International Agency for Research on Cancer
Centre International de Recherche sur le Cancer

מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

2009 INTERNATIONAL P53 MARATHON

4th International Workshop on Mutant p53

"In vivo effects of mutant p53: experimental data, animal models, clinical consequences"

and by invitation only:

Active p53 Consortium

(26 March 2009)

Mutp53 Consortium

(30 March 2009)

An international meeting organized by the International Agency for Research on Cancer (IARC) and the European Community FP6 program

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

BACKGROUND AND SCOPE

The **4th International Workshop on Mutant p53,** to be held in Akko, Israel, will focus on "In vivo effects of mutant p53: experimental data, animal models, clinical consequences". 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 and 3rd workshops, the annual meetings of two research consortiums working on p53 (Mutp53 and Activep53) and funded by the European Community (FP6) will be held in conjunction with this international workshop.

The workshop will include lectures from top scientists in the field, short presentations selected from submitted abstracts and a poster session.

ORGANIZERS

Pierre Hainaut, International Agency for Research on Cancer, France
Magali Olivier, International Agency for Research on Cancer, France
Varda Rotter, Weizmann Institute of Science, Israel
Moshe Oren, Weizmann Institute of Science, Israel
Klas Wiman, Karolinska Institute, Sweden
Giovanni Blandino, Regina Elena National Cancer Institute, Italy

Administrative Assistants

Vivienne Laufer, Weizmann Institute of Science
Administrative Assistant to Prof. Varda Rotter
Department of Molecular Cell Biology
Weizmann Institute of Science
Rehovot 76100, ISRAEL
Tel: +972-8-934-4072
Fax: +972-8-934-4125
Email: vivienne.laufer@weizmann.ac.il

Michelle Wrisez, International Agency for Research on Cancer
Group of Molecular Carcinogenesis and Biomarkers
International Agency for Research on Cancer
150 Cours Albert Thomas
F-69372 Lyon CEDEX 08
France
Tel: 33 472 738 462
Fax: 33 472 738 322
Email: wrisez@iarc.fr

FUNDING

This meeting is supported by EU FP6.

LAND ARRANGEMENTS ORGANIZED BY:

Bob Kazmer
Target Conferences Ltd.
PO Box 29041, Tel Aviv 61290, Israel
Tel: +972 3 5175150, Fax: +972 3 5175155, e-mail: p53@targetconf.com

OLD ACRE DEVELOPMENT COMPANY LTD.

MARATHON PROGRAM

Wednesday, March 25, 2009
All day — Arrivals of participants of **Active p53 Consortium (Module 1)**
Late afternoon — Registration
Dinner & Overnight: Palm Beach Hotel

Thursday, March 26, 2009
All day — Sessions **Active p53 Consortium (Module 1)** – Palm Beach Hotel
Arrivals of participants of **Mutant p53 Workshop (Module 2)**
Late afternoon — Registration – Palm Beach Hotel
Evening — Get-together reception (Module 1 and Module 2)
Overnight — Palm Beach Hotel

Friday, March 27, 2009
All day: — Sessions **Mutant p53 Workshop (Module 2)** – Prisoners' Hall – Old City
Module 1 departures
Dinner & Overnight: Palm Beach Hotel

Saturday, March 28, 2009
Morning: — **Mutant p53 Workshop (Module 2)** - Old City
Morning sessions include Poster Session as well as presentation of Selected Short Papers
Afternoon — Tour of Old City of Akko
Evening — At leisure/dine around
Overnight — Palm Beach Hotel

Sunday, March 29, 2009
All day — **Mutant p53 Workshop (Module 2)** - Old City
Arrivals of participants - **Mutp53 Consortium (Module 3)**
Evening — Crusader Dinner for participants of Module 2 and Module 3 - **Mutant p53 Workshop and Mutp53 Consortium**
Overnight — Palm Beach Hotel

Monday, March 30, 2009
All day — Departures of participants of **Mutant p53 Workshop (Module 2)**
Mutp53 Consortium (Module 3) – Sessions (Palm Beach Hotel)
Dinner & Overnight: Palm Beach Hotel

Tuesday, March 31, 2009
All day — Departures **Mutp53 Consortium (Module 3)**

WORKSHOP SCIENTIFIC PROGRAM – Day 1 -

Friday, 27 March 2009

9h00 ***Introduction & Welcome***

Session 1 – p53 mutants and the p53 pathway

9h15 ***Curtis Harris,*** *Bethesda, USA* – (L1)

9h45 ***Moshe Oren,*** *Rehovot, Israel* – (L2)

10h15 ***Coffee break***

10h45 ***Wolfgang Deppert,*** *Hamburg, Germany* – (L3)

11h15 ***Genrich Tolstonog,*** *Hamburg, Germany* – (L4)

11h45 ***Varda Rotter,*** *Rehovot, Israel* – (L8)

12h15 ***Lunch***

14h00 ***Michelangelo Cordenonsi,*** *Padua, Italy* – (S7)

14h30 ***Carol Prives,*** *New York, USA* – (L7)

15h00 ***Giannino Del Sal,*** *Rome, Italy* – (L10)

15h30 ***Coffee break***

16h00 ***Mike Resnick,*** *Research Triangle Park, USA* – (L9)

16h30 ***Ygal Haupt,*** *Jerusalem, Israel* – (L11)

17h00 ***Giovanni Blandino,*** *Rome, Italy* – (L12)

17h30 ***- end of day -***

WORKSHOP SCIENTIFIC PROGRAM – Day 2-

Saturday, 28 March 2009

Session 2 – Short communications selected from abstracts

9h00	***Kanaga Sabapathy,*** *Singapore* – (S1)
9h15	***Gianluca Bossi,*** *Rome, Italy* – (S2)
9h30	***Job de Lange,*** *Leiden, The Netherlands* – (S3)
9h45	***Virginie Marcel,*** *Lyon, France* – (S4)
10h00	***Andrea Bisso,*** *Trieste, Italy* – (S5)

Session 3 – Posters

10h15	***Poster exhibition + Coffee break***
13h00	***Lunch + Poster exhibition***

Leisure time

Afternoon	***Tour in Akko***

WORKSHOP SCIENTIFIC PROGRAM – Day 3-

Sunday, 29 March 2009

Session 4 – From structures to drugs

9h00	***Andreas Joerger,*** *Cambridge, UK* – (L13)
9h30	***Zipi Shakked,*** *Rehovot, Israel* – (L14)
10h00	*Coffee break*
10h30	***Assaf Friedler,*** *Jerusalem, Israel* – (L15)
11h00	***Silvia Soddu,*** *Rome, Italy* – (L16)
11h30	***Galina Selivanova,*** *Stockholm, Sweden* – (L17)
12h00	***Klas Wiman,*** *Stockholm, Sweden* – (L18)
12h30	*Lunch*

