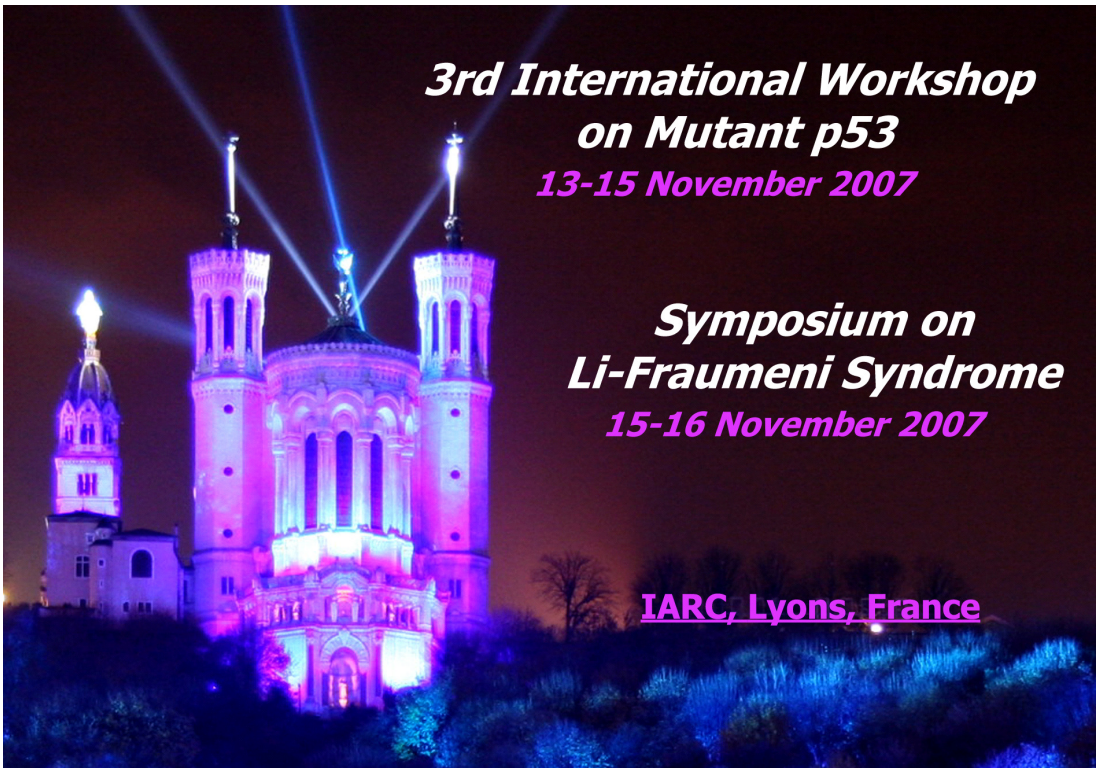
INTERNATIONAL P53 MARATHON

Deregulating the p53 Network: Origin and Consequences of TP53 Mutations



An international meeting jointly organized by the International Agency for Research on Cancer (IARC) and the European Community

And

by invitation only:

Mutp53 consortium, 12 November, 2007
Active p53 consortium, 15-16 November, 2007

IARC TP53 Mutation Database
http://www-p53.iarc.fr/index.html

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MEETING SCOPE

The **3rd International Workshop on Mutant P53,** held at IARC in Lyon, focused on "The origin and consequences of *TP53* mutations". Understanding the biological role of mutant p53 and its clinical impact requires the development of a global approach that integrates the structural biology of mutant proteins, the evaluation of their functional properties, the distribution of mutations in human cancers, and the correlation between mutations and the clinical and pathological parameters of cancer. The objective of this workshop is to review and discuss the state of the art and the experimental methods and models available for such global approaches. The workshop will cover topics ranging from the causes of mutations to the evaluation of their functional and clinical impacts in human cancers.

As for the 2nd workshop, the annual meetings of two research consortiums working on p53 (Mutp53 and Active p53) and funded by the European Community (FP6) was held in conjunction with this international workshop.

This year the workshop included a symposium on **Li-Fraumeni syndrome (LFS).** LFS is a rare autosomal disorder characterized by a familial clustering of early-onset tumors that is caused by *TP53* germline mutations. The objectives of this symposium were to (1) revisit this syndrome in light of recent accumulated knowledge on the biological impacts of *TP53* mutations, (2) stimulate the creation of an international registry of families with *TP53* germline mutations, (3) set-up a framework for supporting genetic counseling and *TP53* genetic testing for Li-Fraumeni and Li-Fraumeni like families in low resource countries.

The workshop included 34 lectures, 8 short presentations selected from submitted abstracts, a poster session with 42 posters. In the LFS symposium, a group of 13 experts hold a round-table discussion on key issues related to management of patients affected by Li-Fraumeni syndrome.

ORGANIZERS

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FUNDING BODIES

International Agency for Research on Cancer
European Community, FP6

VENUE

International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon, France, http://www.iarc.fr/.

Marathon Website: http://www-p53.iarc.fr/P53meeting2007/P53meeting2007.html

MARATHON 2007 PROGRAMME

Monday, 12 November
All day **Mutant p53 Consortium**

Tuesday, 13 November
All day **3rd International Workshop on Mutant p53**
End of day Cocktail

Wednesday, 14 November
All day **3rd International Workshop on Mutant p53**
Evening Dinner

Thursday, 15 November
Morning **3rd International Workshop on Mutant p53**
Afternoon **LFS symposium**
Active p53 Consortium

Friday, 16 November
Morning **LFS round-tables**
Active p53 Consortium

	Monday 12	Tuesday 13	Wednesday 14	Thursday 15	Friday 16
Mutp53 consortium					
3rd International Workshop					
LFS round-tables				LFS symposium	
Active p53 consortium					
Social events		Cocktail	Dinner		
Registration	*7:30 - 9:30 AM* *4:00 - 6:00 PM*	*7:30 - 1:00 PM* *4:00 - 5:30 PM*	*8:00 - 9:00 AM* *4:00 - 6:00 PM*	*10:00 - 2:00 PM*	

WORKSHOP SCIENTIFIC PROGRAMME

Tuesday, 13 November

Introduction and welcome

Peter BOYLE, *Lyon, France*

Session 1: TP53 mutations in Molecular Carcinogenesis
Chair: Alan FERSHT

Lecture 1: **Curtis HARRIS**, *Bethesda, USA*
INFLAMMATION AND CANCER: INTERACTIONS OF THE MicroRNA, p53 AND CYTOKINE PATHWAYS -- *Aaron Schetter, Krista Zanetti, Ewy Mathe, S. Perwez Hussain, Carlo Croce and Curtis C. Harris*

Lecture 2: **Pierre HAINAUT**, *Lyon, France*
REVISITING "INITIATION" IN CARCINOGENESIS: PLACE OF *TP53* MUTATIONS IN EARLY STEPS OF CANCER

Short communication 1: **Catherine WHIBLEY**, *Leeds, UK*
MOUSE MODELS TO STUDY SENESCENCE BYPASS AND CANCER GENE MUTATIONS CAUSED BY ENVIRONMENTAL MUTAGENS -- *Whibley C., Hollstein M.*

Session 2: p53 structures and activities
Chair: Curtis HARRIS

Lecture 3: **Sir Alan FERSHT**, *Cambridge, UK*
THE STRUCTURE OF p53

Lecture 4 : **Jean-Christophe BOURDON**, *Dundee, UK*
p53 ISOFORM EXPRESSION MAY ABROGATE P53 MUTATION AND IS ASSOCIATED WITH GOOD PROGNOSIS IN BREAST CANCER -- *A. Diot, K. Fernandes, S. Bray, P. Quinlan, L. Baker, C.Purdie, D. Kellock, A. Thompson and JC Bourdon*

Short communication 2: **Ella KIM**, *Göttingen, Germany*
SPLICE ISOFORM p53-BETA HAS ANTI-APOPTOTIC ACTIVITY AND ANTAGONIZES APOPTOTIC SIGNALING MEDIATED BY p53 -- *Eva-Maria Bücker, Sven Hanson, Kira Erber, Nadine Pettkus, Alf Giese, and Ella Kim*

Lecture 5: **Sumitra DEB**, *Richmond, USA*
GAIN OF FUNCTION MUTANT P53 PROTEINS ALTER CHEMOSENSITIVITY OF CELLS VIA NF-KB2 -- *Scian M.J., Nesheiwat I., Stagliano K.E.R., Anderson M.A.E., Dumur C., Garrett C., Deb S.P. and Deb S.*

Session 3: Mutant p53 functions I

Chairs: Wolfgang DEPPERT & Giannino DEL SAL

Lecture 6: **Mike RESNICK**, *Research Triangle Park, USA*
NONCANONICAL SEQUENCE MOTIFS AS TARGETS FOR TRANSACTIVATION BY WT AND MUTANT p53 -- *Michael A. Resnick, Daniel Menendez, Jennifer J. Jordan, Maher Noureddine, Douglas Bell, Alberto Inga*

Lecture 7: **Giannino DEL SAL**, *Trieste, Italy*
CHARACTERIZATION OF THE ROLE OF PIN1 AS A REGULATOR OF P53 FUNCTION AND DYSFUNCTION -- *Mantovani F., Girardini J., Tocco F., Guida E., Bisso A., Napoli M. and Del Sal G.*

Short communication 3: **Genrich TOLSTONOG**, *Hamburg, Germany*
SYSTEMATIC APPROACHES TO STUDY TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL FUNCTIONS OF MUTANT P53 IN HUMAN AND MOUSE TUMOR CELLS -- *Annette März, Lars Tögel, Marie Brazdova, Christine Loscher, Wolfgang Deppert, and Genrich V. Tolstonog*

Short communication 4: **Gianluca BOSSI**, *Rome, Italy*
EXPLORING MUTANT P53 GAIN OF FUNCTION IN VIVO THROUGH CONDITIONAL RNA INTERFERENCE -- *Gianluca Bossi, Francesco Marampon, Bianca Zani, Giovanni Blandino,Ada Sacchi*

Lecture 8: **Varda ROTTER**, *Rehovot, Israel*
MUTANT P53 ATTENUATES THE SMAD-DEPENDENT TGF-B1 SIGNALING PATHWAY BY REPRESSING THE EXPRESSION OF TYPE II TGF-B RECEPTOR -- *Eyal Kalo, Yosef Buganim, Keren E. Shapira, Hilla Besserglick, Naomi Goldfinger, Lilach Weise, Perry Stambolsky, Yoav I. Henis, and Varda Rotter*

Lecture 9: **Jiri BARTEK**, *Copenhagen, Denmark*
TUMOR SUPPRESSORS IN DNA-DAMAGE CHECKPOINTS PATHWAYS

Short communication 5: **George HINKAL**, *Houston, USA*
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Short communication 6: **Ygal HAUPT**, *Jerusalem, Israel*
REGULATION OF P53 BY PML IN RESPONSE TO DNA DAMAGE -- *Sue Haupt, Osnat Alsheich Bartok, Inbal Mizrahi and Ygal Haupt*

Lecture 10: **Jeffrey LAWRENCE,** *Pleasanton, USA*
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Wednesday, 14 November

Session 4: Mutant p53 functions II
Chair: Moshe OREN & Jo MILNER

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RECIPROCAL REGULATION BETWEEN P53 AND SIRT1 -- *Jo Milner, Jack Ford, Shafiq Ahmed and Ming Jiang*

Lecture 12: **Karen VOUSDEN**, *Glasgow, UK*
ROLE OF P53 IN APOPTOSIS AND AUTOPHAGY

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ONCOGENIC FUNCTIONS OF MDMX, AN ESSENTIAL REGULATOR OF P53 ACTIVITY

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Lecture 16: **Giovanni BLANDINO**, *Rome, Italy*
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Session 5: Posters

Special announcement

p53 Gateway, a new source of information on basic and translational research activities on *TP53* (www.p53gateway.org)

Session 6: p53 and beyond
Chair: Geoffrey WAHL

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E4F1: AN ATYPICAL UBIQUITIN E3-LIGASE MODULATING THE p53 FAMILY -- *Matthieu Lacroix, Laetitia K. Linarès, Amelie Thepot, Soline Estrach, Elodie Hatchi, Conception Paul, Guillaume Bossis, Pierre Hainaut, Pierre Dubus, Claude Sardet and Laurent Le Cam*

Lecture 18: **Ute MOLL**, *New York, USA*
REGULATED NUCLEAR IMPORT OF p53 BY BINDING TO IMPORTIN ALPHA 3 CONTRIBUTES TO STRESS-MEDIATED NUCLEAR ACCUMULATION -- *Natasha D. Marchenko, Kerstin Becker and Ute M. Moll*

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INACTIVATION OF FAILSAFE PROGRAMS BY TWIST ONCOPROTEINS -- *Stéphane Ansieau, Jeremy Bastid, Sandrine Valsesia-Wittmann and Alain Puisieux*

Lecture 20: **David MALKIN**, *Toronto, Canada*
SNPs, CHIPs AND TELOMERE TIPS: GENETIC MODIFICATION OF THE TP53-LI-FRAUMENI SYNDROME PHENOTYPE -- *Malkin D., Shlien A., Feuk L., Tabori U., Marshall C., Nanda S., Druker H., Novokmet A., Feuk L., Scherer S.W.*

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Session 7: From mouse models to clinical applications
Chairs: Klas WIMAN & Gigi LOZANO

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MUTANT p53 GAIN OF FUNCTION PHENOTYPE IN A MOUSE MODEL FOR ONCOGENE-INDUCED MAMMARY CARCINOGENESIS -- *Heinlein C., Krepulat F., Löhler J., Speide D., Tolstonog G. and Deppert W*

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Lecture 24: **Anne-Lise BØRRESEN-DALE**, *Oslo, Norway*
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TARGETING p53 BY T CELLS -- *Carina Lotz, Arjen Sloots, and Matthias Theobald*

Lecture 26: **Galina SELIVANOVA**, *Stockholm, Sweden*
MOLECULAR MECHANISMS OF PREFERENTIAL INDUCTION OF APOPTOSIS AND DOWNREGULATION OF ONCOGENIC PATHWAYS BY PHARMACOLOGICAL REACTIVATION OF p53 -- *Martin Enge, Vera Grinkevich, Joanna Zawacka-Pankau, Fedor Nikulenkov, Ying Zhao, Wenjie Bao, Eisabeth Hedström, Natalia Issaeva and Galina Selivanova*

Lecture 27: **Klas WIMAN**, *Stockholm, Sweden*
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Li-Fraumeni symposium

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MOLECULAR BASIS OF THE LI-FRAUMENI SYNDROME: AN UPDATE FROM THE FRENCH LFS FAMILIES -- *Thierry Frebourg and Gaëlle Bougeard for the French LFS working group*

Lecture 31: **Magali OLIVIER**, *Lyon, France*
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LI-FRAUMENI SYNDROME – A NEW LOOK AT OLD PROBLEMS

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SUPPORT FOR POTENTIAL SURVEILLANCE STRATEGIES FOR MEMBERS OF LFS KINDREDS -- *Sapna Syngal, MD, MPH, Serena Masciari, MD, Akriti Dewanwala, MD, Annick D. Van den Abbeele, MD, Lisa R. Diller, MD, Iryna Rastarhuyeva, MD, Jeffrey Yap, PhD, Katherine Schneider, MPH, Lisa Digianni, PhD, Frederick P. Li, MD, Joseph F. Fraumeni, Jr. MD, Elena Stoeffel, MD, Judy E. Garber, MD, MPH*

Lecture 34: **Maria-Isabel ACHATZ**, *Sao Paolo, Brazil* **&**
Patricia ASHTON-PROLLA, *Porto Alegre, Brazil*
HIGH POPULATION IMPACT OF A LOW PENETRANCE *TP53* GERMLINE MUTATION CAUSES HIGH INCIDENCE OF LFL FAMILIES IN SOUTHERN BRAZIL

Friday, 16 November

9:00-10:45 AM *Li-Fraumeni Round-table 1*

10:45-11:15 AM Coffee break

11:15-1:00 PM *Li-Fraumeni Round-table 2*

1:00 PM Lunch

--- End ---

ABSTRACTS

Lecture 1

INFLAMMATION AND CANCER: INTERACTIONS OF THE MicroRNA, p53 AND CYTOKINE PATHWAYS

Aaron Schetter, Krista Zanetti, Ewy Mathe, S. Perwez Hussain, Carlo Croce and* ***Curtis C. Harris***

Laboratory of Human Carcinogenesis, CCR, NCI, NIH, Bethesda, MD 20892-4255 USA; and *Institute of Genetics, Ohio State University, Columbus, OH 43210

Free radicals are ubiquitous in our body and are generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic micro organisms. Because free radicals can also inflict cellular damage, several defences have evolved both to protect our cells from radicals–such as the p53 pathway and antioxidant scavengers and enzymes–and to repair DNA damage. Free radicals can cause an adaptive increase in certain of the protective base excision repair enzymes. Understanding the relationship between chronic inflammation and cancer provides insights into the molecular mechanisms involved. In particular, we highlight the interaction between nitric oxide and p53 as a crucial pathway, and the role of microRNAs and cytokines in inflammatory-mediated carcinogenesis. For example, the microRNA profile of colon, lung and oesophageal cancer can predict cancer diagnosis and patient's survival.

Lecture 2

REVISITING "INITIATION" IN CARCINOGENESIS: PLACE OF *TP53* MUTATIONS IN EARLY STEPS OF CANCER

Pierre Hainaut

International Agency for Research on Cancer, Lyon, France

The chemical carcinogenesis model of initiation-promotion-progression proposes that carcinogenesis begins with a mutagenic "initiation" hit into one or a small set of critical genes, which induce cells to switch on new programmes enhanced by reversible biological modifiers, a process termed "promotion". In many cancers, mutations in *TP53* have been identified as early events detectable in non-cancer tissues, in particular those exposed to environmental carcinogens. Our studies on lung, liver and oesophageal cancers show that *TP53* mutation may occur early in the natural history of these cancers. Rather than initiating events, they contribute to the emergence of clonal cell populations as adaptative mechanisms that facilitate tissue remodelling in response to a large spectrum of stresses. In hepatocellular carcinomas (HCC) in a context of Hepatitis B (HB) chronic carriage, the aflatoxin-related R249S mutation is detectable at high copy numbers in the plasma of chronic carriers exposed to aflatoxins with temporal variations reflecting the seasonal patterns of dietary aflatoxin intake. Based on copy numbers in the plasma, several tens of millions of liver cells may acquire such a mutation, without being obviously primed to develop into cancer. Furthermore, the mutation is not found in non-carriers exposed to the same diet. This suggests that R249S may become selected in chronically infected cells, perhaps as an adaptative mechanism to resist HB-induced cell death. In lung cancers of never-smokers, p53 function is systematically inactivated either by *TP53* mutations or by loss of p14Arf expression. This observation suggests that in these cells, the p14Arf/Mdm2/p53 connection functions as a protective mechanism to prevent the untimely proliferation in response to excessive growth signals. Inactivation of this mechanism is required to allow sustained proliferation of cells with activated EGFR. In oesophageal cancers, *TP53* mutation occurs in a context of tissue remodelling in response to environmental (squamous cell carcinoma, SCC) or acid/bile (adenocarcinoma, ADC) stress. The main factor responsible for these remodelling processes is *TP63*, which regulates cell adhesion and proliferation in the squamous mucosa. *TP63* amplification enhances squamous hyperplasia, the first step of the histopathological sequence of SCC. In contrast, degradation of p63 isoforms by the proteasome occurs in cells acid/bile stress, the main condition inducing the formation of intestinal metaplasia and ADC in the lower oesophagus. In conclusion, in the three examples developed here, *TP53* mutation may help cells and tissues to cope with stress-induced tissue remodelling constraints, providing a short-term proliferation advantage which ultimately backfires as enhanced risk of progression to cancer.

Short presentation 1

MOUSE MODELS TO STUDY SENESCENCE BYPASS AND CANCER GENE MUTATIONS CAUSED BY ENVIRONMENTAL MUTAGENS

Whibley C.[1], *Hollstein M.*[1]

[1]Leeds Institute of Genetics, Health and Technology, University of Leeds, UK

Senescence bypass is an important step in tumourigenesis, and possibly in tumour recurrence following chemotherapy. In mice, senescence control is regulated by the p19ARF/p53 tumour suppressor pathway, and escape from this control can occur when point mutations arise in the p53 gene that obliterate function. There is thus strong selective pressure to inactivate p53 in murine senescent cultures, which can be exploited to capture mutations in p53 following exposure to environmental mutagens: immortal cell lines will arise from cultures liberated from the senescence block, and they can be sequenced for the presence of a p53 mutation. This approach, using cells from a knock-in mouse model harboring a humanized p53 gene (the Hupki mouse), revealed a remarkable degree of concordance between mutations that allow senescence bypass in vitro, and mutations that arise in vivo during human tumorigenesis. Examples will be given of p53 signature mutations in cell lines derived from carcinogen-treated cultures that strengthen the link between environmental exposures and cancer.

