-2 with the membrane and catalytic domains of diphtheria toxin, binds to and potently kills cells that express the IL-2 receptor (IL-2R). One component of that receptor is CD25. High level IL-2R expression is a characteristic of immunosuppressive regulatory T lymphocytes (Tregs), which many types of solid tumors are known to utilize for immune evasion. We found that a 1–2 hour exposure to DD dose-dependently depleted CD4+CD25+FoxP3+ murine splenocytes or CD4+CD25^{hi}FoxP3+ human blood leukocytes *in vitro*, while largely sparing CD4+CD25- splenocytes. The same brief DD exposure that led to depletion of Tregs (as measured by flow cytometry) also inhibited suppressive activity of murine Tregs (as measured by suppression of [^{3}H]thymidine uptake) towards stimulated non-Treg T cells. *In vivo* exposure to DD at 4.5 μg/mouse (Q7dx2) led to stasis of established subcutaneous CT26 colon tumors in BALB/c mice. Of interest, we also found that DD at 4.5 μg/mouse (Q7dx2) was completely without anti-tumor effect towards CT26 tumors if tumors were implanted in immunocompromised nude mice. Transcriptional profiling of excised tumors from DD-treated and untreated mice revealed a strong immune activation response to DD treatment. In syngeneic mouse models of solid tumors, we conclude that DD exerts its major anti-tumor effect against T cells, and in particular against Tregs.

Poster No. 210

Clusterin Knockdown Inhibits FAK Phosphorylation and Attenuates Migration in Prostate Cancer Cells

Anousheh Zardan[1,2], Amina Zoubeidi[2], Michael E. Cox[1,2,3], Martin E. Gleave[2,3]
[1]*Department of Experimental Medicine, University of British Columbia, Vancouver, BC, Canada,* [2]*Prostate Center, Vancouver General Hospital, Vancouver, BC, Canada,* [3]*Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada*

Acquisition of migratory capacity of prostate cancer cells is an essential event for metastatic disease progression; however, the molecular mechanism underlying acquisition of a metastatic capacity remains unresolved. Clusterin (CLU) is a secreted chaperone protein, over-expressed in many cancers that has been previously reported as up-

regulated during Castration Resistant progression of prostate cancer (CRPC). We used an antibody array to identify changes in protein expression and phosphorylation of PC3 prostate cancer cells in which CLU expression was suppressed by siRNA knockdown. We observed that CLU siRNA knockdown leads to decreased focal adhesion kinase (FAK) phosphorylation as well as its downstream targets. FAK is a member of a family of non-receptor protein-tyrosine kinases that acts as a key regulator of cell migration and whose expression level correlates with CRPC progression. Validating the antibody array results, we confirmed that CLU siRNA knockdown decreases FAK phosphorylation in PC3 cells without affecting total FAK levels by immunoblot analysis. We have gone on to show that CLU siRNA treatment suppresses serum- and VEGF-inducing FAK phosphorylation, and attenuates PC-3 cell migration and invasion capacity in wound healing and matrigel invasion assays. All together, these observations implicate CLU as an important regulator of cell motility and FAK activation in PC3 cells.

Poster No. 211

Radiation-induced re-distribution of Tumor-associated CD11b Positive Cells in a Murine Prostate Cancer Model

Chi-Shiun Chiang[1], Sheng-Yung Fu[1], Fang-Hsing Chen[1], Chun-Chieh Wang[2], Ji-Hong Hong[2]
[1]*Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu, Taiwan, Taiwan,* [2]*Department of Radiation Oncology, Chang Gung Memorial Hospital, Taoyuan, Taiwan, Taiwan*

Our recent study in murine prostate cancer cells, TRAMP-C1, found that radiation therapy (RT) by either 25 Gy in a single dose or 60 Gy with 15 fractions in 3 weeks resulted in the development of chronic and persistent hypoxia, which allured the aggregation of CD68 positive TAMs to these regions. To further study the distribution of tumor-infiltrating cells in the irradiated tumors, flow cytometry and immunohistochemistry were used to characterize the distribution and association of these cells in TRAMP-C1 tumors with hypoxic and necrotic regions following RT. Tumor-infiltrating cells in control, undisturbed tumors were randomly located and no specific distribution pattern can be identified. In irradiated tumors, except the aggregation of CD68 positive macrophages at chronic hypoxia region, we further found that CD11b and Gr-1 positive cells were concentrated in central necrotic region and F4/80 positive macrophages were distributed along the junction of necrotic and chronic hypoxic region. Flow cytometry assay demonstrated that total CD11b cells were not altered, but there are more CD11b and Gr-1 positive cells in the necrotic region of irradiated tumor than control tumor, no matter the size of tumor or necrotic area. The re-distribution pattern of different subsets of CD11b positive cells into different microenvironments in irradiated tumors suggest irradiated tumors form subcomponent which has factor(s) to attract specific subset of CD11b positive cells. The illustration of the role and function of these cells in particular regions may provide a new strategy to improve the effectiveness of radiation therapy. (This work is supported by grants of NHRI-EX98-9827BI and NTHU-98N2425E1 to Chi-Shiun Chiang)