Session 5 – Phenotypes and clinical impacts of mutations

14h15	***Monica Hollstein,*** *Leeds, UK* – (L19)
14h45	***Gigi Lozano,*** *Houston, USA* – (L20)
15h15	***Hein Te Riele,*** *Amsterdam, The Netherlands* – (L21)
15h45	*Coffee break*
16h15	***Jeffrey Myers,*** *Houston, USA* – (L22)
16h45	***Magali Olivier,*** *Lyon, France* – (L23)
17h15	***Pierre Hainaut,*** *Lyon, France* – (L24)
17h45	***-end of scientific sessions-***

Evening – Crusader Dinner

- End of workshop -

LECTURES (L) and SHORT COMMUNICATIONS (S)

Lecture 1

TELOMERES "COMING UP SHORT": p53-DEPENDENT SENESCENCE

Izumi Horikawa[1], Kaori Fujita[1], Abdul Martin[1], Qin Yang[2], Borivoj Vojtesek[3], Jean-Christophe Bourdon[4], David P. Lane[4], and ***Curtis C. Harris[1]***

[1]Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD, USA; [2]Department of Radiation Oncology, Washington University in St. Louis, St. Louis, MO, USA; [3]Department of Clinical and Experimental Pathology, Masaryk Memorial Cancer Institute, Brno, Czech Republic, [4]Department of Surgery and Molecular Oncology, University of Dundee/Inserm U858, Dundee, Scotland, United Kingdom

The p53 pathway is an intrinsic monitor and response pathway of telomeric attrition involved in cellular aging and senescence. Cellular senescence is tumor suppressive that can be activated by p53 in cancer cells. We are currently studying the molecular mechanisms of cellular senescence in normal human cells and the role of the telometric multiprotein complex, shelterin, that includes TRF2 and POT1. Our ongoing studies have revealed that p53 regulates both specific microRNAs and TRF2 expression as endogenous mechanisms of replicative senescence. In addition, POT1 isoforms are functionally diverse in both maintaining telomeric integrity and preventing p53-dependent senescence induced by telomeric shortening.

Lecture 2

INVOLVEMENT OF p53 IN TUMOR-STROMA INTERACTIONS

Neta Moskovits[1], Jair Bar[1,3], Yoseph Addadi[2], Michal Neeman[2], Varda Rotter[1] and ***Moshe Oren[1]***

Departments of [1]Molecular Cell Biology and [2]Biological Regulation, The Weizmann Institute of Science, Rehovot 76100, Israel
[3]Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Israel

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

Lecture 3

TUMOR INITIATING CELLS IN ONCOGENE-INDUCED MOUSE MAMMARY CARCINOMAS: MOLECULAR PATHWAYS AND PRACTICAL APPLICATIONS

C. Heinlein[1], C. Mänz[1], S. Babu[1], F. Wegwitz[1], K. Gruner[1], B. Otto[2], T. Streichert[2], B. Ylstra[3], L. Baumbusch[4], G. Tolstonog[1], ***W. Deppert[1]***

[1]Heinrich-Pette-Institute, Hamburg, Germany
[2]University Clinics Eppendorf (UKE), Dept. of Clinical Chemistry, Hamburg, Germany
[3]VUMC Cancer Center Amsterdam, The Netherlands
[4]Biomedical Research Group, Dep. of Informatics, University of Oslo, Norway

We have constructed and characterized transgenic mice expressing the SV40 early gene region under the control of the WAP-promoter, which allows induction of oncogene expression specifically in the mature mammary gland (WAP-T mice). WAP-T mice were crossed with WAP-mutp53 mice (mice transgenic for mutant *p53* minigenes, also under control of the WAP-promoter) to assess the effect of *p53* mutations on top of SV40 early gene expression, which already mimics a variety of genetic lesions observed in human mammary carcinogenesis. We found that co-expression of mutp53^{R270H} leads to an increase in tumor incidence, an aggravated tumor phenotype, and strongly enhanced metastasis.

The transgenic mice allow the parallel analysis of the effects of loss of wtp53 function (WAP-T mice) and the postulated mutp53 gain of function (WAP-T x WAP-mutp53 mice). Concerning the latter aspect we were able to demonstrate that, at least at the level of p53 target gene expression, the gain of function effects of co-expressed mutp53 are not due to a dominant-negative effect of mutp53 over a possible residual wtp53 activity. The most important wtp53 loss effect is the activation of c-Met expression by SV40 large T (LT), due to abrogation of *c-met* repression by wtp53. Our data indicate that expression of c-Met not only is essential for mammary carcinoma initiation, but also important for tumor progression, as molecular analyses of the SV40-induced tumors revealed that the *c-met* gene is amplified and strongly expressed in nearly all undifferentiated tumors.

We identified multipotent mammary epithelial stem/progenitor cells as the tumor initiating cells in our transgenic mice. These tumor initiating cells can be recovered from tumors and, after orthotopic transplantation, form tumors in syngeneic mice with a phenotype indistinguishable from endogenously growing mammary carcinomas. From a bi-transgenic WAP-T x WAP-mutp53 tumor, we established a primary culture of SV40-transformed mammary epithelial cells with stem cell and tumor initiating characteristics, which can be serially passaged *in vitro* (GeTo cells). Upon orthotopic transplantation into syngeneic mice as low as 10^2 GeTo cells form mammary carcinomas that closely resemble primary tumors. GeTo cells thus provide a convenient tool for the analysis of molecular and biological properties of tumor initiating cells, of tumor cell dissemination and metastasis, and are suitable for preclinical testing of drugs intended to discriminate between effects on tumor cell growth and metastatic behavior.

Lecture 4

HIDDEN FORCE OF P53: DIFFERENTIATION AND ASYMMETRIC DIVISION OF PROGENITOR CELLS

Mark-Andreas Kluth, Rudolph Reimer, Heinrich Hohenberg, Wolfgang Deppert, ***Genrich Tolstonog***

Heinrich-Pette-Institute, Hamburg, Germany

Tumorigenesis is often accompanied by inactivation or alteration of p53 function. As tumorigenesis also involves the conversion of normal stem/progenitor cells to cancer initiating cells, we explored the potential role of p53 in controlling proliferation and differentiation of stem/progenitor cells. We used mouse F9 teratocarcinoma cells, as they constitute a well studied model system for embryo-derived cancer cell differentiation. In culture, F9 cells comprise a heterogeneous population of stem and committed progenitor cells, which upon induction differentiate into extraembryonal parietal and visceral endoderm. Enrichment of stem/progenitor cells by sorting F9 cells for high activity of the stem cell marker aldehyde dehydrogenase using the Aldefluor® reagent ($ALDE^+$ cells) revealed that $ALDE^+$ cells also contain high levels of p53, suggesting that p53 might play a role in controlling stemness. In support, shRNA mediated knock-down of p53 in F9 cells resulted in inductor-independent cell differentiation, while p53 overexpression inhibited F9 cell differentiation by inductors. An important physiological property of stem cells is their ability for asymmetric cell division, which experimentally can be detected by BrdU-label retention. We were able to show that asymmetrically dividing, BrdU-label retaining F9 cells express significantly higher levels of p53 than the rest of the F9 cell population, thereby providing phenomenological evidence for a role of p53 in the regulation of asymmentric cell division. Thereby our data establish a link between the loss or alteration of p53 function and the transformation of normal stem/progenitor cells to cancer cells, as loss of the tight control over cell fate regulation in such cells leads to their expansion via symmetric cell divisions.