Lecture 3

THE STRUCTURE OF p53

Alan Fersht

Cambridge University and MRC Centre for Protein Engineering, Hills Road, Cambridge CB2 2QH, UK

p53 is directly inactivated by mutation in some 50% of human cancers. Some 30-40% of the mutations simply lower the stability of the core domain so it melts close to or below body temperature. We have shown in principal that it is possible to reactivate p53 by small molecules that bind to and stabilize it. To understand further the structure of the protein and hence the rational design of drugs, we are solving its structure at high resolution. We are faced with twin problems: the tetrameric protein consists of 1572 residues, some of which are in well-structured domains but others are natively unfolded; and the important core domain is intrinsically unstable and not well suited to systematic study. We have solved the structure of the core domain in solution by state-of-the-art NMR methods and found structural reasons for its instability. We have engineered a more stable variant, which is biologically active and have solved the crystal structures of oncogenic mutants in this framework. Some cancer mutations cause surface cavities that are drug targets. We solved the quaternary structure of the full-length tetrameric complex by combining high-resolution structural information on the folded individual domains with NMR, small angle x-ray scattering and electron microscopy, which should be a paradigm for solving other complex proteins that are involved in the cell cycle. We are refining these structures to high resolution, and studying their complexes with partner proteins.

Lecture 4

p53 ISOFORM EXPRESSION MAY ABROGATE P53 MUTATION AND IS ASSOCIATED WITH GOOD PROGNOSIS IN BREAST CANCER

A. Diot, K. Fernandes, S. Bray, P. Quinlan, L. Baker, C.Purdie, D. Kellock, A. Thompson and ***JC Bourdon***

University of Dundee, Ninewells hospital, Dept of Surgery &Molecular Oncology, Inserm-European Associated Laboratory U858, Dundee, DD1 9SY (UK)

p53 gene is the most frequently mutated gene in human cancer. Intriguingly, the frequency of p53 gene mutation is particularly low in primary breast tumours. Moreover, it is difficult to link p53 status with breast cancer diagnosis and prognosis. We recently published the identification of p53 isoforms and established that they are differentially expressed in breast tumours. Here we report the analysis of p53 isoform expression in relation with clinical data and outcomes in 174 primary breast tumours.

Our data indicate that some p53 isoforms are associated with Estrogen Receptor (ER) status while others are associated with p53 gene mutation. Moreover, loss of p53 isoform expression can be associated with poor survival.
Therefore, differential expression of the p53 isoforms in primary breast tumours may help to link p53 status to biological properties and drug sensitivity.

Short Communication 2

SPLICE ISOFORM p53-BETA HAS ANTI-APOPTOTIC ACTIVITY AND ANTAGONIZES APOPTOTIC SIGNALING MEDIATED BY p53

Eva-Maria Bücker, Sven Hanson, Kira Erber, Nadine Pettkus, Alf Giese, and ***Ella Kim***

Translational Neurooncology Research Group, Department of Neurosurgery, Georg-August-University of Goettingen, Robert-Koch-Strasse 40, 37075 Göttingen, GERMANY

P53 isoforms produced by alternative splicing have emerged as important constituents of the p53 regulatory network. p53beta is one of the C-terminally truncated p53 isoforms expressed in normal and transformed cells. Biological functions of p53beta remain elusive and have been interpreted distinctly. In contrast to the previously proposed view that p53beta functions have little relevance for the biology of proliferating cells (Flaman et al 1996) recent findings have suggested that p53beta may play important roles in modulating the apoptotic response in tumour cells presumably by augmenting transcriptional activation of bax by p53 (Bourdon et al 2005). Among the possible reasons for the apparent discrepancy between different views on p53beta functions one is that until now, an in-depth assessment of cellular functions of p53beta has been difficult due to the low expression levels of endogenous p53beta and the lack of experimental systems allowing constitutive expression of p53beta. To overcome these drawbacks we established experimental systems allowing constitutive expression of p53beta in the presence or the absence of wild type p53 and assessed the roles of p53beta in regulation of transcription and apoptosis. We show that p53beta possesses intrinsic anti-apoptotic activity independent from p53; is susceptible to stress-inducible post-translational modifications; becomes stabilized and accumulates at the mitochondria under conditions of cytotoxic stress; inhibits transcription of the canonical p53 targets bax and mdm2 but not of p21. Our findings reveal for the first time that p53beta acts as functional antagonist of p53 in the apoptotic response. To further elucidate the mechanisms underlying transcriptional inhibition by p53beta we have characterised mutant forms of p53beta produced in tumour cells with hot-spot mutations in the TP53 gene. The impact of hot-spot mutations on molecular and biological activities of p53beta will be discussed.

Lecture 5

GAIN OF FUNCTION MUTANT P53 PROTEINS ALTER CHEMOSENSITIVITY OF CELLS VIA NF-KB2

*Scian M.J.[1], Nesheiwat I.[1], Stagliano K.E.R.[1], Anderson M.A.E.[1], Dumur C.[3], Garrett C.[2, 3], Deb S.P. [1, 2] and **Deb S.**[1, 2]*

[1]Department of Biochemistry and Molecular Biology, and [2]Massey Cancer Center, [3]Department of Pathology, Virginia Commonwealth University, Richmond, VA 23298

Over-expression of mutant p53 is a common theme in tumors suggesting a selective pressure for p53 mutation in cancer development and progression. To determine how mutant p53 expression may lead to survival advantage in human cancer cells, we generated stable cell lines expressing p53 mutants -R175H, -R273H and -D281G using p53-null human H1299 (lung carcinoma) cells. Compared to vector transfected cells, H1299 cells expressing mutant p53 showed a survival advantage when treated with different chemotherapeutic drugs; however, cells expressing the transactivation deficient triple mutant p53-D281G (L22Q/W23S) or p53-R175H (L22Q/W23S) had significantly lower chemoresistance. Gene expression profiling of cells expressing transcriptionally active mutant p53 proteins revealed a striking pattern that all three p53 mutants induced expression of approximately 100 genes involved in cell growth, survival, and adhesion. The gene NF-κB2 is a prominent member of this group whose over-expression in H1299 cells also leads to chemoresistance. Treatment of H1299 cells expressing p53-R175H with siRNA specific for NF-κB2 made these cells more sensitive to etoposide. We have also observed activation of the NF-κB2 pathway in mutant p53-expressing cells. Thus, one possible pathway through which mutants of p53 may induce loss of drug sensitivity is via the NF- κB2 pathway. A number of lung and breast cancer cell lines with mutant p53 show chemoresistance that is dependent on the level of p53 as the chemoresistance decreases with siRNA against p53. Also, a preliminary screen of human lung cancer specimens shows co-existence of p53 mutation and over-expression of NF-κB2 suggesting that, in the clinic, there is a subset of patients with p53 mutant, NF-κB2 over-expressing tumors.

Lecture 6

NONCANONICAL SEQUENCE MOTIFS AS TARGETS FOR TRANSACTIVATION BY WT AND MUTANT p53

Michael A. Resnick[1], *Daniel Menendez*[1], *Jennifer J. Jordan*[1], *Maher Noureddine*[1], *Douglas Bell*[1], *Alberto Inga*[2]

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The human genome contains a vast number of sequences that might be bound by the p53 master regulator and mediate transcriptional regulation of associated regions. In vitro studies have demonstrated binding of a tetramer of p53 molecules to DNA sequences corresponding to the following loose consensus: RRRCWWGYYY (n) RRRCWWGYYY, where n = 0–13 bp and W = A or T. While such studies provide a framework for understanding p53 function, there are clearly additional factors that could influence p53 activity as a sequence-specific transcription factor in vivo. We have created yeast, human and semi-in vitro systems to address the combined roles that DNA sequence, p53 levels and p53 mutations can play in transactivation.

Recently, we identified a promoter SNP that places the angiogenesis-related gene Flt1 under p53 control. Surprisingly, the p53 responsiveness is greatly increased by the presence of estrogen receptor (Mol Cell Biol 27, 2007). In fact, we established that the transactivation was regulated through a half-site p53 response element (RE) and a half-site estrogen receptor response element (ERE) upstream from the p53 RE. There are hundreds of such motifs in the human genome. We found that the response from a weak RE could be greatly enhanced by a nearby half-site ERE.

Since the non-canonical half-site RE appeared to be responsive to p53 on its own, we are deconstructing the canonical p53 consensus sequence and motif in order to identify other potential non-canonical REs. Contrary to early reports for in vitro binding, increases in spacer length of only a few bases between decamer half-sites greatly reduces p53 transactivation in yeast and human cells, and the extent of transactivation is strongly influenced by p53 levels. This has been confirmed using human cell nuclear extracts in a newly developed microsphere binding assay that recapitulates in vivo binding by wild type and mutant p53. Transactivation at a natural RE with a 2-base spacer is greatly increased when the spacer is removed, suggesting that variation in spacer size may play an important evolutionary role in modulating p53 responsiveness.

We found that half-site REs could be nearly as responsive as well-known weak REs. Many of the altered-spectrum p53 mutants as well as a dimer-forming mutant, but not a monomer mutant, could transactivate from the half-sites.

Thus, the non-canonical RE sequences are likely to figure prominently in the p53 master regulatory network and influence the biological responses to mutant p53 proteins. We propose that the p53 master regulatory network is much larger than estimated strictly from the known consensus sequence and it may be intricately related to other master regulators.

Lecture 7

CHARACTERIZATION OF THE ROLE OF PIN1 AS A REGULATOR OF P53 FUNCTION AND DYSFUNCTION

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Coordinated cell signaling is crucial for normal cell behavior and tissue homeostasis. In order to cope with the overwhelming complexity of signaling events, cells have evolved efficient mechanisms that allow proper regulation of signal transduction. These mechanisms relay substantially on post-translational modifications in order to transfer information within signaling pathways. Recently, a crucial role has emerged for phosphorylation-dependent conformational changes caused by the prolyl-isomerase Pin1 on several highly interconnected substrates. Pin1 binds and catalyzes cis/trans isomerization of prolines on S/P or T/P motifs in many proteins involved in different processes. Our previous work has established a crucial role for Pin1 in the regulation of the p53 and p73 apoptotic response, supporting the idea that Pin1 contributes to checkpoint mechanisms essential for normal cell physiology. However, Pin1 is over-expressed in several human tumors, suggesting that the presence of other alterations may subvert the contribution of Pin1 to mechanisms that restrain uncontrolled proliferation. We have further characterized the mechanisms underlying p53 functional activation showing that Pin1 is able to enhance p53 function by modulating different events that prelude its full activation. Pin1 is required for efficient loading of both p53 and p300 on p53-target promoters upon stress and In addition, Pin1 mediates its dissociation from the apoptosis inhibitor iASPP, leading to cell death. However, mutations in the p53 gene frequently result in expression of p53 point mutants that accumulate in human tumors, which may actively collaborate with tumor progression through the acquisition of gain of function properties. We found that Pin1 interacts with several cancer-associated p53 mutants, and that this interaction is relevant for mutant p53 function. Therefore, Pin1 could be part of a checkpoint system that surveys cell proliferation in normal cells, but it may also contribute to the amplification of proliferative signals in a pathological context. The design of anti-cancer therapies should take into account cancer-specific alterations that may condition tumor progression and clinical outcome. Our results would predict that inhibition of Pin1 may counteract the cytotoxicity and hence the clinical activity of drugs that induce p53-dependent apoptosis. Conversely, Pin1 inhibition could be beneficial at later stages in tumors that may have also acquired mutant p53. Interfering with mutant p53 function may represent a valid alternative to block tumor growth and development of aggressive phenotypes. In this context, we have isolated peptide aptamers (PAs) able to interact with p53R175H using a two-hybrid strategy. These PAs are able to efficiently recognize several different p53 point mutants, but not wt p53. Transient expression of PAs induces apoptosis only in cells expressing mutant p53.

Short Communication 3

SYSTEMATIC APPROACHES TO STUDY TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL FUNCTIONS OF MUTANT P53 IN HUMAN AND MOUSE TUMOR CELLS

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To study the molecular mechanism of mutant p53 (mutp53) action, we used tumor cell lines expressing endogenous and inducible mutp53 proteins as models and applied techniques for decipher the mutp53 -DNA, -RNA, and -protein interactomes. Genome-wide ChIP-cloning, ChIP-on-chip and expression profiling allowed us to map the genomic locations of functional mutp53 binding sites. These sites are frequently localized in the regulatory first intron, and enriched in Alu-type SINEs which together with other repetitive sequences organize chromatin into functional domains. Non-random, clustered distribution of genes co-regulated by mutp53 as well as interaction of mutp53 with protein and RNA components of transcription factories points to its global role in regulation of gene expression. Our data provide a basis for a working model according to which mutp53 is a structural component of transcription/ processing factories and is involved in the regulation of synthesis and stability of transcripts providing a growth and survival advantage to tumor cells.

Short Communication 4

EXPLORING MUTANT P53 GAIN OF FUNCTION IN VIVO THROUGH CONDITIONAL RNA INTERFERENCE

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Mutant p53 proteins are though to have acquired a "gain of function" (GOF) activity that mainly contributes to tumor aggressiveness [1, 2]. Previously we reported that constitutive RNA interference [3] of mutant-p53 proteins reduces tumorigenicity of cancer cells, but molecular determinants of this effect *in vivo* have not been highlighted [4]. To address this point, mimicking physiological conditions, a conditional lentiviral-based system of conditional RNA interference was exploited [5] and through microarray analysis putative mutant p53 target genes were evidenced. *In vivo* studies assessed the efficacy of conditional RNA interference in inhibiting gain of function activity of mutant-p53 proteins by: *a)* reducing tumor growth ability and *b)* modulating putative mutant p53 target gene expression. Results are confirmatory that mutant p53 protein depletion impacts on tumor malignancy and validated the inducible lentiviral-based system as an efficient tool to study the gain of function activity of human tumor derived p53 mutants.

[1] Aas *et al*., 1996
[2] Blandino, *et al*., 1999
[3] Brummelkamp, *et al*., 2002
[4] Bossi *et al*., 2006
[5] Wiznerowicz *et al*., 2003

Lecture 8

MUTANT P53 ATTENUATES THE SMAD-DEPENDENT TGF-B1 SIGNALING PATHWAY BY REPRESSING THE EXPRESSION OF TYPE II TGF-B RECEPTOR

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Both TGF-β and p53 have been shown to control normal cell growth. Acquired mutations either in the TGF-β signaling pathway or in the p53 protein were shown to induce malignant transformation. Recently, a crosstalk between wild type p53 and the TGF-β pathway has been observed. The notion that mutant p53 interferes with the wild type p53 induced pathway and acts by a "gain of function" mechanism prompted us to investigate the effect of mutant p53 on the TGF-β induced pathway. In this study we show that cells expressing mutant p53 lost their sensitivity to TGF-β1 observed by lower cell migration and a reduction in wound healing. We found that mutant p53 attenuates TGF-β1 signaling. This was exhibited by a reduction in SMAD2/3 phosphorylation and inhibition of both the formation of SMAD2/SMAD4 complex and translocation of SMAD4 in to the cell nucleus. Furthermore, we found that mutant p53 attenuates the TGF-β1 induced transcription activity of SMAD2/3 proteins. In searching for the mechanism that underlies this attenuation, we found that mutant p53 reduces the expression of TGF-βR II. These data provide important insights into the molecular mechanisms that underlie mutant p53 "gain of function" pertaining to the TGF-β signaling pathway.

Lecture 9

Jiri BARTEK

No abstract.

Short Communication 5

THE CELLULAR AND MOLECULAR CHARACTERIZATION OF A P53 MUTANT MOUSE MODEL OF ACCELERATED AGING

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The stress response transcription factor p53 has been described as the guardian of the genome due to its roles in the many pathways of tumor suppression. Most p53 mouse models have focused on mutations which result in a loss of this tumor suppression. In contrast, our lab has developed a p53 mutant mouse model with enhanced tumor resistance as compared to wild-type. It exhibits persistent increased p53 activity via a heterozygous N-terminal truncated p53 called the M protein; the resulting heterozygous mouse is known as $p53^{+/m}$. Surprisingly, this p53 hypermorph exhibits a broad spectrum of accelerated ageing characteristics. Using transfection experiments we have shown the M protein can directly interact with wild-type p53 and drive it into the nucleus in the absence of stress, even when the nuclear localization sequence of p53 is mutated. By increasing nuclear translocation, wild-type p53 is potentiated to activate downstream targets. This capacity for M to activate p53 even in the absence of stress appears to be the route to accelerated ageing. Kidneys, livers, and spleens from old (20-27 month) $p53^{+/m}$ mice show increased levels of several senescence markers and increased accumulation of mutations as compared to age-matched wild-type tissues. Thymocytes and splenocytes from young and old $p53^{+/m}$ mice are resistant to apoptosis, exhibiting deficient caspase cleavage, decreased TUNEL staining, and/or decreased PERP activation in response to stress. After exposure to low levels of ionizing radiation (IR), $p53^{+/m}$ spleens exhibit enhanced DNA damage repair but, more importantly, an increased senescence response to high levels of IR (3-10 Grays). Consequently, $p53^{+/m}$ cells that would usually be eliminated by apoptosis instead enter senescence allowing unrepaired DNA to accumulate as mutations. As yet, it is not clear why apoptosis is reduced in $p53^{+/m}$ mice. Cumulatively, we have shown that apoptotic resistant tissues are shunted toward senescence-mediated tumor suppression. The accumulation of these senescent cells may then lead to a loss of tissue homeostasis and an ageing phenotype. This work also presents the conundrum of using persistently activated p53 as a cancer therapy as it may lead to premature ageing and additional negative downstream consequences.

Short Communication 6

REGULATION OF P53 BY PML IN RESPONSE TO DNA DAMAGE

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Upon exposure to DNA damage p53 is protected from negative regulation, mostly by the Mdm proteins: Mdm2 and Mdmx. This protection of p53 involves multiple mechanisms, where post-translational modifications of both p53 and Mdm proteins play an important role. Of particular interest are modifications that modulate the p53/Mdm2 interaction. Within the Mdm2 binding site of p53 Ser20 and Thr18 play key roles. We have previously described a role for PML in the regulation of Ser20 phosphorylation in response to DNA damage. This modification is enhanced by the action of PML, which recruits both p53 and Chk2 into the PML nuclear bodies. Phosphorylation of p53 on a nearby site, Thr18, has been proposed to be even more critical in the modulation of the p53/Mdm2 interaction. We found that PML enhances the phosphorylation of p53 on Thr18 by CK1 in response to DNA damage, and this phosphorylation is important for the protection of p53 from Mdm2. Further, PML and CK1 interact in response to stress.

Since mutant p53 is also modified in response to DNA damage, we ask whether PML also regulates the post-translational modifications of mutant p53, and whether this regulation differs between wt and mutant p53. We will report that PML also regulates the phosphorylation of mutant p53 in response to DNA damage, and that PML interacts with mutant p53 in human cancer cell lines.