Poster No. 212

Single-Chain Antibodies against the HGF/SF Receptor

Danielle DiCara[1,3], Zhe Sun[2], John McCafferty[2], Ermanno Gherardi[1]
[1]*Growth Factors Group, MRC Centre, Cambridge, UK,* [2]*Department of Biochemistry, University of Cambridge, Cambridge, UK,* [3]*Department of Oncology, University of Cambridge, Cambridge, UK*

Dysregulation of the Met receptor tyrosine kinase and of its cognate ligand Hepatocyte Growth Factor / Scatter Factor (HGF/SF) occurs frequently in cancer, and Met overexpression indicates poor prognosis in several cancers such as breast and head and neck. HGF/SF binding triggers signalling that promotes cancer cell migration, proliferation and invasion. We have generated Met-binding single-chain fragment variable (scFv) antibodies by phage display, using the 'McCafferty' library, which has a diversity of 10^{10} clones. After two rounds of biopanning, 76/182 clones bound Met in ELISA, of which 72 were found to be unique. Preliminary data indicates isolation of several clones capable of inhibiting HGF/SF-induced scatter of the pancreatic cancer line BxPC-3. Affinity maturation and selection strategies directed towards antibodies that bind the same epitopes as HGF/SF may yield clones with higher activity. Met-blocking scFv may be useful for cancer therapy.
This work is funded by Cancer Research UK / Cancer Research Technology.

Poster No. 213

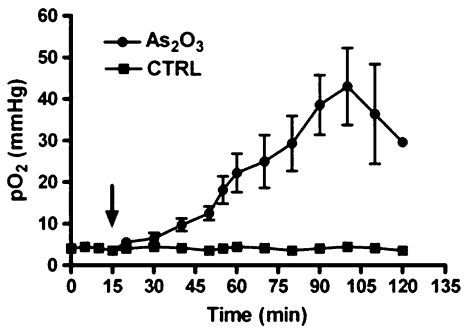
MR Characterization of the Tumor Microenvironment after Arsenic Trioxide Treatment: Evidence for an Effect on Oxygen Consumption that Radiosensitizes Solid Tumors

Caroline Diepart[1], Bénédicte Jordan[1], Bernard Gallez[1]
[1]*Université catholique de Louvain, Brussels, Belgium*

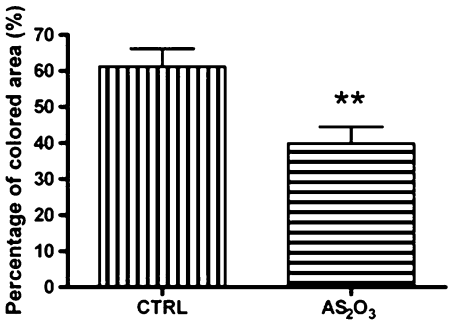
Introduction
The pO_2 is a crucial factor affecting the response of tumors to irradiation and other cytotoxic treatments. It has been predicted that modification of oxygen consumption is much more efficient at alleviating hypoxia than modification of oxygen delivery.
Arsenic has been reported to have anti-tumor effect in acute promyelocytic leukemia and in solid tumors. As_2O_3 seems also to inhibit mitochondrial respiratory function in human leukemia cells. Thus, we hypothesized that As_2O_3 could be an important modulator of tumor oxygenation by affecting the oxygen consumption of tumors.
Materials and methods
The effect of As_2O_3 (5 mg/kg) was studied in TLT tumor model. Local pO_2 was measured in vivo using low frequency EPR (1) and ^{19}F-relaxometry (2). The oxygen consumption rate was measured in vitro using high-frequency EPR. At the maximum pO_2 (after 1 h30) perfusion and radiation sensitivity were also studied by Patent Blue staining assay and regrowth delay experiment after X-Ray irradiation (10 Gy), respectively (Fig.4).