Lecture 5

EXPLORING MDMX ONCOGENIC FUNCTION IN HUMANS AND IN CONDITIONAL TRANSGENIC MICE

Anja Böhnke[1,#], Jessika Wynendeale[2,#], Sarah De Clercq[2], Agnniezka Gembarska[2], Eleonora Leucci[3], Irina Lambertz[2], Nadja Sbrzesny[1], Dana Kubitza[1], Anja Wolf[1], Elise Gradhand[4], Katarina Balschun[4], Oliver Kuß[5], Christoph Thomssen[6], Steffen Hauptmann[4], Anders Lund[3], Frank Bartel[1], ***Jean-Christophe Marine[2]***

[1] Junior Research Group, Fyaculty of Medicine, University of Halle-Wittenberg, 06097 Halle/Saale, Germany
[2] Laboratory for Molecular Cancer Biology, VIB-UGent, B9052 Ghent, Belgium
[3] Biotech Research and Innovation Centre, University of Copenhagen, DK-2200 Copenhagen, Denmark
[4] Institute of Pathology, Faculty of Medicine, University of Halle-Wittenberg, 06097 Halle/Saale, Germany
[5] Institute for Medical Epidemiology, Biometrics and Computer Science, Faculty of Medicine, University of Halle-Wittenberg, 06097 Halle/Saale, Germany
[6] Department of Gynecology, Martin-Luther-University Halle-Wittenberg, Halle, Germany

MDM4 (or *MDMX*) is amplified and over-expressed in various human cancers. Evidence suggests that MDM4 contributes to tumor formation and/or progression via its ability to bind and inactivate the p53 tumor suppressor protein. To further study the oncogenic properties of Mdmx (and Mdm2) *in vivo*, we have generated Mdmx and Mdm2 conditional transgenic mouse lines. These conditional transgenic mice are being used to study the contribution of Mdmx and Mdm2 to cancer formation and/or progression. Data from these studies will be presented.

Importantly, even modest decrease in Mdm4 levels compromises p53-dependent tumor suppression in mice, suggesting that genetic variants of MDM4 might affect carcinogenesis in humans. We will also report a single nucleotide polymorphism (SNP34091) in the 3'-UTR of MDM4 that creates a new illegitimate target site for has-miR-191, a microRNA (miR) that is highly expressed in normal and tumor tissues. This causes down-regulation of *MDM4* expression and hence significantly delays ovarian carcinoma progression and increases sensitivity to chemotherapy. This is the first demonstration of the clinical relevance of *MDMX* genetic alterations in human cancer and that mutations creating miR-target sites are important modifiers of cancer risk.

Cancelled

Lecture 6

GAIN OF FUNCTION MUTANT p53 EXPRESSION IN CANCER CELLS ALTER MOBILITY, INVASIVE PHENOTYPE AND CHEMOSENSITIVITY OF CANCER CELLS: IMPLICATION OF ACTIVATION OF AXL AND ERK PATHWAYS.

***Deb, S*[1]*, Sankala, H.*[5]*, Graves, P.*[2,5]*., Nesheiwat, I.*[1]*,Hafiz, Ali, Vaughan, C.*[1]*, Yeudall, A.*[2]*; Dumur, C.*[3]*, Garrett, C.*[2,3]*, and Deb, S.P.*[1,2]*.*

Department of Biochemistry and Molecular Biology[1], and Massey Cancer Center[2], Department of Pathology[3], Philips Institute [4], Department of Radiation Oncology[5], Virginia Commonwealth University, Richmond, VA 23298

Cancer cells with p53 mutations, in general, grow more aggressively than those with wild-type p53, and cells expressing p53 mutants show reduced sensitivity towards chemotherapeutic drugs. Here, using a number of breast and lung cancer cell lines with mutated p53 we show that lowering the level of p53 is accompanied by a significant increase of chemosensitivity. Using RNAi against p73 and p63 we demonstrate that the gain of function observed by the expression of mutant p53 in reducing chemsensitivity cannot be completely explained by mutant p53's ability to neutralize p73 and p63. 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. One of them is the protein tyrosine kinase receptor Axl, which is involved in cell growth and survival. We show that mutant p53-induced chemoresistance is at least in part dependent on Axl. We also show that mutant p53 expressing cells leads to enhanced phosphorylation of Erk2, perhaps indicative of mutant p53's involvement in receptor protein tyrosine kinase pathway. Furthermore, gain-of-function p53 mutants enhanced cell migration on extracellular matrix proteins, which was reversed by transactivation-deficient mutants or p53-targeted RNA interference. Finally, in cells with either engineered or naturally-occurring p53 mutants, RNA interference revealed that enhanced migration was dependent upon expression of NF*κ*B2, previously reported as a target of gain-of-function mutant p53. The data support a role for gain-of-function mutant p53 proteins in altered cell-matrix interactions and enhanced cell motility, in part through transactivation of NF-*κ*B2.

Cancelled

Lecture 7

THE ROLE OF LYSINE RESIDUES IN p53 TRANSCRIPTIONAL ACTIVITY AND TARGET GENE SELECTION.

***Carol Prives**, Rachel Beckerman, Melissa Mattia, Andrew Zupnick, Wei Gu[1], Susan Keezer[2] and Oleg Laptenko.*

Department of Biological Sciences and [1]Institute for Cancer Genetics, Columbia University. New York, N.Y. 10027 [2] Cell Signaling Technology, Inc., Danvers, Massachusetts 01923.

It is well established that wild-type p53 is extensively modified on several lysine residues. We have been studying two aspects of p53 lysine (K) modification that are relevant to its function as a sequence specific transactivator. Two projects will be discussed. Although several groups have investigated the mode by which p53 interacts with naked DNA fragments, few have examined the interaction of p53 with DNA that is wrapped around a histone octamer. We have generated mononucleosomes consisting of different extents of DNA in which a strong p53 binding site (RE) was placed at varying distances from the end of the DNA and examined their interaction with either unmodified or acetylated p53. Our data imply that in the text of chromatin, unmodified p53 is likely to bind to its Res when they are present (either transiently or permanently) within naked DNA such as within the linker region between nucleosomes. Nevertheless, our data also indicate that acetylation of p53 may increase the scope and availability of sites within nucleosomal DNA. Relevantly, mutation of the C-terminal lysines to arginines produces a version of p53 that, when expressed at physiological levels in H1299 cells, is partially impaired in activating some of its target genes. We also generated H1299 cell lines that express inducible p53 variants in which the only two lysines that are found within the p53 tetramerization domain, K351 and K357 were changed either to arginine (R, to conserve charge), or to glutamine (Q, to neutralize charge and possibly mimic acetylation. P53 (K351R/K357R) displayed similar DNA binding, transcriptional abilities and cell cycle arrest did wild-type p53. On the other hand, p53 (351Q/357Q) was selectively transcriptionally impaired and could not induce cell cycle arrest even though its full expression led to even higher levels of p21 than wild-type p53. Interestingly, p53 (351Q/357Q) displayed defective activation of and binding to both cyclin G1 and miR34a promoters. Further, our preliminary data obtained with modification-specific antibodies, indicate that one or both tetramerization domain lysines are in fact basally acetylated *in vivo* and such acetylation is reduced when cells are stressed by DNA damage. This is the first indication that loss of acetylation of certain p53 residues is required for activation of the full
transcriptional repertoire required for cell cycle arrest.