Lecture 10

DETECTION OF P53 MUTATIONS IN CANCER BY THE AMPLICHIP p53 TEST, A MICROARRAY-BASED RESEQUENCING ASSAY

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The tumor suppressor gene p53 is one of the most frequently mutated genes in human cancer. Most p53 mutations are missense mutations involving a single-base nucleotide substitution. Although mutations can occur in the entire coding region of exon 2-11 of p53 gene, most are found in the core DNA-binding domain. Somatic p53 mutations have been shown to be an independent prognostic indicator in breast cancer (Olivier et. al. Clinical Cancer Research 2006), and may also be predictive to therapy response in many cancers including breast and esophageal cancer. Thus the status of the p53 gene in cancer may be a important parameter in patient management in the near future. Detection of somatic p53 mutations in cancer samples, especially in formalin-fixed paraffin-embedded tissues (FFPET), can be very challenging due to the often poor quality of genomic DNA. The AmpliChip p53 test is a microarray-based re-sequencing test in development which is designed to detect single nucleotide substitutions and 1 bp deletions in the entire coding region and the flanking splice sites of exon 2-11 of the p53 gene in either FFPET or fresh frozen tissues. The AmpliChip p53 test queries for the presence of sequence alterations through comparative analysis of the hybridization pattern of a series of probes to sample DNA and wild-type reference DNA. The AmpliChip p53 Test is comprised of 6 major steps: 1) genomic DNA extraction from tissue, blood or bone marrow; 2) PCR amplification of purified DNA; 3) fragmentation and labeling of the amplified products; 4) hybridization of the labeled products to the microarray and staining of the bound products; 5) scanning of the microarray; and 6) determination of the sequence of the p53 gene. We will show results of three clinical research studies using the AmpliChip p53 test. First, we analyzed over 700 breast cancer FFPET samples from the North Carolina Breast Cancer Study, a population-based study of women diagnosed from 1993 to 1996. p53 mutations were found in ~40% of patients. 272 samples out of this cohort were also analyzed by SSCP-sequencing. The results of 256 out of 272 patients (94.1%) were concordant between AmpliChip p53 test and SSCP-sequencing. 16 samples (6%) were discordant, mainly due to insertion mutations or to > 2-bp deletions that AmpliChip p53 is not designed to detect. Secondly, we are investigating the predictive value of p53 in a clinical trial of capecitabine and docetaxel with or without trastuzumab in locally advanced breast cancer. Samples of 78 of 157 patients contained p53 mutations (49.6%), which were widely distributed in exon 2, 4, 5, 6, 7, 8, 9, and 10, with the highest number seen in exon 5, 6, and 8. p53 mutations were found most frequently in so-called "triple negative" breast cancer patients (ER-, PR- and Her2-). The correlation of p53 mutations with clinical and pathological outcomes is ongoing. Lastly, we analyzed p53 mutations in 193 patients with chronic lymphocytic leukemia (CLL). The overall incidence of p53 mutations in this cohort was 13.5% (26/193). FISH analysis detected deletion of 17p in 18 cases; interestingly 17 out the 18 del 17p samples carried a p53 point mutation on the second allele, suggesting that loss of p53 function plays a key role in the poor prognosis of CLL cases with del 17p. The 26 patients with p53 mutations detected by AmpliChip p53 test had a significantly increased disease progression compared to other CLL patients. In conclusion, the AmpliChip p53 research test offers the potential as an accurate and standardized way for detecting p53 mutations for routine use in a clinical laboratory.

Lecture 11

RECIPROCAL REGULATION BETWEEN P53 AND SIRT1

***Jo Milner**, Jack Ford, Shafiq Ahmed and Ming Jiang*

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SIRT1 belongs to the sirtuin family of mammalian de-acetylases (SIRT1 – 7). It regulates chromatin structure and gene expression via histone de-acetylation (resulting in chromatin compaction, gene silencing and formation of heterochromatin) and also via de-acetylation of specific transcription factors and co-factors, including p53 and p300.

SIRT1 functions are linked with ageing and age-related disease processes including type II diabetes, inflammation, neurological disorders including Parkinsons disease, and cancer.

The functioning of SIRT1 is NAD-dependent. Over-expression of SIRT1 confers survival benefits to affected cells. Expression is regulated at the levels of transcription and of transcript stability. In the former case complexes of SIRT1 with HIC-1 (hypermethylated in cancer-1) repress SIRT1 gene expression: in cancer loss of HIC-1 due to hypermethylation disrupts this negative feed-back transcriptional regulation of SIRT1 expression. SIRT1 transcript stability is prolonged by mRNA complexing with HuR.

We now show that SIRT1 is also regulated via stability of the SIRT1 protein, and that increased protein stability results in some 20-fold accumulation of SIRT1 in cancer cells. Treatment of cells with the anti-cancer drug 5-fluorouracil (5-FU) results in decreased SIRT1 protein stability and is accompanied by apoptosis.

Three independent lines of evidence indicate that regulation of SIRT1 protein stability is p53-dependent, thus linking p53 with SIRT1 functional capacity.

Lecture 12

REGULATION OF MUTANT p53 STABILITY AND ACTIVITY BY UBIQUITINATION

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While wild type p53 is normally a rapidly degraded protein, mutant forms of p53 are stabilised and accumulate to high levels in tumor cells. We have shown that mutant and wild type p53s are ubiquitinated and degraded through overlapping but distinct pathways. Mutant p53 is heavily ubiquitinated in an MDM2-independent manner – this ubiquitination does not promote the degradation of mutant p53 but is associated with cytoplasmic localization. Interestingly, MDM2 can drive the degradation of mutant p53 through a mechanism that does not depend on the E3 activity of MDM2. This separation of ubiquitination and degradation of p53 is also seen in MDM2 mutants that retain the former but not the latter activity. The contribution of MDM2 to the degradation of mutant p53 may reflect an ability of MDM2 to deliver the ubiquitinated mutant p53 to the proteasome.

Lecture 13

THE IMPACT OF MDM2 AND MDMX SUBCELLULAR DISTRIBUTION AND ABUNDANCE ON p53 PATHWAY RESPONSES

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Current models of p53 regulation indicate that post-translational modifications fine-tune its activity. By contrast, control of the stability of its core negative regulators, Mdm2 and MdmX is envisioned to control p53 abundance and transcriptional output. In vitro studies, mouse models and human genetic studies emphasize that subtle changes in the relative abundance of each of these factors can profoundly affect p53 function. Therefore, substantiation of such models requires the quantitative analysis of their endogenous levels and subcellular distribution. We will present data quantifying p53, Mdm2 and Mdmx in normal and tumorigenic human cells. Our data indicate the nuclear abundance of Mdm2 and Mdmx relative to p53 limits its activity in cells growing in culture. Surprisingly, while substantial increases of Mdmx level attenuate basal p53 activity, genotoxin-induced signaling remains dominant, resulting in similar p53 activation kinetics and functional output. These and other data lead us to propose that damage-induced Mdm2 and Mdmx destabilization limits their ability to antagonize p53.

Defining the determinants of the cellular response to p53 activation has significant therapeutic importance. We previously showed that Mdmx abundance can affect p53-mediated execution of cell cycle arrest versus apoptosis. We will present data addressing whether this occurs at the level of p53-dependent transactivation, or whether additional levels of control exist.

Short Communication 7

CONTROL OF RHO/ROCK SIGNALLING BY P53: CONSEQUENCES ON CELL MIGRATION AND INVASION.

Gadea Gilles, de Toledo Marion, Anguille Christelle and ***Roux Pierre***

We have previously presented evidence that the contribution of the tumour suppressor p53 to the control of tumorigenesis is not restricted to its anti-proliferative activities, but is extended to the modulation of cell migration. Identification of the mechanisms by which p53 modulates cell migration will be important to understand how invasive cells arise. Here we show that p53 deficiency induces a switch in mouse embryonic fibroblasts cultured in a 3D matrice from an elongated, spindle-shaped morphology to a markedly spherical and flexible one associated with highly dynamic membrane blebs that strikingly resemble amoeboid movement. This transition is characterised by a polarised distribution of integrin β1 and ezrin in the direction of movement and requires the RhoA/ROCK pathway. These rounded, motile cells show higher velocity and a significant increase in invasive properties. This type of p53-mediated transition is also observed in melanoma A375P cancer cells.Our data demonstrate that p53 is an important determinant of the mode of cell motility. This connects the regulation of proliferation to the control of cell migration and defines a new concept of p53 function as a tumour suppressor gene, suggesting that the genetic alterations of p53 in tumours are sufficient to promote motility and invasion, thereby contributing to metastasis.

Short Communication 8

NEW FINDINGS ON THE IMPACT OF C-TERMINAL LYSINES ON p53 TRANSACTIVATION AND CELLULAR OUTCOMES

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Functional in a tetrameric state, the p53 protein confers its tumor-suppressive activity by transactivating genes which promote either cell-cycle arrest or programmed cell death. How p53 decides between these two divergent cellular outcomes is still largely unknown, although a number of functional mutations, interacting proteins, and post-translational modifications have been implicated. Along these lines, the C-terminal region of p53, which contains the tetramerization domain, is highly modified following genotoxic stress although the precise in vivo consequences of these modifications are controversial. We have generated H1299 cell lines that express specific sets of lysine mutations in p53's C-terminal region that are inducibly expressed at physiological levels. In the first case we mutated the two lysines that are found within the p53 tetramerization domain, K352 and K357. These mutants confer unique properties onto p53. By blocking possible lysine modifications but conserving charge, (K351R and K357R) p53's DNA binding and transcriptional abilities are unchanged, yet a strong G1 arrest is initiated and cells are protected from death. On the other hand, by changing these residues to glutamine (Q), thereby possibly mimicking lysine acetylation, p53 is transcriptionally impaired and is unable to arrest cells yet can still effectively induce apoptosis.

In the second case, the six lysines within the extreme C-terminus of p53 were changed to glutamine (6KQ). This 6KQ mutant was found to be completely defective in binding and transactivating several p53 target genes. On the other hand, blocking modification by mutating these residues to arginines (6KR) enabled p53 to bind and transactivate its target promoters, although to a lower extent than wild-type p53. These same C-terminal lysines are implicated in a novel interaction of p53 with its main negative regulator, Mdm2. This suggests that a basic CTD charge, and to a lesser extent, a "native" CTD structure, are important for p53 function in vivo. Finally, when two additional KQ mutations in p53's linker domain (K305 and K320) are present in the background of the 6KQ mutant (8KQ) p53 cannot enter the nucleus. However, when forced into the nucleus by adding the SV40 NLS the 8KQ mutant p53 can bind to and transactivate its targets as efficiently as the wild-type p53 protein. We also examined the impact of altering C-terminal lysines on the function of a common tumor derived mutant (R248Q) and have potentially interesting findings to report. Taken together, we suggest that modifications of the lysines within the p53's C-terminal domain can serve as a "molecular switch" to direct p53 towards either its arrest or apoptotic functional pathway.

Lecture 14

ONCOGENIC FUNCTIONS OF MDMX, AN ESSENTIAL REGULATOR OF P53 ACTIVITY

Aart Jochemsen

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Keeping the activity of p53 in check is essential for normal development. Two key inhibitors of p53 activity are the structurally related proteins Mdm2 and Mdmx. While Mdm2 is the main regulator of p53 half-life in normal cells, Mdmx also critically contributes to the regulation of p53 activity.
Activation of p53 function upon various forms of stress enables cells to respond to these insults. Therefore, cells have developed stress-induced regulatory mechanisms that quickly abrogate the inhibitory effect of Mdm2 and Mdmx on p53.
One important activation mechanism of p53 is aberrant mitogenic signaling, which can be caused by initial oncogenic events, like loss of pRB expression or oncogenic mutation in e.g. H-Ras. Therefore, it is assumed that all human tumors have a somehow attenuated p53 response pathway. In approximately half of the human tumors the p53 gene itself has been mutated. In the cases retaining wild-type p53, upstream regulators or down-stream targets of p53 are usually aberrantly expressed. It had been shown that the human Mdm2 gene is over-expressed in a significant proportion of human tumors, in general correlating with expression of wild-type p53. More recently it has become increasingly clear that the human Mdmx gene is also aberrantly expressed in a high proportion of human tumors, with a particular high percentage in a tumor type that virtually never shows p53 mutation, i.e. retinoblastoma. In my presentation I will present our data on the effects of manipulation of Mdmx expression in human cell lines on their growth properties and their sensitivity to p53-activating agents.

Lecture 15

MODULATION OF THE VITAMIN D3 RESPONSE BY CANCER-ASSOCIATED MUTANT p53

*Perry Stambolsky[1], Yuval Tabach[1], Lilach Weiss[1], Ira Kogan[1], Eyal Kalo[1], Itamar Simon[2], **Moshe Oren**[1], and Varda Rotter[1]*

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TP53 gene mutations, which occur frequently in human cancer, often endow the mutant p53 (mutp53) protein with new oncogenic functions, manifested by accelerated growth and protection from apoptosis. This is generally referred to as mutp53 gain of function (GOF). In an attempt to elucidate the mechanistic basis of mutp53 GOF, and in light of previous work implicating transcriptional effects in this process, we performed ChIP-on-chip analysis on human SKBR3 breast cancer cells, harboring endogenous mutp53. The results revealed that VDR-RXR response elements (VDRE) are significantly over-represented in gene promoters bound by mutp53. This conclusion gained independent support from expression microarray analysis of tumor cells overexpressing mutp53.

VDRE elements confer transcriptional regulation by Vitamin D3 (VitD3), which is mediated by the Vitamin D receptor (VDR). We therefore assessed the impact of mutp53 on VDR-dependent transcription. We found that mutp53 can augment the transcriptional activity of a promoter carrying multiple VDRE elements. Furthermore, we found that mutp53 interacted physically with the VDR protein and increased its nuclear retention following VitD3 treatment.

It has been extensively documented that VitD3 can facilitate cancer cell death. Nevertheless, in cells overexpressing mutp53, VitD3 was actually found to exert a cytoprotective effect in the face of chemotherapy agents; this was in contrast to its ability to induce increased death of tumor cells carrying wild type p53. Expression microarray analysis revealed that mup53 alters the transcriptional response of cancer cells to VitD3, not only quantitatively but also qualitatively. The identities of the genes whose VitD3-regulated expression is markedly affected by mutp53 suggest that some of them may contribute to the observed cytoprotective effect of VitD3.

Together, our data imply a cross talk between mutp53 and VDR on several molecular and functional levels. This cross talk has profound effects of the transcriptional output of VitD3 and on the consequent biological outcome. Specifically, our findings raise the possibility that p53 status may be a critical determinant of tumor response to VitD3, which should be taken into consideration when contemplating the use of VitD3 analogs for cancer treatment.

Lecture 16

MUTANT p53: AN ONCOGENIC TRANSCRIPTION FACTOR

Giulia Fontemaggi[1,2], Silvia Di Agostino[1], Stefania Dell'Orso[1,2], Sara Donzelli[1], Francesca Biagioni[1], Francesca Fausti[1], Tal Shay[3], Eytan Domany[3], Varda Rotter[3], Moshe Oren[3], Sabrina Strano[1], and ***Giovanni Blandino[1,2]***

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Inactivation of tumor-suppressor genes is one of the key hallmarks of a tumor. Unlike other tumor-suppressor genes, p53 is inactivated by missense mutations in half of all human cancers. It has become increasingly clear that the resulting mutant p53 proteins do not represent only the mere loss of wild-type p53 tumor suppressor activity, but gain new oncogenic properties favoring the insurgence, the maintenance, the spreading and the chemoresistance of malignant tumors. One of the major mechanisms underlying mutant p53 gain of function is the ability to regulate gene expression. In order to identify novel mutant p53 transcriptional targets we performed a microarray analysis using H1299 cells overexpressing inducible p53R175H. Among the modulated genes we investigated the molecular mechanisms controlling ID4 expression. ID4 is a member of the ID (inhibitor of DNA binding) family of proteins (ID1-ID4), which function as dominant-negative regulators of basic helix-loop-helix transcription factors. ID factors are involved in numerous cell processes, including cell proliferation, differentiation and tumorigenesis. We found that ID4 is transcriptionally up-regulated by mutant p53. ID4 expression is driven by mutant p53 in cancer cell lines and ID4 promoter is recruited by transcriptionally active complexes containing mutant p53 and two other transcription factors, p65 (NFkB) and E2F-1. The net biological output of ID4 transactivation is the increase in the angiogenic potential of mutant p53-expressing cancer cells.

Aimed at identifying *in vivo* the pattern of direct transcriptional target promoters of gain of function mutant p53, in parallel to the microarray analysis, we performed a ChIP-chip approach. The ChIP-chip technique is a very powerful technology that combines Chromatin Immunoprecipitation (ChIP) and microarray analysis allowing genome-wide evaluation of *in vivo* promoter occupancy of a certain transcription factor. We have generated the first dedicated ChIP-chip slide for mutant p53. The slide is a low-density array enclosing 50-mer oligonucleotides complementary to the promoters of all the so far known mutant p53 target genes. The hybridisation of this slide with DNA derived from mutant p53-immunoprecipitated chromatin from SKBR3 cells, carrying p53His175, identified a signature of promoters that interacts with mutant p53 in this cell line. The parallel analysis of chromatin from the same cell line immunoprecipitated for methylated histone H3 and acetylated histone H4 enabled us to assign an active or inactive state to the chromatin of the mutant p53-recruited promoters. Finally, through the analysis of chromatin immunoprecipitated for p65 (NFkB) we observed that the promoters of a subset of genes involved in the TNF-α response are bound by both NFkB and mutant p53 even in the absence of TNF-α stimulation in SKBR3 cells. The future analysis of the *in vivo* promoter occupancy of those transcription factors (like NF-Y and E2F1) that mediate mutant p53's binding to promoters will shed light on the role of the various mutant p53-containing transcriptional complexes that control the expression of different subsets of mutant p53 target genes.

Lecture 17

E4F1: AN ATYPICAL UBIQUITIN E3-LIGASE MODULATING THE p53 FAMILY

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E4F1 was originally discovered more than 20 years ago as an ubiquitously expressed cellular transcription factor regulated by the viral oncoprotein E1A during adenoviral infection. While most other cellular targets of E1A discovered at the same time (E2F/pRB, CBP/p300, PCAF etc…) became « famous », investigated in depth, and recognized as central regulators of cell proliferation and survival, E4F1 functions remained, inexplicably, poorly investigated until recently. However, several observations indicate that E4F1 plays important roles in the proliferation/survival balance of somatic, stem and cancer cells through its implication in essential oncogenic pathways including the p53 pathway. Our data indicate that E4F1 is a novel and atypical E3-ligase for p53 that modulates its effector functions (Le Cam et al., 2006). Strikingly, we found that, in contrast to hitherto described E3-ligases of p53 (such as Hdm2), E4F1 stimulated oligo-ubiquitylation of p53 does not lead to its proteasomal degradation but regulates its transcriptional activities. E4F1-stimulated ubiquitin-conjugated forms of p53 are associated with chromatin, and their stimulation coincides with the induction of a p53-dependent transcriptional program involved in cell cycle arrest but not apoptosis. Interestingly, we recently found that E4F1 also regulates another p53 family member: p63. In contrast to p53, E4F1-mediated post-translational modification of p63 implicates Mdm2. Our analysis of E4F1 conditional knock-out mice suggest that E4F1-mediated regulation of p63 plays important roles in epidermal homeostasis. We will present our unpublished data regarding the importance of p63 regulation in E4F1 functions in the homeostasis of stratified epithelia.

Le Cam L, Linares LK, Paul C, Julien E, Lacroix M, Hatchi E, Triboulet R, Bossis G, Shmueli A, Rodriguez MS, Coux O, Sardet C. (2006). E4F1 is an atypical ubiquitin ligase that modulates p53 effector functions independently of degradation. Cell. Nov17;127(4):775-88.

Le Cam L, Lacroix M, Ciemerych MA, Sardet C, Sicinski P. (2004) The E4F protein is required for mitotic progression during embryonic cell cycles. Mol Cell Biol. 24(14):6467-75.

Lecture 18

REGULATED NUCLEAR IMPORT OF p53 BY BINDING TO IMPORTIN ALPHA 3 CONTRIBUTES TO STRESS-MEDIATED NUCLEAR ACCUMULATION

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The function of p53 as an inducible transcription factor naturally depends on its rapid stabilization in the nucleus upon stress. However, the exact mechanism of p53 nuclear accumulation remains unclear. While stress-induced block of nuclear export plays a role, the possible contribution of import has not been studied. Here we suggest stress-induced nuclear import of p53 as a previously unrecognized determinant of nuclear accumulation after DNA damage.

1) We demonstrate that Importin alpha 3 specifically facilitates nuclear import of p53 via a strong direct interaction. p53 failed to bind to Importin alpha 1 and bound only weakly to Importins alpha 5, 6 and 7.

2) We identified NLS I of p53 as the major site of interaction with Importin alpha 3. This interaction strongly depends on the positive charges contributed by lysine residues 319, 320 and 321. Conversion of these lysines to arginines partly reduced the interaction with Importin alpha 3, although still enabled some nuclear accumulation. In contrast, p53 NLS I mutation to alanines at residues 319-321 exhibit a profound disturbance, disrupting the interaction with Importin alpha 3 and locking p53 into the cytoplasm.

3) We identified lysines 319-321 of p53 as novel cellular targets of Mdm2-mediated ubiquitination. Thus, their ubiquitination may result in neutralization of the positive charges at NLS I, opening the possibility that nuclear import might be regulated by Mdm2.

4) In support, careful fractionation experiments under various conditions revealed that independent of stress the nucleus harbors almost exclusively non-ubiquitinated p53. In contrast, unstressed cytoplasm harbors both ubiquitinated and non-ubiquitinated p53, while stressed cytoplasm contains mostly non-ubiquitinated p53. Upon stress, endogenous p53 shows enhanced binding to Importin alpha 3 that coincides with stress-induced disruption of Mdm2-mediated ubiquitination of p53. We are currently testing the possibility that upon stress non-ubiquitinated cytoplasmic p53 is selectively imported by Importin alpha3 and contributes to rapid nuclear accumulation. Implications for mutant p53 will also be discussed.