Results

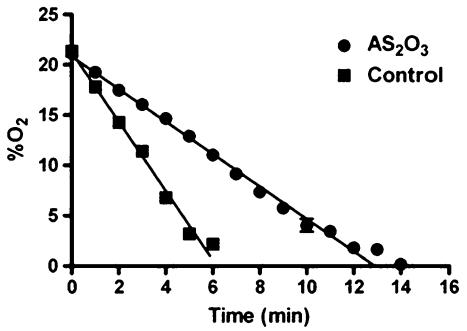
The administration of As_2O_3 increases significantly the pO_2 in TLT tumors, an effect that was not observed for the control group (Fig.1). The results were confirmed by ^{19}F NMR. The increase in pO_2 induced by As_2O_3 was not due to an increase in tumor perfusion as shown by the Patent blue staining assay (Fig.2). As the increase in pO_2 was not due to an increase in perfusion, the tumor oxygen consumption was investigated. The administration of As_2O_3 significantly decreased the oxygen consumption (Fig.3). Finally, the irradiation (10 Gy) of tumors showed a regrowth delay that was significantly increased in arsenic-treated mice.



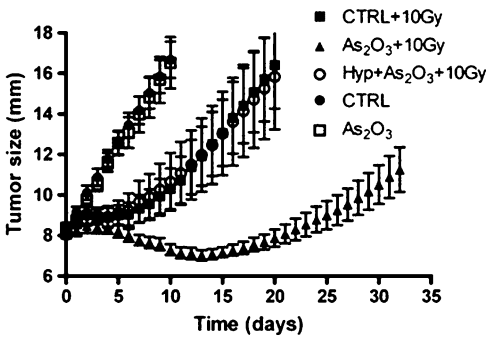
Tumor pO_2 measured by EPR oximetry in TLT tumors as a function of time. Arrows,injection time of the drug.



Effect of As_2O_3 on blood perfusion time measured by the Patent blue staining.



Effect of As_2O_3 administration on tumor oxygen consumption rate in TLT tumor cells.



Effect of As_2O_3 and radiation on TLT tumor regrowth.

Conclusion

As_2O_3 is an important modulator of pO_2 by decreasing oxygen consumption and enhances the response of tumors to radiotherapy.

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Poster No. 214

Zinc-α2-glycoprotein: A New Biomarker of Breast Cancer?

Virginie Dubois[1,2], Laetitia Delort[1,2], Hermine Billard[1,2], Thierry Jardé[2], Florence Mishellany[3], Charlotte Lequeux[5], Odile Damour[5], Frederique Penault-Llorca[3], Marie-Paule Vasson[2,4,6], Florence Caldefie-Chezet[1,2,6]

[1]Laboratoire SVFp, Clermont-ferrand, France, [2]Departement of pharmacy, EA 4233 "Nutrition, Cancérogenèse et Thérapie anti-tumorale", CRNH Auvergne, Clermont-ferrand, France, [3]Laboratoire d'anatomopathologie, Clermont-ferrand, France, [4]Unité de Nutrition, Centre Jean-Perrin, Clermont-ferrand, France, [5]Banque de Tissus et de Cellules, Hôpital Edouard-Herriot, Lyon, France, [6]Cancéropole Lyon Auvergne Rhône-Alpes (CLARA), Lyon, France

It is now established that adipose tissue secretions, i.e. adipokines, may play a role in mammary carcinogenesis development. We have shown that two major adipokines, leptin and adiponectin, had stimulating and inhibiting effects on cell proliferation respectively and were expressed in mammary adenocarcinoma[1,2]. At present, we evaluated Zinc-α2-glycoprotein (ZAG) expression, which is known to be overex-

pressed in prostate tumors[3], in tumor or healthy breast tissue and examined whether it was correlated with that of major adipokines (leptin, adiponectin), usual tumor biomarkers (sex steroids receptors, *i.e.* estrogen (ER) and progesterone; Ki67 proliferation factor; cErb2 growth factor receptor), or apoptosis markers (Bcl2 and Bax).

Biopsies (n=55) of human ductal breast carcinoma (Jean-Perrin Anti-Cancer Center) and mammary tissues (n=6) of healthy women (Edouard-Herriot Hospital), were used to investigate ZAG expression by immunohistochemistry (ABC technique, biotin-avidin-peroxidase). Statistical analysis was realized with Spearman correlation.

ZAG expression was detected in ductal carcinoma and in normal epithelial adjacent tissue (87% and 94% of cases studied respectively) but was not found in normal tissue of healthy women. In cancer tissue, its expression was positively correlated to leptin receptor ($p=0.01$, $r=0.459$) and negatively to adiponectin receptor ($p=0.03$, $r=-0.371$) and ER ($p=0.04$, $r=-0.279$). We did not show statistically significant correlation between ZAG and the other studied markers.

These preliminary results suggest both a close relationship between ZAG expression and pathways involving major adipokines or estrogen and, that ZAG may be a potential breast cancer biomarker, which requires further investigations.