Lecture 8

IDENTIFICATION AND CHARATERIZATION OF p53-ASSOCIATED "GENE SIGNATURES" INVOLVED IN CELLULAR TRANSFORMATION

Varda Rotter, *Yossi Buganim, Ira Kogan, Hilla Solomon, Eyal Kalo, Ran Brosh, Alina Molchadsky, Shalom Madar, Ido Goldshtein, Osnat, Ezra, Noa Rivlin, Perry Stambolsky, Rachel Sarig,and Naomi Goldfinger.*

Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot, Israel

As it is well accepted that malignant transformation is a stepwise process, it is challenging to discover which of these steps involve the p53 protein. To that end we have established several in-vitro transformation models in which normal cells were transformed into cancer cells by well-controlled genetic alterations. In our experiments, we have immortalized various human primary cells of lung and prostate origin and engineered into them several defined cancer associated genetic alterations. These included inactivation of the p53 tumor suppressors by several methods, over-expression of mutant p53, over-expression of the Ras oncogene and various combinations of these modifications. As a result we have obtained transformed cells that are capable of developing into tumors in mice, suggesting that the in-vitro developed system represents authentic model of cancer development. To characterize the gene networks that are associated with the defined malignant steps, we have used a genome-wide approach, which permits the identification of gene signatures that are associated with the individual steps of malignant transformation.
In general it seems that the clusters that we have identified and analyzed using this in-vitro model agree with specific steps of transformation and thus may serve as specific hallmark signatures of tumorigenesis.

Milyavsky, et al, Cancer Research 63, 7147-7157.
Milyavsky et al., Cancer Res. 65:4530-43.
Tabach et al., Molecular System Biology, 2005 Oct 18.
Milyavsky et al., Cancer Cell, 11:133-146.
Brosh, et al., Mol. Sys. Biol. (in press).
Molchadsky et al., PLoS ONE. 2008;3(11):e3707. Epub 2008 Nov 12

Lecture 9

p53 MUTANTS IN AN EXPANDING p53 UNIVERSE

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

[1]National institute of Environmental Health Sciences (NIEHS), NIH, Research Triangle Park, NC 27709, USA; [2]University of Trento, Italy

The p53 master regulatory transcription factor can influence many cellular stress responses through direct regulation of hundreds of target genes. The p53 transcription targets are mainly regulated through response elements that are generally considered to be variants of a 20 base consensus sequence. Given the broad importance of p53 as a master regulator, we have investigated the depth and diversity of its regulated network. Diversity within the p53 transcriptional network can arise from a matrix of changes that include the target response elements, levels of p53 expression, as well as transcriptional changes associated with functional mutants that retain transcriptional capability. Using a combination of model yeast and human cell systems, we have established that noncanonical sequences composed of 1/2 and 3/4 site response elements can be targeted for p53 transactivation, as found for the Flt1 and the RAP80 genes. Importantly, estrogen receptor (ER) acting *in-cis* from a nearby target response element can greatly enhance the p53 response at a noncanonical site. We have found that the depth of genomic influence of p53 is much larger than originally anticipated due to the "expanding universe" of genes directly targeted by p53 and that functional cancer-associated p53 mutants can change the form of that universe.

Lecture 10

DISSECTING THE GAIN OF FUNCTION (GOF) OF MUTANT p53: THE CRITICAL ROLE OF THE PROLYL ISOMERASE PIN1.

Javier Girardini, Marco Napoli, Alessandra Rustighi, Carolina Marotta and ***Giannino Del Sal***

Molecular Oncology Unit. Laboratorio Nazionale CIB (LNCIB). Padriciano 99,Trieste, Italy.

A vast body of evidence from clinical and basic research studies has demonstrated that in response to a wide variety of stress signals the tumor suppressor p53 and its signal transduction pathway act as an essential barrier in preventing cancer onset and development. p53 exerts its role mainly at the transcriptional level and its timely activation/inactivation depends on a complex repertoire of post-translational modifications and interactions with proteins. In this context our laboratory has provided, over the years, key answers by identifying new p53 post-translational modifications and co-factors, in particularly by dissecting the essential role of the prolyl isomerase Pin1. Upon stress signals Pin1 acts in a phosphorylation-dependent manner to produce conformational changes required for the full transcriptional activation of both p53 and p73 (Zacchi et al. 2002 Mantovani et al. 2004, Mantovani et al. 2007). However p53 is frequently mutated in tumor tissues and the majority of these mutations are missense, leading to the expression of full-length mutant proteins. Several lines of evidence have established that a subset of these p53 mutants gain new oncogenic functions and concur to the development of invasive and metastatic phenotypes (gain of function, GOF). However the mechanisms responsible for mutant p53 function remain still elusive. Many signalling pathways may impinge on p53 as a consequence of different stress stimuli and some of them may also contribute to modulate mutant p53 function. We and others have shown that Pin1 is over-expressed in breast cancers (Rustighi et al 2009) and binds to mutant p53. Therefore it is conceivable that Pin1 could also contribute to the gain of function of mutant p53. Evidences supporting the role of Pin1 in potentiating mut p53 activity will be presented.
Overall, our results are consistent with the hypothesis that Pin1, under physiological conditions, may act as a fine tuner of the tumor suppressor function of wt p53, however, during the transformation process, it can change its role becoming a dangerous amplifier of mutant p53 oncogenic activity.

Zacchi et al., Nature, 2002.
Mantovani et al., Mol Cell, 2004.
Mantovani et al., Nat Struct Mol Biol, 2007.
Rustighi et al., Nat Cell Biol, 2009.

Lecture 11

REGULATION OF WT AND MUTANT p53 BY PML: IMPLICATIONS FOR CELL TRANSFORMATION

Sue Haupt[1], Osnat Alsheich-Bartok[2], Silvia di Agostino[3], Igal Louria-Hayon[2], Inbal Mizrahi[2], Yaara Levav-Cohen[2], Tamar Grossman[2], Stefan Muller[4], Martin Scheffner[5], Giovanni Blandino[3], and ***Ygal Haupt***[1,2].

[1]The Peter MacCallum Cancer Centre, St. Andrew's Place, East Melbourne 3002, Victoria, Australia. [2]Lautenberg Center, The Hebrew University, Jerusalem Israel. [3]Translational Oncogenomics, Regina Elena Cancer Institute, Rome, Italy. [4]Max Planck Institute of Biochemistry, Martinsried, Germany. [5]University of Konstanz, Konstanz, Germany.