Lecture 19

INACTIVATION OF FAILSAFE PROGRAMS BY TWIST ONCOPROTEINS

Stéphane Ansieau, Jeremy Bastid, Sandrine Valsesia-Wittmann and ***Alain Puisieux***

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A major obstacle to the expansion of abnormal cells with significant proliferative potential is the induction of innate defense mechanisms that initiate programs leading to senescence or apoptosis. The control of these two failsafe programs involves the p53-dependent pathway, thus accounting for the high frequency of TP53 mutations in human cancers. Nevertheless, a significant fraction of human cancers expresses apparently functional p53, suggesting that alternative mechanisms might be involved in the inhibition of oncogene-induced senescence and apoptosis. MYCN-amplified neuroblastoma, a paediatric tumor that derives from primitive sympathetic neural precursors, is a remarkable example of a human tumour with a high expression of mitogenic oncogene and a low prevalence of TP53 mutation. We recently reported the constant overexpression of the Twist1 gene in these tumours. Twist1 protein is a bHLH transcriptional repressor which is a crucial regulator of embryogenesis. We demonstrated that Twist1 acts as an oncogene, by protecting cells from the pro-apoptotic effect of N-Myc through the repression of ARF expression and the inhibition of p53 transcriptional activity. Our ongoing studies further show that Twist1, as well as its structural and functional homolog Twist2, also overrides Ras- and ErbB2- induced premature senescence in both human fibroblasts and epithelial cells through the repression of key regulators of both p53- and Rb-dependent pathways, including the inhibitors of cyclin dependent kinases p16Ink4A and p21WAF1. Escape from oncogene-induced senescence has been shown in vivo to constitute a crucial step determining the progression from benign lesions to malignant tumours. In mammary epithelial cells, this Twist-dependent process is associated with complete epithelial to mesenchymal transition (EMT), a phenomenon which is known to favour invasion and metastasis. Together, these observations demonstrate that Twist transcription factors display a double-edged cooperation with mitogenic oncogenes, by abrogating failsafe programs and favouring metastatic dissemination through induction of an EMT.

Lecture 20

SNPs, CHIPs AND TELOMERE TIPS: GENETIC MODIFICATION OF THE TP53-LI-FRAUMENI SYNDROME PHENOTYPE

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Li-Fraumeni syndrome (LFS) is an autosomal dominant inherited disorder characterized by a strikingly increased risk of multiple early-onset tumors associated with germline *TP53* mutations. Our research program has been interested in exploration of genetic and epigenetic modifiers that impact the biological effect of the variable underlying *TP53* genotype in LFS families.

Analysis of MDM2-SNP309 and *TP53* codon 72 polymorphisms, as well as expression of telomerase and telemore length measurement have been combined with new observations of the role of DNA copy number variations to develop a metric by which characteristic features of LFS may be determined or predicted. This presentation will provide an overview of our findings and relate to the potential to apply these biological markers in a clinically relevant setting and early detection programs.

Lecture 21

FROM BAD TO WORSE: p53 LOSS VERSUS MISSENSE MUTATIONS

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My laboratory has used mouse models to decode the importance of p53 missense mutations in tumorigenesis. We have generated two different p53 alleles, corresponding to mutations in human tumors that encode proteins with partial (p53R172P) or no function (p53R172H) and have compared the tumor phenotypes of these alleles to a p53-null allele. P53R172P homozygous mice retain the ability to arrest the cell cycle and induce senescence, but not apoptosis. These mice exhibit a delayed tumor phenotype. p53-null mice lacking p53 expression exhibit a more severe tumor phenotype. The gain-of-function p53R172H mutant mice show an even worse phenotype than p53-null mice due to the stabilization of that mutant p53 by various mechanisms.

Lecture 22

MUTANT p53 GAIN OF FUNCTION PHENOTYPE IN A MOUSE MODEL FOR ONCOGENE-INDUCED MAMMARY CARCINOGENESIS

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In human breast cancer, mutations in the p53 gene are associated with poor prognosis. However, analysis of patient data so far did not clarify, whether missense point mutations in the p53 gene, in addition to causing loss of wild-type p53 function, also confer a gain of function phenotype to the encoded mutant p53. As heterogeneity of patient material and data might obscure a clear answer, we studied the effects of co-expressed mutant p53 proteins p53R270H and p53R245W in transgenic mice in which SV40 early proteins initiate the development of mammary adenocarcinoma (WAP-T mice). In such tumors the endogenous wild-type p53 is functionally compromised by complex formation with SV40 T-antigen, thereby constituting a loss of wild-type p53 function situation that allowed analysis of the postulated gain of function effects of the mutant p53 proteins. We found that mutant p53 in bi-transgenic mice enhanced the transition from intraepithelial neoplasia to invasive carcinoma, resulting in a higher frequency of invasive carcinoma per gland and per mouse, a more severe tumor phenotype, and more frequent pulmonary metastasis. Surprisingly, mutant p53 proteins in this system do not increase genomic instability. At the molecular level, both mono-transgenic and bi-transgenic mice are characterized by similar genetic alterations, most pronounced an amplification of the Met-locus in tumors of higher histological grades (undifferentiated tumors) accompanied by Met-overexpression, indicating that mutant p53 proteins exert their functions in a quantitative rather than a qualitative manner. The findings might provide an explanation why mutant p53 gain of function is difficult to detect in patient data analyses.

Lecture 23

LI-FRAUMENI SYNDROME: CANCER RISK AND RISK MODIFIERS

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No abstract

Lecture 24

TP53 MUTATION PATTERN IN BREAST CANCER PROGRESSION

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Mutations in the tumor suppressor gene *TP53* are found in approximately 20-30% of breast carcinomas and have been shown to have both prognostic and predictive implications. The frequency and type of mutations varies in different series of breast cancer patients and may be due to factors such as stage of disease and molecular subtype. *TP53* mutations have been suggested to be an early event in carcinomas of the breast, while it seems to be a later event in other types of cancer. To further explore the frequency and type of *TP53* mutations during progression from early to advanced stages of breast cancer, fresh frozen tissue from 781 samples from 6 different breast cancer cohorts were screened for *TP53* mutations in exon 2 to 11, mainly by direct sequencing (ABI 377/3730), some by TTGE/sequencing. The 6 cohorts represent the different stages in development of breast cancer, from premalign tissue, DCIS, DCIS with small invasive components, small invasive, to advanced stage, and with survival data available.

The frequency of *TP53* mutations was found to be 0% in biopsies from mammographic dense tissue (MDG cohort), 13% in Ductal Carcinoma *in situ* (DCIS cohort), 35% and 37% in two series of stage I/II (MicMa and NKI cohort respectively), and finally 52% and 28% in two series of more advanced stage disease (Korean and DBCG cohort respectively).

Small invasive tumors with an *in situ* component showed a higher frequency of mutations than the DCIS only and those with small invasive tumors without an in situ component.

The more advanced stage "DBCG" cohort had higher frequency of deletion/insertion mutations (29%) and lower frequency of missense mutations (55%) as opposed to the stage I/II MicMa cohort (14% and 74% respectively). Among the different nationalities represented (Norwegian, Swedish, Danish, Dutch and Korean) the Korean cohort had the highest frequency of *TP53* mutations (52%), which may reflect ethnical differences.

In conclusion, the *TP53* mutational pattern and frequencies in different stages of breast cancer should be further explored to better understand its role in cancer development and progression.

Lecture 25

TARGETING p53 BY T CELLS

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Since p53 is an intracellular protein, p53-specific antibodies are unlikely to exert immunotherapeutic anti-tumor effects. Targeting of tumors through the p53 antigen thus relies on recognition of p53-derived peptide epitopes by T lymphocytes in the context of major histocompatibility complex (MHC) molecules at the surface of tumor cells. As mutation of p53 is a frequent event in carcinogenesis, one could consider to direct the T cell attack on peptide epitopes that comprise the mutated residue(s). In view of the fact that p53 is an ubiquitously expressed autoantigen, this approach would have the advantage that it avoids various obstacles, such as immunological tolerance and autoimmunity. Mutant p53 peptides would constitute true tumor-specific target molecules. However, unlike mutations in the ras oncogenes, those in p53 are highly diverse. Immunotherapy based on the mutated part of p53 would therefore require prior identification of the p53 mutation in patient tumors, followed by generating an individual, patient-tailored vaccine or T cell induction protocol. It is even far from granted that the mutated region of a given p53 allele will indeed encode a peptide which is not only processed into one of the patient MHC molecules, but is also of sufficient immunogeneicity to serve as target antigen for the T cell immune repertoire. In fact, the examples of mutant p53-derived T cell epitopes are extremely scarce. As a result, research on p53-targeted immunotherapy has primarily been focused on epitopes comprising wild-type (wt) p53 sequences. This is based on the concept that aberrant expression of p53 in tumor cells provides a window of opportunity for T cells to discriminate between malignant and normal somatic cells. A wealth of published data demonstrates the efficacy of class I MHC-restricted cytotoxic T lymphocytes (CTL) in response to tumors. Over the past years, a substantial number of laboratories, including ourselves, has found evidence that wt p53-derived peptides can be presented in the context of both, murine and human MHC class I molecules. Because p53 is subject to ubiquitine/proteasome-mediated degradation, it is conceivable that this process generates a repertoire of p53-derived peptides available for transporters associated with antigen processing (TAP)-mediated translocation into the endoplasmic reticulum (ER) and subsequent binding to nascent class I MHC molecules. Accordingly, it has been shown that tumor cells presenting such epitopes can be recognized by wt p53-specific CTL. Once obtained, p53-specific CTL appear fully efficient in anti-tumor responses. However, self-tolerance to this ubiquitously expressed molecule is a major barrier for generating such immune cells. In fact, we have unambiguously demonstrated that a considerable degree of p53-specific self-tolerance exists in p53-proficient as opposed to p53-deficient mice at the class I MHC-restricted T lymphocyte level. This p53-specific self-tolerance results in a peripheral p53-reactive T cell repertoire that is devoid of high-avidity class I MHC-restricted CTL with only low-avidity, if any, T lymphocytes left. However, we have shown that T cell antigen receptor (TCR) gene transfer can be used to circumvent self-tolerance of autologous T lymphocytes to p53 and thus provide the basis for a TCR gene transfer-based broad-spectrum immunotherapy of malignant disease. The ins and outs of therapeutic p53-specific TCR gene transfer will be presented.

Lecture 26

MOLECULAR MECHANISMS OF PREFERENTIAL INDUCTION OF APOPTOSIS AND DOWNREGULATION OF ONCOGENIC PATHWAYS BY PHARMACOLOGICAL REACTIVATION OF p53

Martin Enge, Vera Grinkevich, Joanna Zawacka-Pankau, Fedor Nikulenkov, Ying Zhao, Wenjie Bao, Eisabeth Hedström, Natalia Issaeva and ***Galina Selivanova***

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Reactivation of p53 in tumors might open new therapeutic avenues against cancer. A more rigorous determination of the mechanism of action, targets and specificity of small molecules reactivating p53 is crucial for the development of novel therapies. We have previously identified a wild type p53-reactivating compound RITA in a cell-based screen. Notably, we found that RITA can restore the apoptosis-inducing function of at least some p53 mutants, including hot-spot His273 mutant. Our preliminary data suggests that the molecular mechanism of mutant p53 reactivation by RITA involves induction of a conformational change upon binding of RITA to mutant p53. Indeed, our experiments demonstrate direct interaction of 14C-labelled RITA with purified p53, as well as with endogenous p53 in cells. Moreover, using DNA microarray analysis we show that the response of tumor cells to RITA is entirely p53-dependent, suggesting high specificity of targeting p53 by RITA. Pathway analysis revealed preferential induction of p53 apoptosis pathway by RITA, consistent with our observation that apoptosis is major response to RITA treatment in various types of tumor cells. Importantly, we identify p21 as a major switch between survival and death induced by p53 and uncover a previously unrecognized role of HDM2 in p53 choice between growth arrest and apoptosis. Furthermore, we show that reactivation of p53 can overcome survival signaling in tumor cells and identify the blueprint for oncogenic pathways suppressed by RITA-activated p53. p53 unleashes a transcriptional program which inhibits the expression of anti-apoptotic proteins Bcl-2, MAP4, Mcl-1 and survivin, blocks IGF1R-PI3K-Akt-eIF4E pathway by targeting several of its components and downregulates a set of downstream oncogenes including c-Myc, cyclin E and β-catenin. Moreover, p53 ablates the expression of c-Myc via several mechanisms including transcription, translation and protein degradation. Thus, hierarchical and robust downregulation of key oncoproteins by p53 reactivation might offer a potent therapy regardless of the particular combination of mutations in a given tumor.

Lecture 27

MECHANISMS OF PRIMA-1-MEDIATED MUTANT p53 REACTIVATION AND APOPTOSIS

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Restoration of wild type p53 expression in vivo has been shown to trigger cell death and rapid elimination of tumors. The identification of mutant p53-reactivating small molecules such as PRIMA-1 opens possibilities for the development of more efficient anticancer drugs. We have investigated the molecular mechanism by which PRIMA-1 targets mutant p53 and triggers mutant p53-dependent apoptosis in human tumor cells. PRIMA-1 treatment induces the pro-apoptotic p53 target genes Bax and PUMA and activation of caspase-2, leading to loss of mitochondrial membrane potential, cytochrome c release, and activation of downstream effector caspases. Microarray analysis revealed that PRIMA-1 induces changes in expression of a relatively limited number of genes in mutant p53-expressing cells. These genes include known p53 target genes that regulate apoptosis as well as genes that are associated with cell cycle regulation and senescence. A better understanding of the mutant p53-dependent cellular events triggered by PRIMA-1 may facilitate the design of more potent and specific mutant p53-targeting anticancer drugs.

Lecture 28

GENOTYPE & PHENOTYPE IN FAMILIES WITH LI-FRAUMENI & LI-FRAUMENI-LIKE SYNDROMES

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Li and Fraumeni (1969) described 4 families in which there were siblings or cousins with childhood soft tissue sarcoma associated with sarcomas, breast and other cancers in young adult relatives. They suggested that these findings constituted a syndrome and subsequently proposed a clinical definition based on a proband with sarcoma and 2 close relatives with cancer under 45 years of age (Li-Fraumeni syndrome, LFS) (Li et al 1988). 70-80% of LFS families carry germline *TP53* mutations. Families with related patterns of cancer may also carry *TP53* mutations. These are termed Li-Fraumeni-like (LFL). The main cancers associated with germline *TP53* mutations are bone and soft tissue sarcomas, pre-menopausal breast cancer, brain tumours and adrenocortical carcinoma but other cancers also occur to excess.

In a study of 19 *TP53* positive families, we found that missense point mutations within the DNA- binding domain of TP53 conferred a more highly penetrant cancer phenotype than other *TP53* mutations (Birch et al 1998). Hwang et al (2003) applied similar comparisons to 7 kindreds and did not find such differences. However, Olivier *et al* (2003) analyzed the IARC *TP53* database and found correlations between cancer phenotype and structural/functional mutation group. We have now updated our analyses on a series of 35 families with *TP53* mutations and in addition, have applied the structure/function groupings defined by Olivier *et al*. Predicted numbers of cancers diagnosed aged 0-44 years among the cohort of families were estimated from age, morphology, site, sex and calender period specific UK cancer statistics. Methods took account of selection biases in the families. Families were partitioned according to mutation type and their cancer incidence patterns compared.

Considering the groups of Olivier *et al*, there was a highly significant difference in cancer incidence ($p<0.001$), with the highest incidence in families with mutations in the L2 and L3 loops (Group 1). Grouping according to whether mutations were: proven gain of function/transdominant (group A); splicing (B); other truncating (C); point mutations shown not to be transdominant (D), there was no overall significant difference ($p=0.13$) but the highest incidence was seen in group A. When hotspot, non-hotspot and other mutations were compared, the difference was significant ($p=0.008$). The highest incidence occurred in families with hotspot point mutations. Differences by cancer type will also be presented. We conclude that penetrance and phenotype are influenced by type of germline mutation.

LFL cancer clusters in *TP53*-negative families may be due to a variety of other genes including NF1, heterozygous BRCA2 mutation and bi-allelic BRCA2, MMR and PALB2 mutations, among others. However, it is now apparent that CHEK2 is not a LFS gene. Given these observations, it seems appropriate that the diagnosis of Li-Fraumeni Syndrome should be based on the presence of a germline *TP53* mutation in an individual or family. The original clinical criteria are useful in selecting families for TP53 testing but should be re-visited in the light of recent findings.

Lecture 29

CLINICAL EFFICACY AND SAFETY OF ADENOVIRAL p53 (ADVEXIN) IN THE TREATMENT OF TUMORS WITH INHERITED AND ACQUIRED p53 ABNORMALITIES

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Advexin® is comprised of a replication-incompetent Adenovirus type 5 vector containing the normal p53 tumor suppressor gene as its therapeutic component. We investigated the predictive biomarkers influencing efficacy of adenoviral p53 gene therapy. To evaluate abnormal p53 protein over expression by immunohistochemistry as a biomarker to predict Advexin therapeutic efficacy in different histological types of cancer, we evaluated Advexin monotherapy treatment responses across different multiple tumor types--squamous cell carcinoma of the head and neck (SCCHN); non-small cell lung cancer (NSCLC); prostate and Li-Fraumeni Syndrome (LFS) tumors, with and without p53 protein abnormalities. In the summary analyses provided below, abnormal p53 protein detected by IHC was associated with a statistically significant increase in tumor response following Advexin treatment in different solid tumors sharing p53 protein defects.

Tumor Histology	**Number of Patients**	**Tumor Response**	
		p53 Abnormal	**p53 Normal**
SCCHN	27	38% (6/16)	0% (0/11)
NSCLC	7	20% (1/5)	0% (0/2)
Prostate	19	33% (3/9)	0% (0/10)
Li-Fraumeni	1	100% (1/1)	NA
Total	**54**	**35% (11/31)**	**0% (0/23)**
P = 0.001, *Fisher's Exact Test*			

Importantly, all responders by Choi criteria [1] ($\geq$ 10% reduction in tumor size--SCCHN, NSCLC and prostate cancer or > 50% reduction in SUV by PET scan—LFS patient) in a variety of solid tumor types had abnormal p53 protein by IHC while no responders had normal p53 evaluations. Our findings indicate that the abnormal p53 protein biomarker is predictive of Advexin efficacy across a wide range of tumor types. In the recurrent SCCHN patients treated with Advexin for whom survival data was evaluated, abnormal p53 detected by IHC was a molecular predictive biomarker associated with statistically significant increases in survival. The median survival of patients with the abnormal p53 biomarker was 11.6 months compared to only 3.5 months in patients with normal p53 tumors (p=0.0007; log-rank test). In conclusion, abnormal p53 protein detected by IHC was found to be a molecular predictive biomarker associated with statistically significant increases in tumor response and survival following Advexin therapy in a variety of tumor histological types. These findings support its future application in the treatment of inherited and non-inherited tumors characterized by defective p53 pathways.

[1] Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA, Benjamin RS. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol. 2007 May 1;25(13):1753-9.