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Poster No. 215

TP53 Mutations in CFDNA from Egyptian Patients, as Biomarkers for Cancer Prevention

Gihan Hosny[1], Pierre Hainaut[2]

[1]*Environmental Health & Molecular Carcinogenesis Division, Dept of Environmental Studies, Institute of Graduate Studies and Research, University of Alexandria, Alexandria, Egypt,* [2]*Molecular Carcinogenesis, International Agency for Research on Cancer, Lyon, France*

Background:

It is well known that chronic infections affect the microenvironment with a high proportion of cancer incidence.In Egypt, chronic infection with hepatitis C,HCV, is a widespread infection among Egyptian population, and has been associated with increased incidence of Hepatocellular Carcinoma,HCC, and in some studies with increased risk of non-Hodgkin lymphoma,NHL.P53 protein plays an important role in the maintenance of genome stability in mammalian cells;it acts in many processes including cell-cycle checkpoint, DNA repair, apoptosis, and angiogenesis. Mutations of P53 have been reported as common mutations in solid tumors, including HCC and NHL, and have been implicated in drug resistance, aggression and poor prognosis. Circulating free DNA (CFDNA) has been shown to be a good source of liver tissue derived DNA in African and Asian patients with chronic liver disease or HCC.

Objective:

We have examined the presence of p53 mutations from exons 5 to 9 in CFDNA of patients with HCC or chronic liver disease, and of patients with NHL from Alexandria,Egypt, in two separate case-control studies.

Results:

The DNA concentrations were significantly higher in HCC patients compared to HCV individuals. TP53 249 mutations were shown in 5% of CFDNA and 10% of tumors of HCC,with underlying HCV. Also, concentrations of CFDNA were significantly higher among NHL patients compared to the negative control individuals. Mutations of p53 determined in NHL cases (30%) were of Arg-176(1/20:5%), Phe-238(1/20:5%), Ser-249(2/20;10%), Lys-249(1/20:5%) and Phe-250(1/20:5%).No mutations were detected among controls.

Conclusion:

Our findings of higher DNA concentrations with some p53 mutations in CFDNA from cancer patients that match the previous reported p53 mutations from tumor DNA may hold promises that CFDNA may serve as a convenient source of tumor-derived DNA to serve as a promising tool of a non-invasive, low-cost new strategy for earlier detection, diagnosis and follow-up of the disease.

Poster No. 216

In Vivo Targeted Delivery of Members of the TNF Superfamily to RIP-Tag Tumours Enhances T Cells Penetration and Function

Anna Johansson[1], Juliana Hamzah[1], Ruth Ganss[1]

[1]*University of Western Australia, Centre for Medical Research, Western Australian Institute for Medical Research, Perth, WA, Australia*

Solid tumours maintain a barrier that prevents 1) adequate delivery of anti-tumour drugs and 2) immune cells penetrating the tumour microenvironment and exerting their effects. In clinical trials, this is reflected by the large proportion of patients where systemic anti-cancer vaccines or adoptive transfer of anti-cancer immune cells ultimately fail to induce a strong anti-tumour response. In a mouse model where SV40 Large T antigen is expressed in the β cells of the pancreas (RIP1-Tag5), studies have shown that the inflammatory environment and the tumour vasculature can be modulated as to allow T cell penetration and tumour rejection [1–3]. Recently, a peptide was identified (CRGRRST) that specifically homes to RIP1-Tag tumour vessels [4]. We have used this peptide to produce fusion proteins using the TNF family members, TNFa and LIGHT (LIGHT; Homologous to Lymphotoxins, shows inducible expression, and competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed by T lymphocytes). These compounds are of particular interest for tumor-targeting because of their documented anti-tumor effects and their potential but unexplored dual actions on tumor stroma and immune effector cells. The activity of our fusion proteins was verified in vitro using FACS analysis, followed by demonstration of specific homing to RIP1-Tag5 tumour vessels after systemic injection in mice. We

show here that TNFa and LIGHT targeted to the tumour microenvironment simultaneously activate the tumour stroma and CD8+ effector cells, and therefore result in enhanced T cell influx that ultimately leads to tumour destruction.

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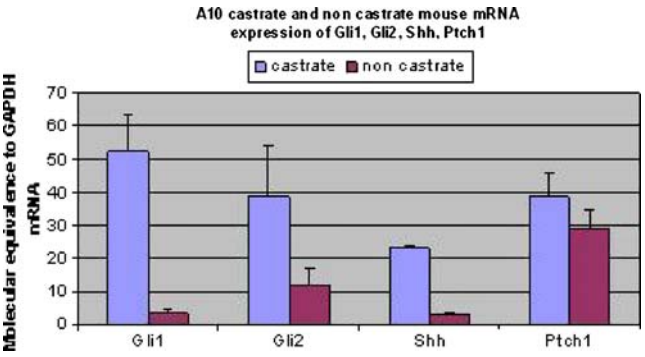
Poster No. 217

Preclinical Model Selection for the Study of Hedgehog Signaling in Androgen Resistant/ Independent Growth in Prostate Cancer. From Bedside to Bench.