The p53 tumour suppressor is tightly regulated under normal and stress conditions. An important regulator of p53 under stress conditions is the promeylocytic leukemia (PML) tumour suppressor. PML is essential for the formation of PML nuclear bodies (NBs), which have been implicated in the regulation of growth inhibition, senescence and apoptosis. We and others have previously shown that PML protects p53 from Mdm2. We have previously shown that this is achieved by facilitating the phosphorylation of p53 on Ser20. More recently we have shown that PML also regulates the phosphorylation of p53 on Thr18 by CK1 in response to DNA damage. In this study we have extended our study of PML regulatory role of p53 to the mutant form of p53. Certain mutations in p53 not only result in a loss of wild type p53 activity, but can also lead to a gain of new oncogenic properties. Here we show that PML interacts with mutant p53 and enhances its transcriptional activity. Unexpectedly, PML is required for the proliferation and colony formation of cancer cells bearing mutant p53. Down-regulation of PML expression inhibits the growth of mutant p53 expressing cancer cells. Our results suggest that the tumour suppression function of PML depends on the status of p53. In the context of mutant p53, PML enhances its cancer promoting activities.

While much has been revealed about the regulatory role of PML, the regulation of PML and the factors governing this regulation are incompletely understood. Here we demonstrate that a catalytically active form of the mammalian E3 ligase E6AP (HPV E6 Associated Protein) acts to reduce the half-life of the PML protein by promoting its degradation in the ubqiquitin proteasome system. E6AP mediates the ubiquitination of PML in vivo and in vitro. E6AP and PML interact at physiological levels and co-localize in PML-NBs. Importantly, PML protein expression and number and intensity of PML-NBs is elevated in multiple cells deficient for E6AP. Our results identify E6AP as an important regulator of PML and PML-NBs.

Lecture 12

MUTANT p53 TRIGGERS AN ONCOGENIC AUTOREGULATORY FEEDBACK LOOP.

DiAgostino S.^, Fontemaggi G.^, Dell'Orso S.^, Biagioni F.^, Donzelli S.^, Fausti F.^, Shay T.°, Muti P.^, Domany E.°, Strano S.^, ***Blandino G****.^.*

^Molecular Medicine Department, Regina Elena Cancer Institute, Rome, Italy.
°Weizmann Institute of Science, Rehovot, Israel.

Growing evidence has shown that gain of function mutant p53 proteins exert their oncogenic activities through the transcriptional modulation of specific sets of genes. Here we are going to present experimental evidence on the biochemical and functional characterization of two novel mutant p53 target genes ID4 and Plk2. The latter have been identified through a microarray analysis of H1299 cells expressing ponasterone-inducible mutant p53His175 protein.
ID4 gene: ID4 (inhibitor of DNA binding 4) is a member of a family of proteins (ID1-ID4), which function as dominant-negative regulators of basic helix-loop-helix transcription factors. Growing evidence links ID proteins to cell proliferation, differentiation and tumorigenesis. siRNA-mediated depletion of mutant p53 protein severely impairs ID4 expression in proliferating tumor cells. The protein complex mutp53/E2F1 assembles on a specific region of ID4 promoter and positively controls ID4 expression. The net biological output of ID4 transactivation is the increase of the angiogenic potential of mutant p53-expressing cancer cells. This occurs through the binding and stabilization by ID4 of mRNAs encoding pro-angiogenic factors. These findings highlight the transcriptional axis mutantp53/E2F1/ID4 as a yet undefined molecular mechanism underlying tumor neoangiogenesis.
Plk2 gene: Here we have identified the Polo-like kinase-2 (Snk/Plk2) as a novel mutant p53 transcriptional target gene after DNA damage. We show that Plk2 protein interacts with mutant forms of p53 and this leads to phosphorylation of mutant p53, giving rise to a transcriptional feedback loop involving mutant p53 and Plk2 in response to DNA damage. Notably, knocking-down of Plk2 expression renders mutant p53 cells more responsive conventional anticancer treatments. This occurs through increased apoptosis down-regulation of cyclin A, cyclin B1, cdk1 and cdc25C expression, as for knocking down of mutant p53 expression.
Data elucidating further what above-mentioned will be presented.

Short communication 1

"KNOCK-IN" MOUSE MODELS TO UNDERSTAND P53 FUNCTIONS IN VIVO

Kanaga Sabapathy

National Cancer Center Singapore, Singapore

Mutations in p53 result in the loss of its ability to block abnormal cell growth, and not surprisingly, almost 50% of all human cancers contain a p53 mutation. Mutations are often found clustered as hot spot mutations, which are the most common mutations found in human tumors. Besides mutations, p53 can be functionally inactivated in cancers by other means, such as rapid degradation, nuclear exclusion and defects in upstream/downstream signaling cascades, regulated primarily through post-translational events such as phosphorylation, acetylation, etc. Thus, a systematic evaluation of the properties of the hot spot mutants in tumor formation and the evaluation of phosphorylation in regulating p53 function in vivo would be of paramount importance in understanding the biology of p53 and in the treatment of cancer.

Mouse models have proved to be very useful in understanding the in vivo properties of tumor-suppressor proteins. The "first generation" p53-/- mice that contain large deletions of the p53 gene develop tumors at high frequencies, providing essential evidence for the importance of p53 as a tumor suppressor. However, essential information on the role of specific phosphorylation sites and hot-spot mutations in the regulation of p53 function in vivo is just beginning to be understood, with the generation of the next generation of specific "knock-in" mice. In this respect, we have generated two "knock-in" mouse strains: one targeting the serine 312 residue (equivalent of serine 315 in human) and the other targeting the arginine 246 residue (equivalent of serine 249 in human). The effects of these mutations on normal development, spontaneous tumorigenecity and specificity, as well as the biochemical functions of the mutant proteins will be discussed. In addition, both irradiation-induced and oncogene-induced tumorigenecity have been evaluated in conjunction with the "knock-in" mutations, of which data will be presented.

Short communication 2

UPREGULATION OF MYTOGEN ACTIVATED PROTEIN KINASE KINASE 3 BY MUTANT p53 IS REQUIRED FOR GAIN OF FUNCTION MUTATIONS

Gianluca Bossi, *Aymone Gurtner, Giulia Piaggio, Giuseppe Starace and Ada Sacchi.*

Department of Experimental Oncology, Regina Elena Cancer Institute, Via delle Messi D'Oro 156, 00158 Rome, Italy.