Lecture 30

MOLECULAR BASIS OF THE LI-FRAUMENI SYNDROME: AN UPDATE FROM THE FRENCH LFS FAMILIES

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We have performed the extensive analysis of *TP53*, based on the complete sequencing of the 11 exons and QMPSF analysis to detect genomic rearrangements, in 474 French families suggestive of LFS, as defined by the Chompret criteria [1] : a proband with a tumour belonging to the narrow LFS tumour spectrum (sarcoma, brain tumour, breast cancer, adrenocortical carcinoma) before 36 years and at least one first- or second-degree relative with a typical LFS tumour before 46 years or with multiple tumours, *or* a proband with multiple tumours, two of which belonging to the narrow LFS tumour spectrum and the first of which occurred before 36 years, *or* a child with adrenocortical carcinoma whatever the family history. We detected in 82 families (17%) a germline alteration of *TP53.* In this series selected according to the Chompret criteria, the median age of tumour onset was 25 years and the most frequent tumours observed in the index cases were pre-menopausal breast cancer (36%) soft-tissue sarcoma (33%), osteosarcoma (23%), brain tumour (18%), and adrenocortical tumour (12%), the frequency of other malignancies being below 10%. The germline *TP53* alterations corresponded, in most of the cases (78/82, 95%), to point mutations or small deletions or insertions, widely distributed between exons 3-11. In 4 families, QMPSF analysis revealed a genomic rearrangement of the *TP53* locus: a complete deletion extending on 44.6 kb; in 2 families, a deletion removing only the promoter and the non coding exon 1 of *TP53* and extending to intron 3 of the *FLJ10385* gene, as shown by subsequent QMPSF scanning; a deletion of exons 2-10. The families harbouring these genomic rearrangements presented a typical LFS syndrome fulfilling the original LFS criteria. The identification of such germline *TP53* deletions in LFS families is of interest for our general understanding of the basis of LFS since it provides the final argument that LFS results from a loss of function at the *TP53* locus. In our series, missense mutations represented 69% of the germline alterations. The missense *TP53* mutations, that we tested in the FASAY (Functional Assay on Separated Alleles in Yeast), were found to inactivate systematically the transcriptional activity of the protein, in agreement with the loss of function mechanism. Nevertheless, this does not explain the remarkable *TP53* germline mutation spectrum characterized by the predominance of missense mutations. The different tumour spectrum observed between *TP53 wt/mt* and *wt/-* mice [2, 3] led us to compare in this series the mean ages of tumour onset between 61 patients harbouring *TP53* missense mutations and 35 mutation carriers with other types of alterations. The mean ages of tumour onset between the two groups (21.6 and 29.1 years, respectively) were significantly different ($p<0.03$). This confirms, as predicted by mouse models, that missense *TP53* mutations have an additional oncogenic effect. The observation of a later age of tumour onset associated with null mutations led us, subsequently, to perform *TP53* analysis in families presenting a tumour spectrum suggestive of LFS but with a first tumour being diagnosed in the index case after 40 years of age. We had indeed the surprise to identify in such families non sense, splicing mutations or in frame deletions of *TP53*. Finally, since the other genetic factor which has been shown by 3 independent studies [4-6] to modulate the age of tumour onset in *TP53* mutation carriers is the *SNP309 G* allele of the *MDM2* gene encoding the main negative regulator of p53, we extended our previous analysis of the *MDM2 SNP309 G* allele to 88 *TP53* mutation carriers. The age of tumour onset (20.9 years) in the

55 G carriers (corresponding to the T/G + G/G genotypes) was clearly different than that observed in the 33 T/T patients (28.9, p = 0.01), the presence of the *G* allele being associated with a 8 years earlier age of tumour onset.

In conclusion, this study performed on 474 patients shows that (i) germline *TP53* alterations can be detected in approximately 20% of the LFS families, as defined according to the Chompret criteria, (ii) genomic rearrangements represent 5% of the germline *TP53* alterations and their identification in LFS families demonstrates that LFS results from a loss of function at the *TP53* locus, (iii) missense mutations, which represent 69% of the germline *TP53* alterations, are associated to a 7 earlier age of tumour onset, and (iv) *the MDM2 SNP309 G* allele is associated to a 8 years earlier age of tumour onset.

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Lecture 31

IARC DATABASE OF LI-FRAUMENI SYNDROME: A RESOURCE FOR THE EXPLORATION OF GENOTYPE-PHENOTYPE RELATIONSHIPS

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A database that compiles information on families carrying a germline mutations in the *TP53* gene and on families affected with Li-Fraumeni syndromes (Li-Fraumeni syndrome, LFS, and Li-Fraumeni Like syndrome, LFL) is maintained as part of the IARC TP53 Database. The IARC *TP53* Database (http://www-p53.iarc.fr/) is a research resource freely accessible on the web that provides comprehensive information on *TP53* mutations associated with human cancers. It compiles data reported in the peer-reviewed literature and provides tools for the analysis of mutation patterns in relation with tumor etiology, tumor phenotype and biological impacts of mutant proteins. Among recent developments, a major effort has been made to document biological activities of missense mutants proteins, the most frequent mutations associated with human cancer. In particular, data from transactivation activities (TA) on p53 response-elements (p53-RE) that have been assessed in yeast and human cells have been integrated in the database. Predicted functional impact of all possible missense mutations were also assessed by two different methods based on protein sequence conservation. Functional classifications of mutations have been derived from these data and can be used to perform systematic "functional" analyses of the database.

Recent analyses revealed several genotype/phenotype associations. For example, in classical LFS families carrying a germline *TP53* mutation the mean age of onset of breast cancer was significantly lower than in LFS families with no *TP53* mutation, and mutations with total loss of trans-activation activity were associated with earlier onset of breast and colorectal cancers compared to mutations that retain partial trans-activation capacity. These results show how the integration of clinical data on LFS families with molecular, epidemiological and biological data allow powerful exploratory analyses that add to our knowledge on the structural and functional properties of p53 that may affect mutation phenotype. With 399 mutations included in the current version of the database, it is important to continue to accumulate data on affected families to generate further knowledge that may help the clinical management of patients.

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Lecture 32

LI-FRAUMENI SYNDROME – A NEW LOOK AT OLD PROBLEMS

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No abstract.

Lecture 33

SUPPORT FOR POTENTIAL SURVEILLANCE STRATEGIES FOR MEMBERS OF LFS KINDREDS

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Guidelines for cancer surveillance in LFS generally focus on physical examination and breast imaging. We undertook a pilot study to gather preliminary data evaluating FDG-PET/CT imaging as a potential surveillance for LFS kindreds. We also studied our data on GI malignancies to inform consideration of endoscopic surveillance for this group.

Prevalence of GI Malignancies: We studied prevalence of gastric cancer among the 367 subjects and 62 families with a known *tp53* mutation in Dana-Farber NCI registry. Thirty-two families (51.6%) were found to have at least 1 gastrointestinal tumor. Twenty-four individuals among 367 (6.5%) from 14 different families (22.6%) had a diagnosis of gastric cancer. Mean age at gastric cancer diagnosis was 46 years (range, 24 -75 y). Of these subjects 4 developed gastric cancer before age 30 (ages, 24, 27, 29, and 29 years). In addition, 9 of the 14 gastric cancer families had an additional gastrointestinal tumor (colon 6, pancreas 3, gastroesophageal junction 1, gastric 6). We have previously demonstrated a similar high prevalence of colorectal cancer in LFS families with a mean age at diagnosis of 33 years including 4 patients developing colorectal cancer before age 21 (ages, 9, 11, 15, and 20 y).

FDG-PET/CT: Members of LFS families with documented germline *tp53* mutations or obligate carrier status, no history of cancer within 5 years of enrollment and no symptoms of cancer or ill-health underwent FDG-PET/CT scanning in a comprehensive cancer center. Scans were centrally reviewed by a nuclear medicine physician experienced in reading FDG-PET/CT. Baseline FDG-PET/CT scan identified 3 asymptomatic cancers in 3 of 15 (20%) subjects: 2 papillary thyroid cancers (stage 2 and stage 3) and a stage 2 esophageal adenocarcinoma.

Conclusions: These data provide the first evidence for potential cancer surveillance strategies that may be worthy of further investigation for patients with LFS. We propose a collaborative effort to study an intensive surveillance strategy including PET/CT scans, breast MRI and upper and lower endoscopic screening in *tp53* mutation carriers.

Lecture 34

HIGH POPULATION IMPACT OF A LOW PENETRANCE *TP53* GERMLINE MUTATION CAUSES HIGH INCIDENCE OF LFL FAMILIES IN SOUTHERN BRAZIL

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Li-Fraumeni syndrome (LFS) is a rare cancer predisposition syndrome transmitted in an autosomal dominant pattern, which predisposes affected individuals to an increased risk of developing a variety of cancers at an early age including childhood. We have screened TP53 germline mutations in 45 unrelated Brazilian subjects, whose family histories matched LFS or LFL clinical definitions, to characterize the prevalence and type of TP53 mutations associated with these syndromes in the Brazilian population. In 13 index cases, a single mutation was found. Strikingly, 6 of the 12 missense mutations were G to A transitions at the second base of codon 337. In the R337H families, several types of cancers were observed in addition to adrenocortical carcinoma (ADR), contrasting with previous reports showing that this mutation predisposes exclusively to ADR. We have analyzed the possible common ancestry of this mutation by haplotyping TP53 in carriers and non-carriers. All carriers were found to have the same rare allele, demonstrating a founder effect. This mutation had been reported only once in an European series. Further inquiries showed that this family was of Brazilian origin but living in Portugal and referred to a Cancer Center in France at the time of diagnosis. Mutation detection on 21 member of a large pedigree identified 10 mutation carriers, only 5 of whom had previous history of cancer. A survey of 750 healthy subjects from community based Breast Cancer screening program in the area of Porto Alegre identified 2 individuals with R337H (0.3%). Cancer clustering was reported in the two families who were found to be related. Together these results demonstrate that R337H is common in at least parts of Brazilian Population and carries a risk of multiple cancers. Its wide distribution in south Brazil is likely to be due to the particular circumstances of population settlement between 17th and 18th century by Portuguese migrants.

Poster 1

TP53 MUTATIONS IN HEAD AND NECK CANCER – A POSSIBLE INDICATOR OF ENVIRONMENTAL ETIOLOGY?

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Although *TP53* mutations in human tumors generally have been extensively studied, the significance of p53 in head and neck cancers is still incompletely characterized. We analyzed the *TP53* mutations in 46 head and neck squamous cell carcinomas (HNSCC), using a PCR-SSCP, a sequencing and an immunohistochemical staining method (IHC) to detect mutations. We also assessed the structural and functional significance of *TP53* mutations using information IARC p53database. Judging by SSCP the *TP53* gene was mutated in a total of 26 tumors. After extensive control experiment two silent mutations found in codon 170 (AC**G**>AC**A**) and both were found in association with a codon 171 missense mutation (GA**G**>GA**C**). Also the same codon 259 (GA**C**>GA**A**) mutation, which is a very rare mutation, was mutated in 4 samples. Eight of the mutations affected the L2 domain, which is needed for the correct folding and stabilization of the central part of the protein, three affect the LSH (loop-sheet-helix) motif, and three affect the L3 domain, which are directly involved in the interaction between the protein and the DNA. Four missense mutations probably lead to non-functional protein (Cys238Ser, Gly245Asp, Glu258Lys, Arg283Pro). There was an association between p53 immunohistochemical staining and the TP53 mutation status, not statistically significant. Tumors from all seven heavy-smokers (>45 packyears) were positive in p53 immunohistochemistry. Two out of eight nonsmokers (0 pack/years) were positive p53 protein. In persons with exposure to tobacco, alcohol and chemically at work positive result in p53 IHC was more prevalent than in persons without exposure. The fact that patient with negative family history of cancer had TP53 mutation more often than patient with positive family history may also justify further studies on p53 alterations in connection environmental exposure in head and neck cancer.

Poster 2

PROFILING *TP53* MUTATIONS IN SINONASAL CANCER IN A EUROPEAN MULTICENTRE STUDY.

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Molecular mechanisms of carcinogenesis underlying development of the cancer of the nose and paranasal sinuses (SNC) are still largely unknown. In the IARC *TP53* database the information for SNC is sparse and in head and neck cancer (HN), codon distribution profiles with substantial numbers are available only for squamous cell carcinoma (SCC). In this study, we have analyzed *TP53* mutations in paraffin-embedded tissue samples of 358 SNCs from cases with and without occupational exposure to wood dust. Our series of tumours including 123 adenocarcinomas and 212 SCCs is by far the largest analysed for SNC. The SNC cases were identified and collected in collaboration with national cancer registries of Denmark, Finland and France. From the initial collection of >400 tumours, the series analysed for mutations comprised SNC tumours for which histological diagnosis were reviewed by a pathology panel. Tumour DNA samples were analysed with Capillary Electrophoresis Single Strand Conformation Polymorphism (CESSCP) and direct sequencing. Our results show that *TP53* mutations are frequent in SNC; 277 of 358 (77.4 %) cases carried mutations, with 486 mutations detected altogether. Direct sequencing was successful in identifying the sequence change of 158 mutations. More than half of these (92 mutations) were missense mutations; additionally there were 28 frameshift or nonsense mutations, and 38 intronic or silent mutations. Almost all silent mutations occurred in tumours with multiple mutations. Our data indicated the codon 248 as the most often mutated site in SNC; this is in accordance with the HNSCC data in the IARC database. Mutation profile in the IARC database for sinus SCCs is based on a very small number of mutations. Interestingly, this data set recognizes codon 135 mutations; the codon 135 was the second most frequently mutated codon in our study. This site is not commonly mutated in other cancers according to the IARC database. We also assessed occupational exposures by interview and with help of data from national pension security centers for the whole study population. Data analysis indicated, in line with epidemiological studies, that there was a strong association between wooddust exposure and the adencarcinoma cell type, whereas SCC seemed to be more common among smokers. However, smoking did not seem to influence the overall risk of having a mutation, but instead it was associated with multiple mutations, whereas wooddust exposure was associated with the occurrence of *TP53* mutations. We conclude that *TP53* mutation appears to be a common event in the development of the SNC and is related to wood dust exposure.

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Poster 3

TP53 MUTATION PATTERN IN BREAST CANCER PROGRESSION

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Mutations in the tumor suppressor gene *TP53* are found in approximately 20-30% of breast carcinomas and have been shown to have both prognostic and predictive implications. The frequency and type of mutations varies in different series of breast cancer patients and may be due to factors such as stage of disease and molecular subtype. *TP53* mutations have been suggested to be an early event in carcinomas of the breast, while it seems to be a later event in other types of cancer. To further explore the frequency and type of *TP53* mutations during progression from early to advanced stages of breast cancer, fresh frozen tissue from 781 samples from 6 different breast cancer cohorts were screened for *TP53* mutations in exon 2 to 11, mainly by direct sequencing (ABI 377/3730), some by TTGE/sequencing. The 6 cohorts represent the different stages in development of breast cancer, from premalign tissue, DCIS, DCIS with small invasive components, small invasive, to advanced stage, and with survival data available.

The frequency of *TP53* mutations was found to be 0% in biopsies from mammographic dense tissue (MDG cohort), 13% in Ductal Carcinoma *in situ* (DCIS cohort), 35% and 37% in two series of stage I/II (MicMa and NKI cohort respectively), and finally 52% and 28% in two series of more advanced stage disease (Korean and DBCG cohort respectively).

Small invasive tumors with an *in situ* component showed a higher frequency of mutations than the DCIS only and those with small invasive tumors without an in situ component.

The more advanced stage "DBCG" cohort had higher frequency of deletion/insertion mutations (29%) and lower frequency of missense mutations (55%) as opposed to the stage I/II MicMa cohort (14% and 74% respectively). Among the different nationalities represented (Norwegian, Swedish, Danish, Dutch and Korean) the Korean cohort had the highest frequency of *TP53* mutations (52%), which may reflect ethnical differences.

In conclusion, the *TP53* mutational pattern and frequencies in different stages of breast cancer should be further explored to better understand its role in cancer development and progression.

Poster 4

ABERRATIONS OF THE P53 PATHWAY IN REFRACTORY AND RELAPSED NEUROBLASTOMA

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Neuroblastoma (NB) is the most common extracranial paediatric solid tumour. Despite intensive treatment, only 25-30% of children with high-risk disease are curable. Most NB initially respond to therapy but the majority relapse with chemoresistant disease. p53 mutations are rare at diagnosis < 2%. We have previously reported inactivation of the p53/*MDM2/p14*^{ARF} pathway in NB cell lines established at relapse. **Hypothesis:** Inactivation of the p53/MDM2/p14^{ARF} pathway develops during therapy and selects for drug resistance. **Methods:** DNA was analysed from 41 NB tumours (34 paired diagnosis & relapse, 1 pre & post-chemotherapy, 3 post-chemotherapy & relapse), and 3 relapse only, for p53 mutations by automated sequencing, p14^{ARF} methylation & deletion by methylation-specific PCR and duplex PCR, and *MDM2* amplification by fluorescence in-situ hybridisation (FISH). **Results:** Inactivating p53 mutations were identified in 6/41 (15%) cases, (4 relapse, 1 post-chemotherapy & relapse, 1 diagnosis, post-chemotherapy & relapse). 5/6 patients died of disease (p=0.07, log rank). 3/22 (13%) cases had *MDM2* amplification at diagnosis & relapse, 2 patients are alive and disease free, 1 patients died from disease (overall survival – 2.75 years). p14^{ARF} inactivation was detected in 13 (32%) cases: 3 were methylated (1 diagnosis & relapse, 2 relapse), and 10 cases showed homozygous deletion (8 diagnosis & relapse, 2 relapse); 9/13 patients have died from disease. 11 pairs had a p53 abnormality at diagnosis and 7 were acquired. Cases with an acquired abnormality had a worse outcome, $p < 0.05$ (log rank). **Conclusions:** Abnormalities in the p53 pathway were identified in 20/41 (49%) patients with relapsed NB suggesting p53 independent therapies may be useful in the management of high-risk NB.

Poster 5

TP53 MUTATIONS AND HPV INFECTIONS IN TUMOURS OF THE UPPER AERODIGESTIVE TRACT FROM LATIN AMERICA

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We assessed the prevalence and patterns of *TP53* mutations and HPV infections in tumours of the upper aerodigestive tract from Latin America in combination with lifestyle risk factors.
A series of 242 tumours was selected from among 748 cancer cases collected within the framework of a large, multi-center study. DNA was extracted using a standard Qiagen kit protocol and analysed for the presence of *TP53* mutations in exons 5 – 9 by DHPLC and sequencing. The presence of HPV16 E7 DNA in tumour tissue was assessed by PCR with primers amplifying a fragment of the E7 gene. Only squamous cell carcinomas were included in the analysis by lifestyle risk factors.
A total of 138 *TP53* mutations was found in 123 out of 236 analysable tumours (52% mutant tumours) and the prevalence of mutations by tumour subsite ranged from 49% in hypopharynx and larynx cancer to 67% in oesophageal cancer. 57% of all *TP53* alterations were missense mutations and 19% were complex mutations. 91% of the resulting mutant p53 proteins have not retained the wild-type transactivation functions. Smokers were more likely to carry *TP53* mutations than never-smokers and the prevalence of mutations increased from 33% in never-smokers through 53% in former smokers (adjusted OR 3.22, 95% CI: 0.86-12.08) to 59% in current smokers (OR 3.99, CI: 1.15-13.84; P-value for trend 0.050). Smokers carried more G:C>T:A transversions than never-smokers, whereas never-smokers had a higher prevalence of endogenous mutations at CpG sites. Alcohol drinkers carried more G:C>A:T transitions than never-drinkers, whereas never-drinkers had a higher prevalence of mutations at CpG sites.
The prevalence of HPV16 was very low, the E7 DNA being present in only 6 of 242 tumors (0.5%).
The prevalence of *TP53* mutations in tumours of the upper aerodigestive tract in Latin America is high and correlates with a very low prevalence of HPV infections. Tobacco smoking and alcohol drinking result in different *TP53* mutation patterns. Tobacco alone increases the likelihood of *TP53* mutations, and the prevalence of G:C>T:A transversions, known as a tobacco fingerprint, increases down the respiratory tree. The mutations in non-exposed individuals seem to result from endogenous mechanisms, although the numbers are limited.

Poster 6

PATTERNS OF *EGFR*, *HER2*, *TP53*, *KRAS* MUTATIONS AND OF P14^{ARF} EXPRESSION IN NON-SMALL CELL LUNG CANCERS IN RELATION WITH SMOKING HISTORY

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Mutations in the tyrosine kinase domain of the epidermal growth factor receptor *EGFR* are common in Non Small Cell Lung Cancer (NSCLC) of Never-smokers whereas *HER2* mutations are rare. We have analyzed *EGFR* (exons 18 to 21), *HER2* (exons 19 and 20) and expression of the two products of the *CDKN2a* gene, p16^{ink4a} and p14^{arf}, in 116 NSCLC of patients with detailed smoking history. Data on *TP53* and *KRAS* mutations have been reported previously (Le Calvez et al. Cancer Res: 2005 65:5076-5083). *EGFR* mutations were detected in 20/116 tumors (17 %) whereas 5 tumors contained *HER2* mutations (4.3%). No tumor contained both mutations. Of tumors with *EGFR* or *HER2* mutation, 72% were adenocarcinomas, 68% were from Never-smokers and 32% from Former-smokers. *EGFR* but not *HER2* mutations were mutually exclusive with *KRAS* mutation. Among Never-smokers, 11/16 tumors with *EGFR* mutation also had *TP53* mutation, in contrast with 2/17 tumors without *EGFR* mutation (p=0.0008). Expression of p14^{arf}, but not p16^{ink4a}, was more frequently down-regulated in Never-smokers (62.5%) than Ever-smokers (35%) (p=0.008). All tumors with *EGFR* or *HER2* mutations and wild-type *TP53* showed down-regulation of p14^{arf} expression. These observations suggest that functional inactivation of the p14^{arf}/p53 connection is required in tumors with *EGFR* or *HER2* mutations, consistent with the notion that these proteins are part of a failsafe mechanism protecting cells against untimely or excessive mitotic signals.

Poster 7

MDM2 OVEREXPRESSION IN HUMAN LUNG CANCER LEADS TO P53-DEPENDENT UPREGULATION OF NFKB2 EXPRESSION, WHICH IN TURN ENHANCES CELL PROLIFERATION.