Maria Karlou[1], Jun Yang[2], Sankar Maity[2], Nora M. Navone[2], Jing-Fang Lu[2], Xinhai Wan[2], Anh Hoang[1], Christopher J. Logothetis[2], Eleni Efstathiou[1]
[1]*Department of Genitourinary Medical Oncology, David H. Koch Center for Applied Research of Genitourinary Cancers, The Stanford Alexander Tissue Derivatives Laboratory, The University of Texas MD Anderson Cancer Center, Houston, TX, USA,* [2]*Department of Genitourinary Medical Oncology, David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, TX, USA*

Background:
In the tumor microenvironment, activation of tumor-stromal interactions is considered to play a critical role in Prostate Cancer (PCa) progression. Hedgehog signaling, a developmental pathway implicated in cancer, has been associated with resistance to cytotoxic treatment in human samples. Thus hedgehog signaling inhibition is a candidate therapeutic target for combination with maximal androgen ablation. Selection of preclinical models of PCa relevant to the human disease is imperative for development of applicable therapeutic strategies.
Materials and methods:
Xenografts generated by our research team from castrate-resistant PCa specimens were used to screen gene expression of key components in hedgehog signaling. Tumors were examined for the RNA and protein expression of Shh, Gli1, Gli2, Smo, Ptch1 and Sufu by Real Time RT-PCR and IHC in both (human) prostate cancer cells and in host (mouse) derived stromal cells.
Results-Conclusions:
118b is an androgen independent xenograft, not expressing AR, inducing bone formation in the surrounding stroma. This xenograft has a striking overexpression of hedgehog signaling including nuclear expression of Gli1 and Gli2. Xenografts A10, 137, 117, 115 and 79 are expressing AR and some extent of hedgehog signaling. All studied models showed differential gene expression of hedgehog signaling components in stromal compartment compared to tumor cells. Notably, A10 when grown in castrate host has increased expression of the transcription factors Gli1 and Gli2 and the ligand Shh, in the stromal compartment as compared to growth in non-castrate (vide infra). This experiment recapitulates the human condition based on our translational results and therefore might be the most well suited model to test the effect of hedgehog signaling inhibition on blocking androgen-resistant growth.



Poster No. 218

Osteopontin Secreted by Glioma Cells Modulates Behavior of Tumor-Associated Macrophages without Affecting Migration and Invasiveness of Tumor Cells

Magdalena Klocek[1], Pawel Wisniewski[1], Aleksandra Ellert-Miklaszewska[1], Bozena Kaminska[1]
[1]*Nencki Institute of Experimental Biology, Warsaw, Poland*

Recent data have expanded the concept that the tumor microenvironment, largely arranged by inflammatory cells, is an indispensable participant in the neoplastic process. Tumor-associated macrophages represent the major component of the stroma of many tumors, including brain tumors - gliomas, and their high content correlates with malignancy and poor patient prognosis. We have demonstrated that glioma cells release soluble factors which induce accumulation and a non-inflammatory activation of brain macrophages associated with pro-invasive function of these cells[1, 2]. Proteomic analysis of glioma-conditioned medium (G-CM) using HPLC fractionation followed by a tandem mass-spectrometry revealed that one of these factors is Osteopontin (OPN), a metastasis-associated small integrin-binding ligand N-linked glycoprotein family member. Interference with OPN binding to integrins using a blocking RGD peptide, abolished morphological alterations of brain macrophages induced by G-CM. We demonstrate that Osteopontin was abundantly expressed in rat C6 glioma cells, but not in non-transformed glial cells. Using pharmacological inhibitors of many signaling pathways, we found that MEK1/2-ERK and NFκB signaling pathways are

responsible for the high expression of OPN in glioma cells. To evaluate the role of OPN in glioma pathology, Osteopontin expression was efficiently silenced with the commercial siRNA (Qiagen). Silencing of Osteopontin had no impact on proliferation and survival of transfected glioma cells. Furthermore, the migration rate of glioma cells (evaluated with a wound healing assay), as well as glioma invasiveness (determined with the Matrigel invasion assay) were not affected by siRNA OPN. Altogether, our studies indicate that tumor-derived OPN does not affect properties of tumor cells itself, but may be a crucial factor mediating interactions between glioma and tumor-associated brain macrophages and involved into pathogenesis of gliomas.