The p53 gene is the most frequent target for genetic alterations in human cancer. Missense point mutations, often within the conserved DNA binding domain of the protein, is the most prevalent type of p53 alteration. Differently from other tumor suppressor genes, mutant p53 typically maintain the full-length altered protein, frequently present in grossly elevated levels. This has led to the conjecture that the mutant p53 have acquired novel oncogenic functions, through which contributes actively to cancer development and cancer progression. Accordingly to this, studies have shown that mutation of the p53 gene confers additional function (GOF) that can be exerted in a variety of ways, ranging from enhanced proliferation in culture, increased tumorigenicity *in vivo*, and enhanced resistance to a variety of anti-cancer drugs commonly used in clinical practice. Moreover we and others showed that depletion of mutp53 by RNA interference renders cancer cells more sensitive to DNA damaging chemotherapeutics agents in vitro, and reduces tumor malignancy both *in vitro* and *in vivo* environments. Furthermore, we reported in tumor growth-delay analysis by inducible RNA interference in HT29 xenograft model, that mutant p53 protein depletion not only impact on tumor growth but also led to modifications of tumors architecture, with consistent reduction in stromal invasion and tumor angiogenesis. To identify mechanisms through which mutant p53 acquires gain of function activity, we validated microarray data by real time PCR analysis. Our analysis performed both *in vitro* (HT29, SKBR3 cells) and in HT29-xenograft tumors confirmed Mitogen-Activated Protein Kinase-Kinase 3 (*MAP2K3*) as mutant p53 target gene. *MAP2K3* is a specific activator of p38 Mitogen Activating Protein Kinase (p38MAPK). The p38MAPK protein through activation/modulation of specific target genes plays relevant roles in cell proliferation, cell survival and cell differentiation, suggesting that *MAP2K3* modulation might contribute in some mutant p53 GOF cellular responses. Our study shown that MAP2K3 modulation occurred also at protein level, indeed a consistent reduction of MAP2K3 protein was observed in all tested cells, upon mutant p53 depletion. Moreover we observed that reduced MAP2K3 protein reduces phospho-active ATF2 transcription factor, downstream of p38MAPK protein. This data suggested that mutant p53 through MAP2K3 regulates the activation of the entire p38MAPK signal cascade, at least for ATF2 activation. Analogous results were confirmed in other two cell lines MDA-MB468 and MDA-MB231 carrying p53 mutations H273 and K280 respectively. According to this, we observed that specific RNA interference of MAP2K3 protein reproduced similar results in all analyzed cells, confirming MAP2K3 as the major activator of p38MAPK in our tested cancer cells. Chromatin-IP analysis showed that mutant p53 binds to the *MAP2K3* promoter, and Luc assays showed that transcriptional activity of *MAP2K3* promoter was reduced upon mutp53 depletion, suggesting a direct *MAP2K3* modulation mediated by mutant p53. Furthermore consistent with mutant p53 GOF functions we observed that, *MAP2K3* depletion reduces cell growth in all tested human cancer cell lines, meanwhile exogenous *MAP2K3* over-expression increase cell proliferation and cell survival. Together, these findings suggest that one of the mechanisms by which mutant p53 exerts gain of function activity is through the up-regulation of *MAP2K3* expression.

Short communication 3

ONCOGENIC FUNCTIONS OF HDMX IN RETINOBLASTOMA AND UVEAL MELANOMA

Job de Lange *and Aart G. Jochemsen*

Leiden University Medical Centre, Dep. of Molecular Cell Biology, PO Box 9600, 2300 RC Leiden, Netherlands

Mdmx has been shown to act as a crucial inhibitor of p53 activity during embryonic development, and its unwanted overexpression may drive oncogenic transformation. We hope to further elucidate the role of Hdmx in tumorigenesis, and possibly find ways to combat tumors that possess wt-p53 and high levels of Hdmx. To that end, we make use of two eye tumor models, retinoblastoma and uveal melanoma.
Retinoblastomas frequently show Hdmx amplification and overexpression, and p53 mutations hardly occur. By using stable retroviral transductions of Human Embryonic Retinoblasts (HER), a cell culture model for retinoblastoma has been generated to investigate the individual role of putative oncogenes and tumor suppressors. Main focus is the question whether Hdmx overexpression can fully circumvent the need for p53 loss in these cells in developing tumors. The contribution of other cell cycle regulators, like Rb and $p16^{INK4A}$ is also investigated. Using different combinations of retroviral infections, twelve cell lines (with shRb, shp16, or control vector; H-RasV12 or control vector; shp53 or HA-Hdmx) have been established. We have applied several growth assays and *in vivo* tumor growth to monitor the transformed state of the established cell lines. Initial *in vitro* tests indicate that Hdmx overexpression induces growth rate comparable to shp53. H-RasV12, shRb and shp16 all enhance growth. Knocking down p53 completely prevents a Nutlin-3 response, whereas Hdmx overexpression does not, indicating that high Hdmx expression is not sufficient to render transformed cells resistant to Nutlin-3 treatment.
Uveal melanomas, usually lethal once metastasized, usually express wt-p53. Therefore, increased expression of Hdm2 and/or Hdmx is likely to play a role in inactivation of the p53 pathway in these tumors. A number of uveal melanoma cell lines have been used to analyze the impact of Hdmx and Hdm2 expression on proliferation and sensitivity towards drugs. Initial results show that in several cell lines, Hdmx knockdown leads to strong growth inhibition, which is at least partially p53-independent. Furthermore, reducing Hdmx levels enhances Nutlin-3 induced apoptosis. These results suggest a role of Hdmx in uveal melanoma that stretches beyond p53 inhibition. The tumorigenic potential of these cells lines is investigated upon injection of these cells into the anterior eye chamber of immuno-compromised mice.
In conclusion, Hdmx appears to have an important growth promoting role in retinoblastoma and uveal melanoma.

Short communication 4

TP53*PIN3* POLYMORPHISM: GENETIC MODIFIER OF GERMLINE *TP53* MUTATION IN LI-FRAUMENI SYNDROME.

***V. Marcel*[1]**, *El. Palmero[1], P. Falagan-Lotsch[1], G. Martel-Planche[1], P. Ashton-Prolla[2], M. Olivier[1], P. Hainaut[1] and MI. Achatz[3].*

[1]Molecular Carcinogenesis and Biomarkers Group, International Agency for Research on Cancer, Lyon Cedex 08, France; [2]Department of Genetics, Federal University of Rio Grande do Sul and Medical Genetcis Service, Hospital de Clínicas de Porto Alegre, Brazil ; [3]Department of Oncogenetics, Hospital A.C. Camargo, São Paulo, Brazil.

The Li-Fraumeni and Li-Fraumeni-*like* syndromes (LFS and LFL) are characterized by development of multiple and early-onset cancers with heterogeneous tumour pattern. These syndromes are associated with germline *TP53* mutation. However, differences in *TP53* mutation type and functional impact are not sufficient to explain the individual and familial diversity of tumour pattern. Recently, it has been shown that p53*R72P* and *MDM2* SNP309 polymorphisms are genetic modifiers of germline *TP53* mutation.

In this study, we investigated whether the common TP53*PIN3* polymorphism, located in intron 3 (A1: the most frequent non-duplicated allele; A2: the variant 16 bp duplicated allele), may have modifying effect on germline *TP53* mutation in 135 Brazilian LFS/LFL patients. We demonstrated that the TP53*PIN3* polymorphism is associated with a difference of 19 years in the mean age at first cancer diagnosis in *TP53* germline mutation carriers, the A1 allele being associated with an early cancer development (A1A1: 28.0 (±17.7) years; A1A2: 47.0 (±9.2) years; p=0.01). In addition, only A1A1 *TP53* mutation carriers develop their first cancer before the age of 35 years.

The comparison of impact on age at first diagnosis between p53*R72P*, *MDM2* SNP309 and TP53*PIN3* suggest that the modifying effect associated with TP53*PIN3* is the strongest one in this Brazilians series (p53*R72P*: RR *versus* RR+RP, difference of 8.3 years; *MDM2* SNP309: TG+GG *versus* TT, difference of 12.5 years).