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Mutation in p53 and overexpression of the oncoprotein MDM2 are frequent events in human cancer. To determine the frequency of MDM2 overexpression, and to identify co-occurring p53 mutation and other abnormal gene expression, we determined the levels of MDM2, the status of p53 mutation, and the expression of some oncogenes including mutant p53 inducible genes in human non-small cell lung cancer samples. Higher than normal levels of MDM2 was found in approximately 30% of the cancer samples harboring wild-type (WT) or mutant p53. p53 mutation was also detected in 30% of the lung cancer samples. Expression of two mutant p53 inducible genes, NFκB2 and c-myc, showed significant statistical correlation with MDM2 expression in cancer samples that harbored WT p53. Lung cancer samples harboring mutant p53 exhibited elevated levels of NFκB2 although, the extent of upregulation varied from one mutant type to other. Consistent with our finding in lung cancer samples, ectopic overexpression of MDM2 in a cultured lung cancer cell line harboring WT p53 elevated expression of NFκB2 and its transcriptional target c-myc, and upregulated NFκB2 promoter activity. Although mutant p53 can upregulate NFκB2 expression in lung cancer cell lines devoid of p53, MDM2 overexpression in these cells showed a small increase in the NFκB2 levels and did not show increase in c-myc expression. Downregulation of MDM2 expression using siRNA against MDM2 proportionally downregulated NFκB2 expression in lung cancer cells harboring WT p53, and downregulation of NFκB2 expression retarded cell proliferation. We propose that MDM2-mediated NFκB2 upregulation confers growth advantage on lung cancer cells and may constitute one of the steps of oncogenesis.

Key words: MDM2, p53, NFκB2, c-myc, lung cancer

Poster 8

IMPORTANCE OF MONITORING OF BOTH *TP*53 ALLELES IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background and aims: B-cell chronic lymphocytic leukemia is a malignant disease of elderly individuals with highly variable clinical course. Aberrations of *TP53* gene occur in 10-15% of patients and represent one of the most important prognostic factors. The presence of aberrations almost inevitably leads to treatment requirement and poor outcome and the gene thus constitutes the strongest independent marker for disease-related death. Differences in the outcome of patients with both mutation and deletion and patients with one affected allele remain, however, unclear. In this study we determine the proportion of individual *TP53* aberrations (deletions, mutations, combination of both) in our set of unselected B-CLL patients and associate the one allele and both alleles aberrations with therapy requirement and survival rates of the patients.
Methods: The deletions of *TP53* locus (17p13.1) were detected using an interphase FISH and mutations in the gene were searched out using yeast functional analysis (FASAY), coupled to sequencing of templates from yeast colonies bearing the mutated *TP53* genes.
Results: We screened 360 B-CLL patients for the deletions and mutations of the *TP53* gene and 12% of patients manifested a *TP53* alteration. Most of affected patients (58%) exhibited a complete gene inactivation (deletion/mutation). However, single allele missense mutations or deletions also occurred (in 26% or 16% of p53-affected patients, respectively). Patients with deletion/mutation manifested the worst prognosis: 96% of them required chemotherapy; one third of them died within two years after diagnosis and they had the shortest overall survival, which differs significantly from both no *TP53* abnormality subgroup and single allele aberration subgroup ($p < 0,01$). While patients with single allele aberration had markedly better prognosis: 70% of them required a therapy; none of them died within two years after diagnosis and the difference of overall survival was not statistically significant ($p = 0,21$) in comparison to patients without TP53 abnormality
Summary/conclusions: We suggest that monitoring of both p53 alleles might be useful in B-CLL patients, because the correlation between deletions and mutations is not absolute and the course of the disease may be possibly grouped according to complexity of the p53 inactivation.

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Poster 9

AN UNUSUAL P53 MUTATION DETECTED IN BURKITT´S LYMPHOMA: 30BP INSERTION IN CODON 87

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Burkitt´s lymphomas (BL) are aggressive fast growing tumors typified by high expression of c-*myc* protooncogene. This usually results from translocation t(8;14)(q24;q32), t(2;8)(p12;q24) or t(8;22)(q24;q11). p53 tumor suppressor alterations are also relatively frequently detected in BL. Several different methodical approaches have been adopted for detection of the p53 aberrations: immunohistochemical analyses, immunoblotting, DNA sequencing, fluorescent *in situ* hybridization (FISH), and functional assays. We used all these methods to characterize in detail p53 mutation in the case fifty three year old male who underwent surgery in April 2006 as patient suffering from diffuse large B-cell lymphoma with extensively affected abdomen, stomach, surrounding lymph nodes, bone marrow and central nervous system. The disease was finally diagnosed as Burkitt´s lymphoma, clinical stage IVB, with t(8;14) positivity detected by FISH. The patient underwent an intensive chemotherapy and reached complete remission. The remission has lasted up to date.

By immunohistochemical analyses using monoclonal antibody DO-7, we detected high level of the p53 protein in the tumor tissue. By immunoblotting using monoclonal antibody DO-1, we confirmed the high level of p53 expression and revealed that the molecular weight of the p53 protein expressed by tumor cells was higher than that of the standard p53 protein. Similarly, the molecular weight of PCR product prepared by amplification of the tumor p53 cDNA was higher than the PCR product prepared from standard p53 cDNA. Functional analyses of separated alleles in yeast clearly revealed that the tumor variant of the p53 protein is transcriptionally nonfunctional. The yeast colonies expressing this p53 variant possessed unique phenotype: the color of colonies was red with many white spots on their surface. DNA sequencing of tumor cDNA revealed 30 bp insertion in codon 87. The insert represents the intrinsic p53 sequence originating from the region between codons 77 and 87 placed in inverse orientation. Further analyses showed that the insertion is unstable in yeast cells. The FISH analyses did not confer loss of the p53-specific locus 17p13.

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Poster 10

MDM2 SNP309 AND *TP53* CODON 72 POLYMORPHISMS IN GLIOBLASTOMA

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A single nucleotide polymorphism (SNP) in the promoter region of the *MDM2* gene, SNP309, is associated with a younger age of onset of tumours in patients with Li-Fraumeni syndrome and with higher susceptibility to development and poorer survival in several sporadic tumors. Polymorphism at codon 72 (Arg->Pro) of the *TP53* gene decreases its apoptotic potential and may be associated with shorter survival and increased risk of several human neoplasms. In this study, we assessed *MDM2* SNP309 in 360 glioblastomas diagnosed at a population level, and correlated these glioblastomas with patients' age and survival, as well as other alterations in the TP53 pathway (*TP53* mutations, *TP53* codon 72 polymorphism, *MDM2* amplification, $p14^{ARF}$ homozygous deletion/promoter methylation). Frequencies of the *MDM2* SNP309 T/T, T/G, and G/G alleles in glioblastomas were similar to those previously reported in healthy Caucasian individuals. In female patients treated with surgery plus radiotherapy, *MDM2* SNP309 G allele was significantly associated with favourable outcome, with median survival of 16.9 months (G/G), 10.2 months (G/T) and 8.4 months (T/T) (P=0.0037). *MDM2* SNP309 genotypes did not significantly affect age of glioblastoma diagnosis. *MDM2* amplification was detected in 5% of primary glioblastomas, and all of these had *MDM2* SNP309 T/G or G/G alleles. There were significant inverse associations between *TP53* mutation and *MDM2* amplification, and between *TP53* mutation and $p14^{ARF}$ alterations. Glioblastoma patients with *TP53* codon 72 Pro/Pro genotype were significantly younger (mean, 50.2$\pm$15.3 years) than Arg/Arg carriers (56.1$\pm$11 years; P=0.018). Glioblastomas carrying a *TP53* mutation and with codon 72 Arg/Pro or Pro/Pro alleles had a significantly shorter survival (median, 5.9 months) than those with Arg/Arg alleles (9 months; P=0.0083). There was a significant association between *TP53* Pro/Pro genotype at codon 72 and *MDM2* SNP309 T/G or G/G genotypes (P=0088). Glioblastoma patients carrying a *TP53* mutation and with *TP53* codon 72 (Arg/Pro or Pro/Pro) and *MDM2* SNP309 (T/G or G/G) alleles showed significantly shorter survival (median 5.7 months) than those with *TP53* codon 72 Arg/Arg and *MDM2* SNP309 T/T alleles (9.4 months; P=0.0049). These results suggest that common polymorphisms in the *TP53* pathway significantly affect outcomes of patients with glioblastomas.

Poster 11

P53 STATUS IN TUNISIAN NASOPHARYNGEAL CARCINOMA PATIENTS

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The p53 is one of the most extensively studied human genes because of its role as a tumor suppressor It was altered in 50% of human cancer and mutations were clustered in exons 5-8 which were involved in p53-DNA interaction. In contrast to other types of human cancer, p53 is rarely mutated in NPC, although the accumulation of the P53 protein was reported in many NPC cases particularly in the juvenile form.
We showed that in Tunisian NPC patients P53 is also rarely mutated even in young patients
Besides mutations, common polymorphism at codon 72 encoding either Arginine or Proline of the P53 tumor suppressor protein was widely described. These variants may be associated with tumor susceptibility since they interfere with P53 ability to activate apoptosis, and might account for ethnic variation in cancer frequency. Using a PCR-RFLP assay, we tested peripheral blood samples from 115 patients with nasopharyngeal carcinoma and 83 healthy individuals. Patients with NPC (Arg/Arg=*38.26%;* Arg/Pro=*41.73*% and Pro/Pro=*20*%) showed a significant different percentage *(p=0.0307*) of the Pro/Pro genotype compared to the control population (Arg/Arg =39%, Arg/Pro=54% and Pro/Pro=7%). However, no significant difference was observed between P53 codon 72 polymorphism and age, sex, histological grade and metastasis. These results provide evidence that individuals with the Pro/Pro genotype have an increased risk of developing NPC in Tunisia.

Poster 12

MOLECULAR BASIS OF THE LI-FRAUMENI SYNDROME (LFS): AN UPDATE FROM THE FRENCH LFS WORKING GROUP

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The Li-Fraumeni syndrome represents one of the most devastating genetic predispositions to cancers and is characterized by a wide spectrum of early-onset malignancies (sarcoma, brain tumour, adrenocortical tumour, breast cancer, leukemia, lymphoma, gastric tumour, colorectal and lung cancer). We have performed the extensive analysis of *TP53*, based on complete sequencing of the 11 exons and on QMPSF, in 409 families suggestive of LFS, fulfilling the French LFS network criteria (Chompret *et al*., *J. Med. Genet*. 2001). We detected in 76 families (19%) a germline alteration of *TP53* corresponding, in most of the cases (96%), to point mutations or small deletions or insertions, widely distributed from exons 3 to 11 and in 4% of the cases to complete or partial genomic deletions. These results constitute a definitive argument demonstrating that LFS results from a haploinsufficiency at the *TP53* locus. If most of the families presented the classical LFS wide tumour spectrum, the presentation of some kindreds was remarkable, mimicking *BRCA* families. In this series, we confirm that the mean age of tumour onset in *MDMD2* SNP309 G allele carriers (19.2 years) is significantly different from that observed in patients homozygous for the T allele (29.3 years) and found that the mean ages of tumour onset in *MDMD2* G and *TP53* Arg alleles carriers, and in patients with the *MDMD2* T/T and *TP53* Pro/Pro genotype were clearly different (17.6 *vs* 39.2 years). The allele specific tumor spectra observed in *TP53 wt/mt* mice compared to *wt/-* mice (Olive *et al*., Lang *et al*., *Cell* 2004) led us to compare the age of tumour onset between patients harbouring missense mutations (56 patients) and those carrying other alterations (31 patients). As predicted by the murine models, we indeed observed a significant difference between both groups (21.1 years *vs.* 29.2 years). These results confirm that missense mutations do not only inactivate the transcriptional activity of the wild-type protein but also have an additional oncogenic effect.

Poster 13

TWO GERMLINE P53 MUTATIONS IN THE TETRAMERIZATION DOMAIN

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Germline p53 mutations are associated with LFS/ LFL syndromes and an adrenocortical carcinoma development in children. In a search for germline p53 mutations, a new series of pediatric patients both from LFS/ LFL families and recently diagnosed with a sporadic cancer has been studied. p53 exons 2-11 have been screened for mutations by SSCP and sequencing. A novel germline p53 mutation was discovered in a proband of LFS family #630, a boy diagnosed with a synovial sarcoma at the age of 8 years and an osteosarcoma at 12. The heterozygous germline alteration was a missense mutation at nucleotide 17603 G>C, codon 342 CGA>CCA, which caused a substitution of proline for arginine. The mutation was confirmed at RNA level. The second germline p53 mutation was discovered in a boy with a lung metastasis of adrenocortical carcinoma. The mutation was at nucleotide 17602 C>T, codon 342 CGA>TGA, generating a change from arginine to stop codon. Another p53 defect was found in a child from LFS family previously treated for rhabdomyosarcoma and osteosarcoma, and recently diagnosed with MDS (AML). The alteration appeared to be a new p53 somatic mutation detected only at MDS (AML) stage. The heterozygous mutation was in exon 10, codon 337 CGC>GGC, resulting in a substition of glycine for arginine. The defect was confirmed at RNA level, where only the mutant Gly-337 was observed.

In summary, in this new series of pediatric patients screened for p53 alterations, two germline mutations were found: R342X and the new one - R342P, and in addition, the new somatic mutation R337G. It is of note that in polish population, where constitutional p53 mutations are very rare in LFS/ LFL families, the two reported germline mutations were found in the tetramerization domain and the new somatic mutation as well.

Poster 14

COPY NUMBER VARIATION AND CANCER PREDISPOSITION: CHARACTERIZATION OF GERMLINE COPY NUMBER ALTERATIONS IN LI-FRAUMENI SYNDROME

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Unpublished data – abstract withdrawn by author

Poster 15

ESSENTIAL ROLE FOR *SF-1* GENE DOSAGE IN ADRENOCORTICAL CANCER

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Adrenocortical tumor (ACT) in children is a rare form of neoplasm but its incidence is higher in southern Brazil than in the rest of the world. In that region, it is almost invariably found associated with a specific germline TP53 mutation (R337H) and loss of heterozygosity in the other allele. We have shown an increased copy number of the steroidogenic factor 1 (SF-1; NR5A1) gene associated with its overexpression in the majority of childhood ACT compared with normal age-matched adrenal gland. Steroidogenic factor-1 is a nuclear receptor transcription factor playing a pivotal role in adrenogonadal development in both humans and mice. Using an integrated approach comprising human tumor adrenocortical cell cultures, gene expression profiling and transgenic mice analysis, we have defined a critical role for SF-1 dosage in regulating the proliferation of human adrenocortical cells and triggering tumor formation in mice. *SF-1* overexpression can by itself increase human adrenocortical cell proliferation through opposing effects on cell cycle and apoptosis. Also, SF-1 overexpression selectively modulates the steroid secretion profile of adrenocortical cells. By gene expression profiling we identified a novel pro-apoptotic factor for adrenocortical cells, NOV/CCN3, whose levels are significantly downregulated by SF-1 overexpression in human adrenocortical cells and are also reduced in ACT. Moreover, *SF-1* overexpression in mice triggers adrenocortical hyperplasia and development of tumors. Our studies reveal a critical role for *SF-1* dosage in adrenocortical tumorigenesis and constitute a rationale for the development of drugs targeting SF-1 transcriptional activity for ACT therapy.

Poster 16

TRANSCRIPTIONAL FUNCTIONALITY OF GERMLINE P53 MUTANTS INFLUENCES CANCER PHENOTYPE

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Purpose: The TP53 tumor suppressor gene encodes a sequence-specific transcription factor able to transactivate several sets of genes whose promoters include appropriate response elements (REs). While human cancers frequently contain mutated p53, the alleles as well as the clinical expression are often heterogeneous. Germline mutations of TP53 result in cancer proneness syndromes known as Li-Fraumeni, Li-Fraumeni-like, and nonsyndromic predisposition with or without family history. p53 mutants can be classified as partial deficiency (PD) alleles or severe deficiency (SD) alleles depending on their ability to transactivate a set of human target sequences, as measured using a standardized yeast-based assay . We have investigated the extent to which functional features of p53 mutant alleles determine clinical features in patients who have inherited these alleles and have developed cancer.
Experimental design: We retrieved clinical data from the IARC database for all cancer patients with germline p53 mutations and applied stringent statistical evaluation to compare the functional classification of p53 alleles with clinical phenotypes.
Results: Our analyses reveal that PD alleles are associated with milder family history ($p=0.007$), lower numbers of tumors ($p=0.007$) and a delayed disease onset (median: 31yrs *vs.* 15yrs, $p=0.007$) that could be related to distinct tumor spectra.
Conclusions: These findings establish for the first time significant correlations between the residual transactivation function of individual TP53 alleles and clinical parameters in patients with inherited p53 mutations who develop cancer.

Poster 17

IMPACT OF MUTANT P53 FUNCTIONAL PROPERTIES ON *TP53* MUTATION PATTERNS AND TUMOR PHENOTYPE: LESSONS FROM THE IARC *TP53* DATABASE

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Multiple TP53 gene variations have been described so far, including polymorphisms and mutations, both differing in their frequency in human population. Mutations are rare genetic variations, by which *TP53* is frequently inactivated in most human sporadic cancers. Moreover, inherited *TP53* mutations predispose to a wide spectrum of early-onset cancers (Li-Fraumeni Syndrome). All *TP53* gene variations (somatic and germline mutations, as well as polymorphisms) that are reported in the scientific literature or in SNP databases are compiled in the IARC *TP53* Database. They can be searched and retrieved on line at http://www-p53.iarc.fr/.

The most frequent gene mutations are missense substitutions, leading to the production of a mutant protein with a single amino-acid change, which account for 75% of all reported alterations. Over the last ten years, the functional impact of these missense mutations has widely been studied by numerous experimental assays that have been performed in yeast or human cells, and models have been set up to predict the impact of the mutations on protein activity. Annotations on functional and predicted impacts of missense mutations were recently added in the IARC *TP53* Database to provide a framework for the analysis of functional patterns of mutations in cancers and the detection of genotype/phenotype associations.

We performed a systematic analysis of the database to determine the functional properties that contribute to the occurrence of mutational "hotspots" in different cancer types and to the phenotype of tumors. This analysis showed that loss of trans-activation capacity is a key factor for the selection of missense mutations, and that difference in mutation frequencies is closely related to nucleotide substitution rates along *TP53* coding sequence. An interesting new finding is that in patients with an inherited missense mutation, the age at onset of tumors was related to the functional severity of the mutation, mutations with total loss of trans-activation activity being associated with earlier cancer onset cancers compared to mutations that retain partial trans-activation capacity. These results provide new insights into the factors that shape mutation patterns and influence mutation phenotype, which may have clinical interest.

Poster 18

FUNCTIONAL ANALYSIS OF p53 TEMPERATURE-DEPENDENT MUTANTS IN YEAST AND HUMAN CELLS

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More than 1500 different mutations in the *p53* gene are nowadays recorded in IARC TP53 Mutation Database. These mutations differ in their impact on function of the p53 protein. The most common „hot spot" mutations fully disrupt the ability of p53 to bind to specific DNA sequences and consequently to transactivate its target genes. However, there is evidence that many mutations can result in the p53 protein with a new function (e.g. gain of function or superactive mutants) or altered function (e.g. conditional mutants: temperature or pH dependent). The knowledge of functional properties of different p53 mutants could be important for prediction of cancer progression or for development of new therapies aiming to restore the function of p53 protein.

When using functional analysis of separated alleles in yeast (FASAY) for routine detection of p53 mutations from various tumor samples, we recorded 23 temperature-dependent (*td*) p53 mutants. Their transactivation ability is partially or fully restored with increasing or decreasing temperature. First, we analyzed in detail the transactivation ability of these mutants by yeast expression system. FASAY is a semi-quantitative method evaluating the transactivation ability of the p53 proteins according to the color of yeast colonies. The color reflects the level of *ADE2* reporter gene expression. Then, we performed transactivation tests of the p53 mutants in human cell expression system using luciferase as a reporter gene. The results of transactivation tests in both systems were compared and they proved very good correlation. All p53 mutants observed in yeast cells as temperature-dependent and discriminative displayed such phenotype also in human cells. Generally, human cell expression system was found to be more sensitive in addressing the level of p53 transactivation capability. In some cases, relative transactivation rate of mutant p53 in permissive temperature exceeded the activity of wt p53. The yeast system failed to detect this effect. On the other hand, the activity of some *td* p53 mutants was restored in yeast cells at such low cultivation temperature at which it is not possible to cultivate human cells. In this case, the human cell system was unable to confirm such functional restoration.

FASAY is convenient (cheap, fast and non-labor) method for routine analysis of p53 status in clinical material. Our results show that FASAY is reliable not only in detection of the p53 mutations but also in recognition of their different functional impact.