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Poster No. 219

Discoidin Domain Receptor 2 Deficiency Predisposes Hepatic Tissue to Colon Carcinoma Metastasis

Elvira Olaso[1], Iker Badiola[1], Beatriz Arteta[1], Aritz Lopategi[1], Fernando Vidal-Vanaclocha[1]
[1]*Department of Cell Biology and Histology, Basque Country University, Leioa, Bizkaia, Spain*

The transdifferentiation of hepatic stellate cells (HSC) into myofibroblasts is a key event for the development of stroma and angiogenesis during hepatic metastasis development, although regulatory pathways involved in HSC activation are unclear. Discoidin domain receptor 2 (DDR2) is a tyrosine kinase receptor for fibrillar collagen expressed by activated HSC during hepatic fibrosis. Mice lacking DDR2 gene ($DDR2^{-/-}$) have an enhanced susceptibility to carbon-tetrachloride-induced hepatic fibrosis, suggesting that DDR2-dependent genes are anti-fibrogenic. Therefore, we hypothesized that tumor stroma formation by transdifferentiated HSC may be enhanced by DDR2 deficiency, predisposing hepatic tissue to colon carcinoma metastasis. Experimental hepatic MCA38 colon carcinoma metastasis occurrence and development significantly increased in $DDR2^{-/-}$ compared to $DDR2^{+/+}$ mice. Immunohistochemical analysis showed that hepatic metastases in $DDR2^{-/-}$ mice had higher density of HSC-derived myofibroblasts (dual desmin/alpha-smooth muscle actin-expressing cells), neoangiogenic vessels (CD31-expressing cells) and proliferating cells (ki67-expressing) than in $DDR2^{+/+}$ littermates. Consistent with in vivo findings, secretion of endothelial cell adhesion- and migration-stimulating factors, and of MCA38 cell proliferation-stimulating factors significantly increased by 50% in the supernatants of $DDR2^{-/-}$ HSC primary cultures, compared to those from wild-type HSC. These secreted factors further increased by 20% in the supernatants of $DDR2^{-/-}$ HSC cultures pretreated with MCA38 cell-conditioned media. Moreover, compared to wild-type HSC, gene profiling of $DDR2^{-/-}$ HSC showed increased expression of a cluster of genes, associated with inflammation and extracellular matrix remodeling, that have been clinically correlated with hepatic metastasis occurrence, such as IL-10, TGFbeta, syndecan-1, integrin-a2, thrombopoietin and BMP7. These results demonstrate that DDR-2 deficiency predisposes hepatic tissue to colon carcinoma metastasis. The mechanism may depend on a special prometastatic microenvironment operating in the absence of certain DDR2-dependent factors that prevent tumor cell adhesion and proliferation, and endothelial cell migration.

Poster No. 220

Time-Dependent Effects of Aflibercept (VEGF Trap) on Functional Vessels, Tumor Hypoxia, and Distribution of Doxorubicin in Tumor Xenografts

Vithika Sivabalasundaram[1], Krupa Patel[1], Ian F. Tannock[1]
[1]*Division of Applied Molecular Oncology, Princess Margaret Hospital, Toronto, ON, Canada*

Background:
Clinical experience has shown limited benefits when anti-angiogenic agents that target VEGF are used alone, but greater effects when combined with chemo-therapy. Transient vascular normalization has been proposed to explain this unexpected combination effect (Jain, Science 2005;307:58–62), which involves reduced vascular permeability, destruction of immature vessels and increased pericyte recruitment at specific times following anti-VEGF therapy. The resulting improvement of tumor blood flow and oxygenation, and reduction in interstitial fluid pressure, might improve chemotherapy delivery. Evidence to support vessel normalization remains inconsistent. Here we evaluate the effect of aflibercept, a potent soluble receptor for VEGF (undergoing clinical trials), for its effect on vascular physiology and delivery of doxorubicin to solid tumors.

Hypothesis:
During a certain window of time, aflibercept will increase functional blood vessels, decrease hypoxia, and improve delivery and therapeutic effects of doxorubicin.

Methods:
Mice with A431 human squamous cell cancer xenografts were killed at 8, 24, 72 hours and 7 days following administration of aflibercept or its diluent; they received EF5 (to identify hypoxic regions), doxorubicin, and DiOC7 (a marker of blood flow) at 2 hours, 10 minutes and 1 minute respectively prior to death. Excised tumors were frozen, sectioned and stained for blood vessels (with anti-CD31) and hypoxia. The distribution of doxorubicin relative to functional blood vessels was quantified by immunohistochemistry as described previously (Primeau et al, Clin Cancer Res 2005;22:8782–8). Therapeutic effects of doxorubicin, with or without prior VEGF-Trap, were studied by growth delay.

Results:
The table below summarizes median values of various outcomes. Studies to quantify the distribution of doxorubicin, and its therapeutic effects are in progress, and will be reported at the meeting.

Conclusions:
These results suggest a transient improvement in vessel functionality and reduction in hypoxia between 24 and 72 hours in tumors treated with aflibercept lending support for normalization of vessels.