The intronic TP53*PIN3* polymorphism exhibits the strongest modifying effect on germline *TP53* mutation identified to date and may provide a powerful genetic marker in the delineation of cancer screening and intervention guidelines in the LFS/LFL patients.

Short communication 5

PEPTIDE APTAMERS TARGETING MUTANT p53 INDUCE APOPTOSIS IN TUMOR CELLS

*Elisa Guida[1,4], **Andrea Bisso**[1,2], Cristina Fenollar-Ferrer[3], Marco Napoli[1,2], Claudio Anselmi[3], Javier E. Girardini[1,2], Paolo Carloni[3] and Giannino Del Sal[1,2]*

[1] LNCIB, Area Science Park, Padriciano 99, 34012 Trieste
[2] Dipartimento di Biochimica, Biofisica, e Chimica delle Macromolecole (BBCM), University of Trieste, via Giorgieri 1, 34128 Trieste, Italy
[3] SISSA, International School for Advanced Studies and CNR-INFM-DEMOCRITOS Modeling Center for Research in Atomistic Simulation, Via Beirut 2-4, 34014 Trieste
[4] present address: Instituto de Neurociencias de Alicante CSIC-UMH - Unit of Developmental Neurobiology - Av. Ramon y Cajal s/n, 03550 San Juan de Alicante (Spain)

Most tumors are characterized by impairment of the p53 pathway, either by mutations of the p53 gene (TP53), or by deregulation of other components of this pathway. The importance of p53 function as a tumor suppressor is underlined by the fact that at least 50% of human tumors carry mutations in TP53. Interestingly, the majority of the TP53 alterations are missense mutations leading to the expression of full length point mutants that not only have lost wild-type tumor-suppressive functions, but also paradoxically accumulate to high levels in tumor cells and actively collaborate with tumor progression through the acquisition of novel properties. Indeed, p53 mutants were shown to favor tumorigenesis and have been associated with enhanced tumorigenic potential in mice, increased proliferation, and resistance to drugs commonly used in anticancer therapy. In addition, mouse models have provided evidence for a role of mutant p53 in altering tumor spectrum and increasing the metastatic potential of tumor cells. Given that mutant p53 actively contributes to tumorigenesis and is highly expressed in tumor cells, it represents an attractive target for the development of selective anticancer therapies.
The interactions and activities of selected proteins can be specifically modulated by binding of peptide aptamers (PAs). PAs consist of a short variable peptide domain usually expressed in the context of a protein scaffold and they are selected from high-complexity libraries to specifically target proteins and modulate their activity.
We recently reported (Guida et al, 2008) the identification and characterization of short PAs able to bind to different p53 mutants, whereas not to wt p53. The identified PAs specifically interfere with mutant p53 transcriptional functions and are able to trigger apoptosis selectively in tumor cells expressing mutant p53. Of note, ablation of endogenous mutant p53 almost completely abolish PA-induced cell death, confirming the requirement of mutant p53 for PAs pro-apoptotic functions. Moreover, by molecular modeling based on the available structural information, we defined a region on mutant p53 that is predicted to be recognized by PAs.
These PAs could provide a potential strategy to inhibit the oncogenic functions of mutant p53 and improve mutant p53-targeted cancer therapies.

Short communication 6

INTERACTIONS OF MUTANT p53 WITH NON-B STRUCTURES IN GENOMIC DNA AND CONSEQUENCES FOR GENE EXPRESSION *IN VIVO*

Marie Brázdová,1; *Timo Quante,2; Vlastimil Tichý,1; Lucie Navrátilová,1; Christine Loscher,2; Matej Lexa,3; Tomáš Martínek,4; Genrich Tolstonong,2; Miroslav Fojta,1; Emil Palecek,1 and Wolfgang Deppert, 2.*

1 Institute of Biophysics Academy of Sciences CR v.v.i., Královopolská 135, 612 65 Brno, Czech Republic
2 Heinrich-Pette-Institute, Martinistrasse 52, 20251 Hamburg, Germany
3 Masaryk University, Faculty of Informatics, Kotlářská 68a, 60200 Brno, Czech Republic
4 University of Technology, Faculty of Information Technology, Božetěchova 2, 61266 Brno, Czech Republic

Non-B DNA structures were for long time associated with regulatory regions of genes, recognition sites for proteins controlling transcription, and likely other cellular processes such as replication and recombination.
In our analyses we combined molecular and computational approaches to understand the mutant p53 function in specific gene regulation via binding to non-canonical DNA structures in chromatin DNA. We used two glioblastoma cell lines U251 (R273H) and Onda11 (R273C) expressing endogenous mutp53 proteins to isolate natural mutant p53 binding sites (mutp53BS) by genome-wide ChIP-cloning. In our computational work, we developed tools for rapid identification of DNA sequences (among the isolated mutp53BS) tending to form non-B structures (hairpins and triplex DNA) as well as for mapping their genomic locations. These sites are frequently localized in the regulatory first introns of genes and are enriched in repetitive elements. Potential to form triplex and cruciform structures was predicted by developed computational tools and detected by enzymatic and chemical probing. The role of topological status of studied DNA and p53 domains in mutp53BS recognition was investigated by recombinant mutp53 proteins *in vitro* and reporter assays *in vivo*.
Our data suggest that the mutant p53 proteins bind selectively non-B DNA structures not only *in vitro* but also with functional consequences *in vivo*.

This work was supported by EC (FP6-mutp53, QLGA-CT-2001-52001 and MERG-6-CT-2005-014875), MEYSCR (1K04119 and LC06035), GA ASCR (IAA500040701, 204/06/P369 and 204/08/1560).

Cancelled

S7 (Lecture)

A MUTANT-p53/SMAD COMPLEX OPPOSES p63 TO EMPOWER TGF-BETA INDUCED METASTASIS.

***Michelangelo Cordenonsi*[1],** *Maddalena Adorno[1], Marco Montagner[1], Allan Balmain[2] and* Stefano Piccolo[1]

[1]Department of Medical Biotechnologies, Section of Histology and Embryology, University of Padua, viale Colombo 3, 35126 Padua, Italy
[2]Cancer Research Institute, University of California San Francisco, 2340 Sutter Street, San Francisco, CA 94115, USA

TGFβ ligands act as tumor suppressors in early stage tumors but are paradoxically diverted into potent prometastatic factors in advanced cancers. The molecular nature of this switch remains enigmatic. Here we show the workings of a previously undescribed pathway by which TGFβ fosters malignant progression. Mutant-p53 is required to empower TGFβ-dependent cell migration, invasion and metastasis. Mechanistically, TGFβ acts in concert with oncogenic Ras and mutant-p53 to induce the assembly of a mutant-p53/p63 protein complex in which Smads serve as essential platforms. Within this ternary complex, the anti-metastatic properties of p63 are antagonized. Two novel metastasis suppressor genes downstream of this pathway are associated with metastasis risk in a large cohort of breast cancer patients. Thus, two common oncogenic lesions, mutant-p53 and Ras, selected in early neoplasms to promote growth and survival, also prefigure a cellular set-up with particular metastasis proclivity by TGFβ-dependent inhibition of p63 function.