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Poster 19

COMMON, NONCANONICAL DNA MOTIFS THAT ARE FUNCTIONAL TARGETS FOR TRANSACTIVATION BY WILD TYPE AND MUTANT p53

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The DNA binding activity of the master regulator p53 is critical to its tumor suppressor activity in response to cellular and environmental stresses. p53 binds in a sequence-specific manner to sites originally defined as two decameric motifs, or half-sites, defined by the consensus RRRCWWGYYY and separated by 0–13 bp. However the mechanisms by which wild type (WT) and mutant p53 can transactivate to different extents from the many variants of this motif are not well-understood.

We are deconstructing the canonical p53 consensus sequence in order to better understand the role of sequence, organization and level of p53 on transactivation in budding yeast and human model cell systems. Contrary to early reports for *in vitro* binding, increases in spacer length of only a few bases between decamer half-sites greatly reduces p53 transactivation. This has been confirmed using extracts from human cells in a novel microsphere binding assay. These results with separated half-sites contrast with transactivation from pairs of full-sized REs that are weak on their own because of mismatches, but lead to synergistic increases in transactivation even when separated by 10 to 20 bases. While p53 lacked transactivation capacity from many full-sized RE canonical sequences, it functioned to different extents from several noncanonical sites that are frequent in the genome including 3/4 sites (*i.e.*, a decamer and an adjacent pentamer). Surprisingly, there can be substantial transactivation even at half-sites in yeast and mammalian cells depending on sequence and p53 expression level.

To understand functional aspects of p53 required for transactivation from canonical and noncanonical binding motifs, we are analyzing the effect of mutations in key structural domains. Efficient transactivation from canonical and noncanonical elements requires tetrameric p53, based on results with defined amino acid changes in the tetramerization domain yielding either dimeric or monomeric proteins. Regardless of the intrinsic affinity for a sequence, removal of the basic domain (starting at a.a. 368) that is required for non-specific DNA binding reduces transactivation levels. Finally, we show that the p53 family members, p63 and p73 are also greatly affected by spacer length and exhibit overlapping transactivation specificities toward canonical sites when compared to p53.

Our findings demonstrate that sequence and organization of a RE can have a large impact on p53-mediated transactivation. Furthermore, the loosening of a p53 binding element to include noncanonical sequences greatly expands the p53 transcriptional network.

Poster 20

EXPANDING THE P53 TRANSCRIPTIONAL NETWORK: SYNERGY BETWEEN P53 AND ESTROGEN RECEPTOR MASTER REGULATORS

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Previously, we had shown that the tumor suppressor p53 can cooperate with estrogen receptors (ERs) to cause synergistic increases in transcription of the angiogenesis-related gene Flt1 (Mol Cell Biol 27, 2007). Surprisingly, the transactivation of Flt1 was found to be regulated through a half-site p53 response element (RE) and a half-site estrogen receptor response element (ERE) located ~225 bp upstream from the p53 RE. Based on that finding we have proposed that there may be a general synergistic relationship between p53 and estrogen receptor via target canonical and noncanonical (i.e., half-sites) response elements. To address this, the original partial p53RE in the Flt1 promoter-Luciferase plasmid was replaced with canonical weak and strong p53REs of well-established target genes as well as other noncanonical p53 REs, and the resulting plasmids were transfected into human cancer cell lines. p53 was expressed by treating cells with doxorubicin or by co-transfecting with a p53 plasmid. The p53-induced expression was synergistically increased by the presence of active ERα (plus ligand). Site-directed mutations in the original ERE site within the Flt1 promoter disrupted the synergy, confirming the cooperativity *in cis* between p53 and ERα. The identification of synergistic interactions between p53 and ER at canonical and noncanonical response elements greatly expands the transcription master network regulated by p53 both in terms of numbers of genes affected and expression levels. An extensive genome search *in silico* has been initiated to identify putative p53 target genes containing p53 half-sites along with partial or complete associated EREs. We suggest that the synergy in transactivation with ERα may also apply to other transcription factors.

Poster 21

COMPLEX INTERPLAY BETWEEN P53 AND THE ESTROGEN RECEPTORS AT A POLYMORPHIC VARIANT OF THE FLT1 PROMOTER.

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Recently we established that a C>T single nucleotide polymorphism (SNP) in the promoter of the VEGF receptor Flt1 generates a half-site p53 response element (RE-T) that results in p53 responsiveness of the promoter. We also showed that p53 is required but not sufficient for Flt1 transactivation and that there is cooperative interaction with ligand-bound estrogen receptors (ER) *via* an ER half-site response element (ERE) located 225nt upstream the p53 RE-T. Disruption of the ERE in a reporter construct containing a 1kb fragment of the Flt1 promoter resulted in loss of p53 responsiveness in HCT116 (p53 wt, weakly ERb positive) and U2OS (p53 wt, negative for ER) cells. Surprisingly, we have now observed that disruption of the ERE has no impact on transactivation in MCF7 cells (p53 wt, ERa and ERb positive) treated with doxorubicin to induce p53. Promoter pattern searches revealed another putative half-site ERE in the Flt1 promoter located 145bp downstream of the p53 RE. Using site-directed mutagenesis, we showed that while the mutation of this second site has no impact, mutation of both sites greatly reduced transactivation. We also found that ectopic expression of the p53-related transcription factors p73 and p63 could not modulate the Flt1 promoter, regardless of the SNP status. To induce p53 in MCF7 cells we also used 5-FluoroUracil (5FU). Although 5FU was similar to doxorubicin in stabilizing the p53 protein and inducing the *p21* target gene, there was minimal transactivation of the Flt1-T construct, suggesting that doxorubicin might have a specific impact on the p53, ER transcriptional cooperation or might enlist additional transcription factors/cofactors that contribute to the activation of the promoter.

Poster 22

ALGORITHM FOR PREDICTION OF TUMOUR SUPPRESSOR P53 AFFINITY FOR BINDING SITES IN DNA

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The tumour suppressor p53 is a transcription factor that binds DNA in the vicinity of the genes it controls. The affinity of p53 for specific binding sites relative to other DNA sequences is an inherent driving force for specificity, all other things being equal. We measured the binding affinities of systematically mutated consensus p53 DNA binding sequences using automated fluorescence anisotropy titrations. Based on measurements of the effects of every possible single base pair substitution of a consensus sequence, we defined the DNA sequence with the highest affinity for p53 and quantified the effects of deviation from it on the strength of protein-DNA interaction. The contributions of individual nucleotides were to a first approximation independent and additive. But, in some cases we observed significant deviations from additivity. We constructed binding predictor that mirrored existing p53 consensus sequence definition. It predicted the affinity of known naturally occurring response elements. We used it to search for high-affinity binding sites in the genome and to predict the effects of single nucleotide polymorphisms in these sites. Although there was some correlation between the K_d and biological function, the spread of the K_ds by itself was not sufficient to explain the activation of different pathways by changes in p53 concentration alone.

Poster 23

INTERACTION OF MUTANT p53 WITH SUPERCOILED DNA IN CONTEXT OF CHROMATIN

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DNA supercoiled structure of DNA plays an important role in the regulation of various cellular events via specific and non-specific DNA interactions with proteins, in our case mutant protein p53 (mutp53), by formation of DNA/protein complexes. Usually natural negative supercoiling modulates these interactions and hence the efficiency/ specificity/stability of the complexes.

We studied DNA interactions of 'hot spot' mutants (G245S, R273C and R273H) expressed in *E.coli* and in glioblastoma cell lines (U251, Onda10 and Onda11) by electrophoretic and immunoprecipitation techniques.

Mutp53 has been reported to be unable of recognizing p53CON sequences in short linear DNA, but to bind selectively to oligonucleotides in stem-loop forms and to MAR/SAR elements. We found that some of mutp53 proteins recognized also mutant p53 specific genomic sequences obtained by chromatin immunoprecipitation from glioblastoma cell lines in longer DNA (~500bp).

Moreover, they retained ability to interact with natural supercoiled DNA. We studied supercoiled selective (SCS) DNA binding of mutp53 using modulation of activity of the p53 DNA-binding domains by oxidation of cysteine residues and/or by antibodies mapping to epitopes at the protein C-terminus (to block binding within the C-terminal domain). Using p53 deletion mutants, we have shown that the p53 C-terminal DNA binding site is critical for this binding. These data indicate that the C-terminus is primarily responsible for the p53 SCS DNA binding.

Considering the repeatedly proposed active role of the mutant p53 in tumorigenesis these observations may provide important insights into the molecular mechanisms that underlie the mutant p53 gain of function.

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Poster 24

DISSECTING THE ROLE OF p53 PHOSPHORYLATION IN DNA REPAIR PROVIDES NEW CLUES FOR GAIN-OF-FUNCTION MUTANTS.

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Regulation of homologous recombination (HR) represents the best-characterized DNA repair function of p53. The role of p53 phosphorylation in DNA repair is largely unknown. Here, we show that wildtype p53 repressed repair of DNA double-strand breaks (DSBs) by HR in a manner depending on N-terminal phosphorylation by ATM/ATR. Cdk-mediated phosphorylation of serine 315 was dispensable for this anti-recombinogenic effect. However, serine 315 phosphorylation was necessary for the activation of spontaneous, topoisomerase I-dependent HR by p53. Overexpression of cyclin A1, which mimics the situation in tumors, inappropriately stimulated DSB-induced HR in the presence of oncogenic p53 mutants (not Wtp53). This effect required cdk-mediated phosphorylation for stable complex formation with topoisomerase I. p53 mutants have lost the balance between activation and surveillance of HR, which results in a net increase of mutagenic DNA rearrangements. Our data provide new insight into the mechanism underlying gain-of-function of mutant p53 in genomic instability.

Poster 25

STRUCTURE-FUNCTION STUDIES OF P53 MUTANTS

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The function of most p53 cancer-associated mutants is abrogated either by destabilization of the DNA binding domain of the protein or changes in the structural conformation required for DNA binding or both. To investigate the mechanism of restoring function to p53 key cancer-associated mutants we have employed the second-site suppressor mutations approach by introducing mutants from the so-called "cold loop". Specifically, we have used biophysical and biochemical methods to probe the stability and DNA binding activity of select set of p53 mutants from loop 1 and assessed their effect on the global stability of and DNA binding activity of the p53 "hot spot mutant" variants. The significance of our findings may have potential implications for designing small molecules that mimic the effect of the second site suppressors, which can be exploited in cancer therapy.

Poster 26

STRUCTURAL BASIS FOR UNDERSTANDING ONCOGENIC p53 MUTATIONS AND DESIGNING RESCUE DRUGS

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The tumor suppressor protein p53 is directly inactivated by mutation in approximately 50% of human cancers, mainly due to missense-mutation in the DNA-binding core domain of the protein. Reactivating mutant p53 is therefore an important target in the development of novel cancer therapies. We have solved high-resolution crystal structures of numerous cancer-associated core domain mutants to investigate the structural basis of inactivation and provide information for designing drugs that may rescue oncogenic mutants.
We found an intriguingly diverse spectrum of structural consequences upon mutation: (i) just the removal of an essential contact with DNA, (ii) creation of large water-accessible crevices or hydrophobic internal cavities with no other structural changes but with a large loss of thermodynamic stability, (iii) distortion of the DNA-binding surface, and (iv) alterations to surfaces not directly involved in DNA-binding but protein-protein interactions within the p53 tetramer and with a number of signaling proteins [1, 2].
Many destabilized mutants are attractive targets for structure-based drug design. Some have the potential of being rescued by a generic stabilizing drug that restores function by shifting the folding-unfolding equilibrium toward the folded state. In addition, the mutation-induced surface crevice in the cancer hot-spot mutant Y220C is a potential target site for a mutant-selective stabilizing drug.

[1] Joerger *et al.* (2006) Proc. Natl. Acad. Sci. U.S.A. **103**, 15056-15061.
[2] Joerger & Fersht (2007) Oncogene **26**, 2226-2242.

Poster 27

PRIMA-1 SYNERGIZES WITH ADRIAMYCIN AND TRIGGERS AN APOPTOTIC RESPONSE IN NON SMALL CELL LUNG CANCER CELLS.

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p53-dependent apoptosis has an important role for the efficacy of cancer treatment, and tumours carrying mutant p53 are often resistant to chemotherapy. Among tumor types, non-small cell lung carcinomas (NSCLC) exhibit a strong resistance to drug- and radio-theraphy and clinical response to these treatments is very rare. By using PARP cleavage and FACS analysis, we first investigated apoptosis induction by adriamycin or UV-C in three lung cancer cell lines carrying different p53 proteins: A549 (p53wt), LX1 (p53R273H), SKMes1 (p53R280K). After both treatments, A549 and LX1 underwent apoptosis, while SKMes1 did not. Recently, the new molecule PRIMA-1 has been shown to induce apoptosis in human tumour cells by restoration of the transcriptional activity of mutated p53s. Thus, we investigated the apoptotic potential of PRIMA-1 in our lung cancer cells. When PRIMA-1 was given alone, neither PARP cleavage nor an increase of sub G1 cells could be found. However, when given in combination with adriamycin, PRIMA-1 potentiated the adriamycin-induced apoptosis in A549 and LX1, with the PARP cleavage being more efficient in LX1, carrying a mutated p53, than in A549. Interestingly, SKMes1 cells that were not able to develop an apoptotic response following adriamycin alone, showed a strong PARP cleavage at the lowest adriamycin dose when PRIMA-1 was present. To investigate a correlation between the apoptosis observed and a restoration of p53 transactivation activity towards some effector genes, we look at the level of p21, MDM2 and bax proteins. While we did not find induction of p21 and MDM2, the level of bax raised after PRIMA-1 addition. ChIP experiments are in progress to define the role of p53 transactivation in bax induction. Moreover the status of other pro- and anti-apoptotic proteins will be investigated. Our data suggest that in lung tumour cells, normally refractory to apoptosis induction, PRIMA-1 may synergize with classical drugs and trigger apoptosis. However, the role of a p53-dependent transcription in this response remains to be elucidated.

Poster 28

REACTIVATION OF MUTANT P53 BY SMALL MOLECULE RITA

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Mutations in the tumor suppressor p53 have been described in 50% of all human tumors. Restoration of p53 function can be of great value in cancer therapy, as p53 is a potent tumor suppressor. Small molecule RITA has been found to restore the function of wild type p53 in various tumor cell lines through reactivating p53's transcriptional activity and inducing p53-dependent apoptosis (Issaeva et al, Nat Med 2004) and is now being tested against mutant p53.

RITA's ability to restore mutant p53's tumor suppressing function for its effects on mutant p53-expressing cell lines were tested in HT29, SW480, A431 (His273 mutant) and H1299-175 (His175 mutant). Our results show that RITA suppressed growth of all tested mutantp53 cell lines and induced p53 target gene expression, e.g. Gadd45 and p21. RITA restored the wild-type p53 conformation of His273 mutant in SW480 cells. RITA treatment triggered apoptosis as assessed by FACS through induction of subG1 population in HT29 and SW480 cells. In addition to transactivation of target genes, RITA treatment resulted in repression of expression of p53 targets c-myc and IGF1R, two important oncogenes. The sensitivity to RITA differed in various cell lines, depending on the type of the mutant.

This new activity of RITA towards mutant p53 may open a way to targeted therapy against mutant p53-carrying tumors.

Poster 29

PHARMACOLOGICAL REACTIVATION OF P53 INDUCES POTENT DOWNREGULATION OF ONCOGENIC PATHWAYS

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Small molecule RITA has been found to induce apoptosis in tumour cells carrying wt p53 (Issaeva et al, Nature Medicine 2004). Recently we found that RITA also suppresses the growth of mutant p53 cells (see poster 28 by Ying Zhao). p53-dependent effect of RITA is due to its direct binding to the p53 N-terminus, presumably leading to a conformational change which disrupts binding of p53 inhibitors, p53 accumulation and induction of apoptosis of tumor cells. Microarray analysis showed that RITA treatment affects expression of a number of genes involved in cell proliferation and survival in a p53-dependent manner. Transcription of *IGFR-1*, *IRS-1, PIK3Calpha*, *PIK3Cbeta, EIF4E, Bcl-2, Mcl-1* and *c-Myc*, key players in tumorigenesis, was found to be downregulated after RITA treatment specifically in tumor cells expressing p53. We focused on downregulation of c-Myc oncogene by RITA. We found that downregulation of c-Myc occurred both on mRNA and on protein levels. Moreover, we found that inhibition of IGFR/PI3K/Akt signaling pathway after RITA treatment resulted in de-phosphorylation and modulation of activity of mTOR and GSK3β kinases, which leads to the inhibition of translation and proteasomal degradation of GSK3β targets β-catenin and c-Myc, respectively.

Given the extraordinary high frequency of p53 inactivation in tumors, it appears highly desirable to restore the tumor suppressor function of p53 as a strategy to combat cancer. Our results on small molecule RITA suggest that the rescue of p53 function by small molecules might have plejotropic effects on oncogenes critical for tumor cell survival. Thus, targeting p53 can provide a powerful new approach for the therapy of metastatic and chemo- and/or radiotherapy-resistant tumors.

Poster 30

ANALYSIS OF THE MOLECULAR MECHANSIMS OF ACTION OF THE NOVEL P53-REACTVATING COMPOUND RITA

*Martin Enge, **Elisabeth Hedström**, Wenjie Bao, MD PhD and Galina Selivanova, PhD*

p53 is a transcriptional factor which regulates the expression of genes involved in cell growth and cell death. p53-mediated induction of apoptosis, growth arrest and senescence in response to oncogene activation and DNA damage serves to eliminate pre-malignant and malignant cells. Numerous studies demonstrated that the loss of p53 function is causally linked to tumor development. The p53 pathway is inactivated in the majority, if not all, human tumors. Mutations in TP53 occur in about 50% of human tumors and in tumors which retain wild-type p53, p53 function is inactivated due to deregulation of its inhibitors, for example HDM2, a protein which binds to p53, inhibits its transcriptional activity and induces its degradation.
RITA is a low-molecular-weight compound which targets tumors with wild-type p53, which previously had been identified (Issaeva et al,, Nat Med, 2004). RITA binds directly to p53 and displaces its main destructor Mdm2, as well as inducing a conformation change of p53, disrupting its interaction with other inhibitory proteins, like for example iASPP. This is in contrast to the wtp53-reactivating compound Nutlin3a, which targets Mdm2, inhibiting its ability to degrade p53. Using microarray technology we have explored the effect of RITA on the transcriptome of isogenic cell-lines HCT116 with knocked-out (KO) or intact (WT) TP53. Using Quantative Real-Time PCR, it was confirmed that the response on tumor cells is p53-dependent. While the effects on KO cells are below detection limit, the effects on WT cells are profound. The known p53 targets induced by RITA are predominately apoptotic, in contrast to the very few pro-apoptotic genes induced by Nutlin3a. Apoptotic p53 targets in both the intrinsic and extrinsic pathway are activated - Bax, Caspase3, NOXA, PIG3, FAS, KILLER/DR5, and anti-apoptotic targets downregulated – Bcl2, IGF1R. In light of our recent data that RITA can reactivate at least some p53 mutants, these data become even more important, since RITA might be useful in the treatment of a variety of tumors.

Poster 31

RESCUING PROTEIN P53 TETRAMERIZATION BY DESIGNED CALIX[4]ARENES COMPOUNDS

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Mutations in the tetramerization domain of p53 (p53TD) -although not being very frequent- can also result in a non-functional protein, increasing the probability to develop tumours. Thus, molecules able to modulate the stability of the oligomeric structure can be helpful tools for anticancer therapy.
Based on p53TD structure, tetraguanidinium-calix[4]arene ligands were rationally designed. Biophysical studies by Nuclear Magnetic Resonance and Circular Dichroism show how these calix[4]arenes cause spectacular changes in the structure of wild-type p53TD as well as in some of its oncogenic mutants. Saturation Transfer Difference experiments allow determination of the ligand binding mode, which agrees with the rational design. Further thermodynamic characterization by microcalorimetry techniques reveals that the interaction with the calix[4]arenes stabilizes the mutated tetramerization domains up to the level of the wild-type protein. Moreover, cross-linking experiments confirm that the stabilized species is indeed the tetrameric structure.
All these results lead to conclude that the designed calix[4]arene ligands can successfully recover the tetramerization of mutants of p53TD associated to human cancers.