		8 Hours	24 Hours	72 Hours	7 Days
Necrosis (Percent of area)	Control	1.2	2.8 *	4.2	13.0
	VEGF-Trap	1.5	0.4 *	3.9	7.8
Hypoxia (Percent of area)	Control	4.7	5.6	15.1 *	19.2
	VEGF-Trap	3.5	2.3	21.8 *	20.1
Blood Vessels (Percent of area)	Control	2.6	1.6 *	1.5 *	2.0
	VEGF-Trap	3.0	3.4 *	3.5 *	1.9
Proportion of Functional Vessels (Density; Relative to 8 Hr Control)	Control	1	0.71	0.65	0.48
	VEGF Trap	0.88	1.29 *	0.62 *	0.42

*P<0.05 using paired t-test

Poster No. 221

Identification of a Critical Role for Matrix Enzyme LOXL2 in the Creation of the Pathologic Microenvironment in Tumors and a Novel Inhibitory Therapeutic Strategy

Vivian Barry-Hamilton[1], Rhyannon Spangler[1], Derek Marshall[1], Hector Rodriguez[1], Scott McCauley[1], Alison Holzer[1], Carol Wai[1], Miho Oyasu[1], Amanda Mikels[1], Maria Vaysberg[1], Carlos Garcia[1], Arleene Velayo[1], Donna Biermann[1], Daniel Tsai[1], Brett Jorgensen[1], Scott Ogg[1], Peter Van Vlasselaer[1], **Victoria Smith[1]**
[1]*Research and Development, Arresto BioSciences, Palo Alto, CA, USA*

Extensive clinical evidence and mouse models of tumorigenesis support the critical role of the microenvironment in promoting tumor growth and metastasis. We have identified a novel role for extracellular matrix enzyme lysyl oxidase-like 2 (LOXL2) in the creation of the pathologic microenvironment of oncologic and fibrotic diseases through modulation of matrix tension. Our analysis of human tumors and liver fibrosis revealed widespread and conserved expression of LOXL2 by activated fibroblasts and neovasculature. The inhibition of LOXL2, but not LOX, with a specific monoclonal antibody was efficacious in both primary and metastatic xenograft models of cancer, as well as CCl4-induced liver fibrosis. Inhibition of LOXL2 resulted not only in a substantial reduction in fibroblast activation and recruitment, desmoplasia, and vascularization, but also in significantly decreased production of pro-angiogenic growth factors and cytokines such as VEGF and SDF1, as well as reduction of collagen production and LOXL2 expression itself. Tumor cells in anti-LOXL2 treated animals showed significant increases in necrosis and pyknosis. Anti-LOXL2 therapy using a monoclonal antibody, while highly specific, revealed a broad spectrum of pleiotropic effects that impacted tumor viability. The anti-LOXL2 monoclonal antibody outperformed small molecule pan-lysyl oxidase inhibitor b-aminoproprionitrile (BAPN) in all analyses. Unlike BAPN, this antibody acts as a substrate-independent inhibitor of LOXL2, and represents a new therapeutic approach with broad applicability in oncologic and fibrotic diseases. In addition, targeting the genetically more stable stromal cells of the tumor microenvironment offers the potential for reduced likelihood of drug resistance.

Poster No. 222

Impact of Extracellular Matrix Composition on Drug Diffusion and Efficacy

Tiziana Triulzi[1], Gaia Ghedini[1], Patrizia Casalini[1], Cristina Ghirelli[1], Elda Tagliabue[1]
[1]*Department of Experimental Oncology, Fondazione IRCCS, Istituto Nazionale dei Tumori, Milano, Italy*

By microarray supervised analysis on a dataset obtained from breast carcinoma patients treated with docetaxel as neoadjuvant therapy, the foremost variable identified has been SerpinB5, a serine-protease-inhibitor, using disease-progression as supervised variable. SerpinB5 resulted 13 times more expressed in non-responsive in comparison to

responsive tumors ($p<0.0001$). Real Time PCR on 30 core biopsies from patients treated in our Institute with neoadjuvant therapy, revealed 3 times higher SerpinB5 expression in non-responder patients in comparison to responders ($p=0.002$).
To understand the role of SerpinB5 in response to therapy we infected breast carcinoma cells MCF7 with SerpinB5 (MCF7-Ser). Tumors from nude mice xenografted with MCF7-Ser presented reorganized accumulation of collagen fibers. Immunofluorescence analysis by confocal microscopy showed a dramatically decreased localization of doxorubicin (DXR) within tumors from MCF7-Ser in comparison to mock cells, suggesting that resistance to chemotherapy in patients with SerpinB5 overexpressing breast carcinomas could derive from less drug diffusion.
To investigate the importance of extracellular matrix amount in drug diffusion and efficacy, we injected HER-2-overexpressing cancer cells in nude mice, mixed or not with Matrigel. Matrigel-mixed tumors resulted significantly ($p<0.01$) more resistant to DXR and showed lower apoptosis levels compared to those without Matrigel. Analysis by imaging mass spectrometry and immunofluorescence revealed lower uptake of DXR, confirming that dense matrix could be responsible for tumor chemoresistance through drug diffusion inhibition.
Using hydrophilic liposome based DXR formulation, DXR has been detected also in Matrigel-mixed tumors, suggesting that the less free drug diffusion could be due to its physical-chemical properties. Accordingly treatment with hydrophilic-drug Trastuzumab resulted more effective in tumors from Matrigel-mixed cells and the presence of the bio-drug, analyzed by immunofluorescence and radioimmune localization assay, was higher in tumor cells surrounded by dense extracellular matrix.
In conclusion extracellular matrix accumulation impacts drug diffusion according to drug physical properties.
Partially supported by a grant from AIRC)

Poster No. 223

The Spatial Distribution of Various Subtypes of Tumor-Associated Macrophages within a Murine Brain Tumor

Shu-Chi Wang[1], Shao-Hua Lo[1], Chi-Shing Chiang[1]
[1]*Department of Biomedical Engineering and Environmental Sciences., National Tsing Hua University, Hsin-Chu, TAIWAN*

While the roles of tumor-associated macrophages (TAMs) in brain tumors are extensively studied recently, the distinct roles of subtypes of TAMs on tumor progression or caner therapy remain unclear. To define the roles of different subtypes of TAMs within brain tumors, the spatial distribution of CD11b-positive or CD68-positive TAMs within GL261 murine glioma cells grown intracranially in C57/BL6 mice were first examined. We found that CD11b-positive TAMs within the highly cellular tumor were mainly distributed along the tumor border. On the other hand, the CD68-positive TAMs were more centered in tumor core. This indicates that intracranial growing tumors may have two distinct subtypes of TAMs and they may have different origins. To further address this question, bone marrow-derived monocytes from GFP mice were i.v. injected into GL261 tumor-bearing mice. One week after the transplantation, a patch of GFP positive cells were found to be co-localized with CD11b staining in brain tumor region under confocal microscopy. These cells have apparently characteristic of macrophage with kidney-shaped nuclei. These data indicate that not only local microglia proliferation and migration into the tumor, furthermore, the peripheral monocytes can also infiltrate into the brain tumor. To further dissect the origins of CD11b-positive and CD68-positive TAMs within brain tumors, the bone marrow transplantation model is currently undertaken.

Poster No. 224

The Telomeric Complex TRF2-Apollo Protects Tumor Cells from Senescence and Replication Stress

Jing Ye[1], Christelle Lenain[1], Serge Bauwens[1], Simon Amiard[1], Marie-Joseph Giraud-Panis [1], Eric Gilson[1]
[1]*Laboratoire de Biologie Moléculaire et Cellulaire, CNRS UMR5239, IFR128, École Normale Supérieure de Lyon, Lyon, France*

Cells usually respond intrinsically to the perception of DNA damage by initiating the DNA damage response (DDR) that leads to cell-cycle arrest and repair. For instance, critical shortening or chromatin alterations of telomeres activates DDR, thereby inducing senescence or apoptosis. Interestingly, the DDR pathway does not only lead to cell-cycle arrest, repair and senescence but also to an inflammation environment and to the activation of innate immune responses that remove senescent cell from the organism. Therefore, genome integrity is kept in check by both intrinsic and extrinsic mechanisms suggesting unexpected links between DNA alterations, immunity, aging and cancer[1].
Many unknowns remain in the description and understanding of these extrinsic responses to genome injury and in particular in their role during oncogenesis. Our laboratory recently provide evidence that the essential telomere protein TRF2 controls a DDR-independent extracellular anti-tumor program via activation of natural killer cells[2].
We investigate here the function of the TRF2-interacting protein Apollo, which is an ortholog of the Artemis factor. We found that TRF2 and Apollo prevent cells to enter into senescence by preventing breakage during telomere replication. In particular, the expression of a mutated form of Apollo abolishing its 5′-exonuclease activity but preserving its telomeric location does not complement the damaged telomeres resulting from a diminished expression of endogenous Apollo. Moreover, the expression of this nuclease-dead allele of Apollo or of a dominant-negative form of TRF2 triggers the DDR pathway at chromosome ends but also at an interstitial telomeric DNA region. We propose that TRF2 regulates an Apollo-mediated nucleolytic processing of

telomere structures prone to break DNA during replication. We will discuss the possibility that the overexpression of TRF2 and Apollo observed in different types of human cancers protects malignant cells from intrinsic and extrinsic anti-cancer barriers suggesting that these proteins would be valuable therapeutic targets to modulate tumor-microenvironment.

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