Poster 32

IN VIVO SAFETY AND THERAPEUTIC EFFICIENCY OF P53 TCR GENE TRANSFER IN A P53 HUMANIZED MOUSE (TUMOR) MODEL

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One barrier to the development of T cell-based immunotherapies has been the observation that presentation of tumor-associated antigens (TAA) at low copy numbers by normal cells and tissues results in a peripheral T cell repertoire that is devoid of efficient TAA-specific, tumor-reactive cytotoxic T cells (CTL), due to self-tolerance. We have reported that HLA-A2.1 (A2.1) transgenic (tg) mice can be used to bypass self-tolerance to universal human TAA and to generate efficient tumor-reactive CTL. We and others have shown that T cell antigen specificity can be reliably redirected by introducing T cell receptor (TCR) genes. We used A2.1 tg mice, in which the mouse CD8 molecule cannot efficiently interact with A2.1 to generate a high-affinity, CD8-independent TCR specific for a commonly expressed, tumor-associated CTL epitope (LLGRNSFEV) derived from the human p53 tumor suppressor protein. Retroviral expression of this CD8-independent, p53-specific TCR into T cells imparted the $CD8^+$ T lymphocytes with broad tumor-specific CTL activity and turned $CD4^+$ T cells into potent tumor-reactive, p53-specific T helper cells. To improve transduction and expression efficiencies in T lymphocytes and to affect the potentially harmful recombination of transduced and natural TCR chains within recipient T cells, we will follow a codon optimization strategy and insert point mutations to facilitate pairing of the transduced TCR chains. To entirely prevent interchain pairing between transduced and natural TCR chains, we will take advantage of a single chain p53-specific TCR construct. We will evaluate the immunobiology, safety, and therapeutic efficiency of existing and optimized p53 TCR engineered $CD8^+$ CTL and $CD4^+$ T helper cells in a humanized Hupki-A2.1 (Human p53 knock in) mouse model. Hupki-A2.1 and $p53^{-/-}$-A2.1 mouse embryonic fibroblasts transformed by expression of E1A and H-ras and tranduced with recombinant retroviral vectors encoding different p53 mutants were shown to overexpress p53 and to be effectively recognized by p53 TCR-transduced T cells. Tumor cells will be transferred to preconditioned Hupki-A2.1 recipients that will then receive syngeneic T lymphocytes reprogrammed by anti-p53 TCR gene transfer. We will assess p53 TCR^+ T cells not only for induction of tumor regression but also for a potential risk of developing severe adverse effects *in vivo*.

Poster 33

FUNCTIONAL ANALYSIS OF ASSOCIATIONS BETWEEN NOVEL ISOFORMS OF THE P53 TUMOUR SUPPRESSOR AND THE TRANSCRIPTIONAL COACTIVATOR P68

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Until recently it was thought that the p53 gene structure was relatively simple. However our recent work has shown that, as a result of an additional internal promoter and alternative splicing, the p53 gene can be transcribed to yield six different mRNAs, which can encode six different proteins. In a separate study, we discovered that the RNA helicase p68, which had previously been shown to play a role in pre-mRNA and alternative splicing, acts as a specific coactivator of p53.

p68 and splice variants of p53 have been seen to be differentially expressed in breast tumours, and different isoforms of p53 found to exhibit different transcriptional activities. This highlights several potential mechanisms by which p68 may affect p53 function. Different p53 isoforms bind differentially to p53 target gene promoters to regulate p53-dependent transcription and p68 itself appears to coactivate full-length p53 in a promoter-dependent manner. This suggests that, physiologically, p68 may influence promoter selectivity by the p53 isoforms, which could have profound effects on transcriptional activation of endogenous p53 variants in response to stress signals.

We have found that p68 preferentially binds p53 isoforms that contain the C-terminal domain and are currently investigating whether p68 preferentially acts as a coactivator for specific p53 isoforms thus affecting the pattern of p53 downstream target gene expression. We are also investigating potential correlations between expression of the p53 isoforms and p68 in a panel of breast tumours using immunohistochemistry and RT-PCR to determine whether p68 expression is associated with the presence or absence of particular p53 isoforms.

This work is supported by Tenovus Scotland.

Poster 34

Differential expression of tumour suppressor p53 protein isoforms: a new mechanism for genetic susceptibility?

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The tumour suppressor protein p53 is activated by genotoxic stress to regulate proliferation, apoptosis and DNA repair. This protein corresponds to a full-length product, termed TAp53 and is encoded for by the fully spliced p53 mRNA (FSp53). Its suppressor activities are counteracted by a N-terminal truncated isoform, ΔNp53, generated by an alternatively spliced mRNA retaining intron 2 (p53I2) [1].

Several common polymorphisms are found in the *TP53* gene including p53*PIN3*, a 16bp duplication in intron 3 (A1: non-duplicated allele; A2: duplicated allele), which is associated with an altered cancer risk [2]. In order to investigate whether the expression of p53 isoforms depends on the p53*PIN3* status, we analysed the different isoform expression profiles in lymphoblastoid cells either homozygous for the A1 or A2 p53*PIN3* alleles. At both the mRNA and protein level, A2 cells have a lower level of FSp53 expression and a slightly higher expression of p53I2 compared to A1 cells. We also demonstrated using a reverse transcriptase elongation assay that *G-quadruplex* structures overlap the p53*PIN3* sequence and that their topologies are dependent upon the p53*PIN3* status. Site-directed mutagenesis and treatment with TMPyP4, a cationic porphyrin, which modulates *G-quadruplex* formation, showed that disruption of the *G-quadruplexes* favours the retention of intron 2. Taken together, our results suggest that the *G-quadruplexes* favour production of FSp53 mRNA and that their different topologies induce a lower expression of FSp53 in A2 cells.

The polymorphic nature of the *G-quadruplexes* thus provides a mechanism for the genetic susceptibility associated with A2 p53*PIN3* allele.

[1] S. Courtois et *al*, ΔN-p53, a natural isoform of p53 lacking the first transactivation domain, counteracts growth suppression by wild-type p53. *Oncogene* 21, 6722-6728 (2002).
[2] F. Gemignani et *al*, A *TP53* polymorphism is associated with increases risk of colorectal cancer and with reduced levels of *TP53* mRNA. *Oncogene* 23, 1954-1956 (2004).

Poster 35

N-TERMINAL DELETED P53 ISOFORMS CONTROL EPITHELIAL TO AMOEBOID TRANSITION OF COLON CARCINOMA

VINOT Stéphanie, *BOURDON Jean-Christophe and ROUX Pierre*

Epithelial tumorigenesis is a multistep process on which the final step, the conversion of a primary static tumour into an invasive metastasis, is the most critical for patients. The mechanisms that allow cancer cells to acquire the properties of migration and invasiveness remain to be learned. In addition to its anti-proliferative activities, the tumor suppressor p53 can also modulate other cellular functions such as migration and invasion. Identification of the mechanisms by which the regulation of the expression of p53 modulates the final step of tumour progression will be important to understand how invasive cells arise. It has been suggested that an imbalance in the expression of some splice variants of p53 in tumours could favour invasion and metastasis. In our study we show that three N-terminal deleted splice variants of p53 can promote migration and invasion of colon carcinoma cells expressing wt p53. The expression of these isoforms induces a disruption of adherent junctions allowing cells to detach from the epithelium, then to migrate through amoeboid-like movements. This phenotype requires the activity of the Rho Kinase ROCK and is associated with an activation of RhoA which depends on a reciprocal balance of the expression of ECT2 and GEF-H1, two Guanine Exchange Factors (GEFs) of RhoA.
Our data demonstrate that the transcriptional regulation of p53 is an important determinant of tumour progression raising the intriguing possibility that p53 isoforms might control dissemination of metastatic cells.

Poster 36

DOES THE NONSENSE-MEDIATED mRNA DECAY MECHANISM PREVENT THE SYNTHESIS OF TRUNCATED PROTEINS FROM *TP53* AND OTHER BREAST CANCER PREDISPOSING GENES?

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The Nonsense-mediated mRNA decay (NMD) mechanism is an evolutionarily conserved process ensuring the degradation of transcripts carrying premature termination codon(s). NMD is believed to prevent the synthesis of truncated proteins that could be detrimental to the cell. However, although numerous studies have assessed the efficiency of this mechanism at the mRNA level, data are lacking in regards to whether NMD fulfils its expected goal at the protein level. In this study, we have investigated whether endogenous alleles of breast cancer predisposing genes carrying nonsense codons were able to produce detectable amounts of truncated proteins in lymphoblastoid cell lines.

Truncating mutations in the *TP53* gene are expected to both lead to a decrease in the amount of transcripts because of NMD and to an increase in the stability of the protein product, due to the absence of the ubiquitination domain. We therefore wondered whether truncated p53 proteins could be detected in cells carrying a germline truncating mutation.

The 770delT *TP53* mutation was analysed, along with twenty truncating *BRCA1* and the 1100delC *CHEK2* mutations. All the studied alleles triggered NMD, the amount of mutant transcript ranging from 16 to 63% that of the wild-type species. We found that BRCA1 and CHK2 truncated proteins could not be detected, even when NMD was inhibited, while the p53 protein encoded by the 770delT allele is as abundant as the wild-type protein. Removal of the C-terminal p53 domain leads to a stabilized mutant protein, whose abundance is markedly increased when NMD is inhibited. Conversely, the p53β isoform that results from the translation of the alternatively spliced *TP53* transcript containing an additional 133 bp exon derived from intron 9 is efficiently down-regulated by NMD, as it is barely detectable when NMD is not inhibited. The amount of *TP53* PTC+ transcripts produced by alternative splicing is much lower than the amount of PTC+ transcripts expressed from the 770delT allele, which may also explain why there is such a difference in the level of the corresponding proteins. Thus NMD seems to be an important factor in the regulation of the amount of p53β.

Poster 37

p300 STIMULATES HDM2-DEPENDENT DEGRADATION OF p53 BY FAVORING K48-POLYUBIQUITIN-CHAIN FORMATION

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Tightly controlled proteolysis of the oncosuppressive protein p53 by the proteasome is essential for its appropriate regulation. The degradation of p53 usually requires its prior ubiquitylation, which is mediated in particular by the ubiquitylation E3 Hdm2.

When analyzing Hdm2-dependent ubiquitylation of p53, we found that, in addition to promoting mono-ubiquitylation of p53 on multiple lysine residues (multi-mono-ubiquitylation), Hdm2 alone could mediate, both *in vitro* and *in vivo*, poly-ubiquitylation of p53 with ubiquitin (Ub) chains linked through distinct lysine residues of Ub (K6, K63 and K48). However, p53 proteasomal degradation is preferentially supported by K48-Ub-chains, indicating that (i) K6- and K63- Ub-chains built on p53 by Hdm2 might have specific roles in the control of p53 functions, not directly connected to its degradation, and (ii) the functions or fate of p53 might be modulated by factors regulating the type of Ub-chain conjugated to p53 by Hdm2.

Interestingly, Grossman et al. demonstrated in 2003 that p300 possesses, in addition of its acetyltransferase activity, an ubiquitylation activity that promotes Ub-chain conjugation to p53 by Hdm2. We showed later that this function of p300 was critical for p53 turnover, as siRNA directed against p300 strongly stabilized p53. This prompted us to test whether p300 could act in p53 ubiquitylation by modulating the type of Ub-chains added on p53 by Hdm2. We found that, both *in vitro* and *in vivo*, Hdm2 preferentially promoted conjugation on p53 of K48-Ub-chains in the presence of p300. Accordingly, the ubiquitylated forms of p53 made in the presence of both Hdm2 and p300 were most efficiently degraded than the ubiquitylated forms made by Hdm2 alone.

These results show that the stability of p53 can be modulated not only by the extent of its ubiquitylation, but also by the type of Ub-chains conjugated to it, and that an important function of p300 in p53 ubiquitylation is to alter Hdm2 activity to favor p53 degradation. A future challenge will be to understand the specific roles of the various types of Ub-chains conjugated to p53 in the control of the functions of this protein.

Poster 38

ROLES OF THE PROTEIN Rad23/hHR23 IN THE REGULATION OF p53 UBIQUITYLATION.

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The degradation of the oncosuppressive protein p53 is essential for its regulation. It usually requires its prior ubiquitylation, which can be mediated by several ubiquitylation E3s (also called Ubiquitin-ligases). In "normal" cells, the "ring-finger" E3 Hdm2 plays a central role in p53 ubiquitylation/degradation. In the case of infection by the human pappillomavirus type 16 and 18, involved in cervical cancer, Hdm2 functions on p53 are abolished and p53 is ubiquitylated by the E6/E6AP complex (formed by the association of the viral oncoprotein E6 and a cellular ubiquitylation E3, the protein E6AP).
The DNA repair protein Rad23/hHR23 is involved in p53 degradation. Interestingly, Glockzin et al. showed in 2003 that Rad23/hHR23 can inhibit Hdm2- but not E6/E6AP- mediated p53 ubiquitylation. They suggested this differential effect by the fact that Hdm2 promotes only mono-ubiquitylation of p53 on multiple lysine residues (multi-mono-ubiquitylation), while E6/E6AP promotes poly-ubiquitylation on p53 with ubiquitin chains, and that Rad23/hHR23 could inhibit only chain formation. However, we showed that, in addition to mono-ubiquitylation of p53, Hdm2 alone can also mediate, *in vitro*, poly-ubiquitylation of p53. Thus, the mechanism by which Rad23/hHR23 inhibits Hdm2-dependent ubiquitylation of p53 requires more investigation. We analyzed the domains and properties of the protein that are involved in its inhibitory functions. Preliminary results show that Rad23/hHR23 dimerization could be required for these functions, and that Rad23/hHR23 could enhance Hdm2 auto-ubiquitylation. Work in progress aims at clarifying these issues and at understanding the possible underlying mechanisms.

Poster 39

MTBP HAPLOINSUFFICIENCY IN MICE INCREASES TUMOR METASTASIS INDEPENDENT OF P53

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MTBP was previously isolated as an Mdm2 binding protein. MTBP was originally reported to induce p53-independent cell cycle arrest. In contrast, a recent study has found that MTBP overexpression can induce Mdm2 stabilization and subsequent p53 degradation, a process associated with increased cell proliferation. Thus, the function and cellular roles of MTBP remain unclear. To learn more about the *in vivo* physiological functions of MTBP, we generated mice with the *Mtbp* gene deleted. Homozygous disruption of *Mtbp* resulted in early embryonic lethality, which was not rescued by loss of *p53*. $Mtbp^{+/-}$ mice were not tumor-prone. When mice were sensitized for tumor development by *p53* heterozygosity, we found that the $Mtbp^{+/-}p53^{+/-}$ mice developed significantly more metastatic tumors (18.2%) as compared to $p53^{+/-}$ mice (2.6%). Given that $p53^{+/-}$ and $p53^{-/-}$ mice rarely develop metastatic tumors, this phenotype is striking and may be independent of p53. Results of *in vitro* migration and invasion assays support these *in vivo* findings. Down-modulation of MTBP in osteosarcoma cells derived from $p53^{+/-}$ mice resulted in increased invasiveness, and overexpression of MTBP in $Mtbp^{+/-}p53^{+/-}$ osteosarcoma cells, one of which shows LOH for the wild-type *p53* allele, inhibited the invasion potential. These results suggest that MTBP haploinsufficiency in mice increases tumor metastasis independent of p53. These results advance our understanding of the cellular roles of MTBP and raise the possibility that MTBP is a novel therapeutic target for metastasis.

Poster 40

MICROARRAY ANALYSIS TO ELUCIDATE INTEGRIN-DEPENDENT WT p53 INACTIVATION IN MALIGNANT MELANOMAS

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In three-dimensional (3D) conditions and *in vivo* the survival of malignant melanoma cells is dependent on the expression of integrin αv. We found that this survival is due to evasion of apoptosis by induction of the inactive conformation of p53 recognised by antibody pAb240. Thus, there must be an active mechanism utilized from the cell surface molecule integrin αv towards p53. To elucidate this mechanism we utilized genome wide RNA microarrays in order to compare gene expression profiles of melanoma cells with high *vs.* low integrin expression (M21 vs M21L) in 2D vs 3D. Since p53 inactivation by shRNA allows survival of M21L cells in 3D and *in vivo,* also gene expression patterns of M21 and M21L cells stably transfected with p53shRNA are analysed, allowing the identification of the p53 dependent pathways leading to cell death. Specific integrin dependent changes can be identified by comparison of expression profiles of M21 and M21L/p53shRNA cells in 3D. Among these genes we hope to find integrin-dependent modulators of p53 function. The two most promising of the candidate genes revealed by the microarray data are NFkB1 and HDMX.

Poster 41

HIGH-LEVEL EXPRESSION OF HUMAN TUMOUR SUPPRESSOR P53 IN THE METHYLOTROPHIC YEAST: PICHIA PASTORIS

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The human tumour suppressor P53 is a key protein involved in tumour suppression. P53 acts as a ''guardian of genome'' by regulating many target genes involved in cell cycle regulation, DNA repair and apoptosis. We report the P53 expression by the methylotrophic yeast Pichia pastoris using the methanol inducible AOX1 promoter. We have produced the rP53 in intracellular form as well as secreted using the Saccharomyces cerevisiae a-mating factor prepro-leader sequence in two genetic contexts of Pichia, Muts and Mut+. The intracellular P53 was successfully produced by Muts (KM71) as well as Mut+ (X33) strains, however, the secreted form was mainly observed in the Muts strain, despite a higher number of p53 copies integrated in the Mut+ strain. Interestingly, in Muts phenotype, the medium pH influences markedly the rP53 production since it was higher at pH 7 than 6.

Poster 42

EXPRESSION AND ACTIVITIES OF DELTANp63 AND DELTANp73 IN TUMOURS

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The *TP63* and *TP73* genes encode numerous isoforms, generated both by alternative splicing and by the use of two promoters (P1 for TA isoforms, P2 for DeltaN isoforms). TA isoforms act as transcription factors. They are able to activate the expression of genes involved in cell cycle arrest or apoptosis. DeltaN isoforms are devoid of the transactivation domain and exert a dominant negative effect on TA isoforms.

In tumours, the expression of TA and DeltaN isoforms is often unbalanced in favour of the latter. This unbalance could promote tumour initiation and/or progression.

We therefore studied the regulation of *TP63* P2 promoter. Several sites (a TATA box, three CAAT boxes and a SP1 site) were recently identified in this promoter (1). Furthermore, it has been demonstrated that p53 represses P2 promoter through the CAAT boxes (2). As P2 also contains a p53 responsive element (p53RE) and two sites for TCF/LEF transcription factors, we focused on the respective role of p53, DeltaNp63 and beta-catenin (the co-activator of TCF/LEF) in P2 regulation. We confirmed a repression of P2 promoter by p53 and an activation by DeltaNp63alpha itself, both independently of the p53RE. Moreover, we showed the activation of P2 promoter by the beta-catenin, a protein often altered in its activity in tumours, without any involvement of the TCF/LEF sites. Nevertheless, all these effects are direct, *i.e.* they require the binding of p53, DeltaNp63 and beta-catenin on P2, as confirmed by ChIP assay. The identification of the exact binding sites is currently under investigation.

We have also undertaken to characterize the role of the truncated isoforms of the p53 family in tumorigenesis. Preliminary results indicate that DeltaNp63 and DelatNp73 could be considered as actual oncogenes. This hypothesis will be discussed.

In conclusion, our results suggest an active role for DeltaNp63 and DeltaNp73 in tumour formation. For DeltaNp63, this role could be in association with beta-catenin. Indeed, DeltaNp63 overexpression was previously shown to stabilize and activate beta-catenin and here, we show that DeltaNp63 expression is activated by beta-catenin. Taken together, these data suggest the existence of a positive feedback loop between these two proteins, which could promote abnormal cell proliferation and tumorigenesis. This hypothesis is under investigation.

Poster 43

NOVEL POTENTIAL TARGETS of p53(R249S) IN HCC

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Involvement of the mutant p53 proteins in oncogenesis still is an ill-known subject, the mechanism responsible for this phenomenon remains to be uncovered. This study aims to explore how p53(R249S) mutant protein contributes to the oncogenic "Gain of Function" (GOF) phenomenon in the development of hepatocellular carcinoma (HCC) through transcriptomics network analysis using oligonucleotide arrays. Expression profiles of isogenic HCC cell lines, HepG2 (wt-p53) and HepG2-249 having additionally mutant p53(R249S), were compared. Microarray data analysis revealed a molecular signature consisting of 84 differentially regulated genes, showing that the expression of mutant p53(R249S) in HepG2 cells resulted in a distinct expression profile. HNF and fibrinogen pathway involvements were the most significant pathways in Cytoscape generated network with p53 protein. Although there was no significant increase in HNF1α, HNF4α, HNF6 expressions, their target genes were over expressed as quantified by real-time RT-PCR, comparing to wt HepG2 cell line. Our data indicates that involvement of the HNF targets at the transcription level and fibrinogen components at cell communication level can be considered as novel potential targets of p53(R249S) remains to be further investigated. In addition there were a number of novel genes, which may also participate, in mutant p53 oncogenic activities.

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