Lecture 13

p53: FROM STRUCTURE TO DRUG DISCOVERY

Andreas Joerger

MRC Centre for Protein Engineering, Hills Road, Cambridge, CB2 0QH, UK

The mutation Y220C occurs in about 70,000 to 80,000 new cases of cancer per annum. The mutant is highly destabilized and denatures rapidly at body temperature. Our structural studies revealed that the mutation forms a surface cavity that appears a prime target for small molecules to bind to and stabilise the protein. *In silico* design identified a series of molecules that might bind in the cavity. We screened those and the second and third generation derivatives and found small compounds of drug-like properties that raise the melting temperature of the mutants and restore its activity.

Lecture 14

THE UNEXPECTED DIVERSITY IN DNA RECOGNITION MODES BY p53

Zippi Shakked

Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel

p53 binds as a tetramer to DNA targets consisting of two decameric half-sites separated by a variable number of base pairs. To gain insight into the mechanism by which p53 recognize in a sequence-specific manner a wide range of DNA sequences, we elucidated the high-resolution crystal structures of several complexes between the core domain of human p53 and various DNA targets. In all complexes, four p53 molecules associate with two DNA half-sites to form a dimer of dimers stabilized by protein-DNA and protein-protein interactions. The 3-D architecture of the complexes and their stability depend on the specific sequence of the DNA half-site and the spacer between half-sites. In particular, p53/DNA complexes with contiguous half-sites reveal an unexpected geometry of the A/T base-pair doublet at the center of each half-site. This geometry affects the local conformation of the DNA double helix and the relative arrangement of the four p53 molecules on the DNA, resulting in enhanced protein-DNA and protein-protein interactions and hence in higher binding affinity of the corresponding complexes.

Lecture 15

MAPPING THE INTERACTION NETWORK OF ASPP2: INSIGHTS INTO P53-MEDIATED APOPTOSIS

***Assaf Friedler**, Chen Katz, Shahar Rotem, Hadar Benyamini*
Institute of Chemistry, The Hebrew University of Jerusalem, Israel

ASPP2 is a pro-apoptotic protein that specifically stimulates the p53-mediated apoptotic response. The C-terminal Ankyrin and SH3 domains in ASPP2 (ASPP2 Ank-SH3) mediate its interactions with numerous apoptosis-related proteins such as Bcl-2 and NFkB, but the physiological role of these interactions is yet unclear. In addition, ASPP2 contains a proline-rich domain whose function is yet unknown. Understanding the molecular basis of the interaction network of ASPP2 is crucial in order to elucidate its mechanism of action and regulation. This may serve as basis for the design of anti-cancer drugs that stimulate apoptosis of cancer cells by interfering with these interactions. We are using a combination of biophysical, biochemical and computational methods to elucidate the molecular mechanism of the ASPP2 interactions. Our results show that:
(i) ASPP2 binds the three anti-apoptotis Bcl-2 family members Bcl2, BclX and BclW. Using peptide arrays we discovered the interaction sites in both proteins and revealed selectivity between the Bcl2 proteins in ASPP2 binding: The BH4 domain of Bcl2 is the major ASPP2 – binding site in the Bcl2 family members. We constructed a docking model for the complexes between the ASPP2 Ank-SH3 and Bcl2 proteins. Based on our results we propose a mechanism in which ASPP2 induces apoptosis by inhibiting functional sites of the anti apoptotic Bcl-2 proteins [1]. **(ii)** We conducted a computational study of protein docking and molecular dynamics to obtain a structural model of the complex between ASPP2 Ank-SH3 and NFkB p65. We found that ASPP2 Ank-SH3 binds two sites in NFκB p65, which also mediate the binding of NFκB to its natural inhibitor IκB, which also contains ankyrin repeats. Alignment of the ankyrin repeats of ASPP2 Ank-SH3 and IκB revealed highly similar interfaces at their binding sites to NFκB. Protein docking of ASPP2 Ank-SH3 and NFκB p65, as well as molecular dynamics simulations of the proteins, provided structural models of the complex. Our results show that ASPP2 Ank-SH3 binds NFκB p65 in a similar manner to its natural inhibitor IκB, suggesting a possible novel role for ASPP2 as an NFκB inhibitor [2].
(iii) We found that the proline-rich domain of ASPP2 (ASPP2-Pro) is natively unfolded, and interacts intramolecularly with the Ank-SH3 domains. We have characterized in detail the molecular mechanism of this interaction and identified the sites in both domains that mediate the intramolecular interaction. Using peptide arrays derived from known ASPP2 binding proteins we found that only the Ank-SH3 domains, but not the proline-rich domain, mediate the interactions of ASPP2 with its partner proteins. An ASPP2 construct containing both the Ank-SH3 and proline-rich domains (ASPP2 Pro-Ank-SH3) bound a peptide derived from NFkB weaker than ASPP2 Ank-SH3 bound this peptide. Moreover, the NFkB peptide and a peptide from ASPP2 Pro competed for binding ASPP2 Ank-SH3, indicating an overlapping binding site. Based on our results, we suggest a model for the role of the intramolecular interaction between the ASPP2 domains in regulating the intermolecular interactions of the protein [3,4]. Our results provide insight into how ASPP2 interacts with its partner proteins and how these interactions are regulated by an intramolecular domain-domain interaction in the protein. This lays the basis for the design of lead compounds that stimulate ASPP2-mediated apoptosis.

References:

1. Katz C. , Benyamini H. , Rotem S. , Lebendiker M. , Danieli T. , Dines, M. , Bronner, V. , Bravman, T. , Rudiger S. and Friedler A. (2008): Proc. Natl. Acad. Sci. USA, 105(34):12277-82
2. Benyamini H. , Leonov H. , Rotem S. , Katz C., Arkin I.T. and Friedler A. (2009) submitted for publication
3. Rotem S. , Katz C. and Friedler A. (2007): Biochem. Soc. Transac., 35, 966-69
4. Rotem S. , Katz C. , Benyamini H. , Lebendiker M. , Veprintsev D. , Rudiger S. , Danieli T. , and Friedler A. (2008): J Biol Chem 283(27):18990-9

Lecture 16

HIPK2 REGULATION BY MDM2 DETERMINES TUMOR CELL RESPONSE TO THE P53-REACTIVATING DRUGS NUTLIN-3 AND RITA

Cinzia Rinaldo,[1] Andrea Prodosmo,[1] Francesca Siepi,[1] Alice Moncada,[1] Ada Sacchi,[1] Galina Selivanova,[2] and ***Silvia Soddu[1]***

[1]Department of Experimental Oncology, Molecular Oncogenesis Laboratory, Regina Elena Cancer Institute, 00158 Rome, Italy, [2]Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, 17177 Stockholm, Sweden.

In the past few years, much effort has been devoted to show the single-target specificity of non-genotoxic, p53 reactivating compounds. However, the divergent biological responses induced by the different compounds, even in the same tumor cells, demand additional mechanistic insights, whose knowledge may lead to improved drug design or selection of the most potent drug combinations. To address the molecular mechanism underlying induction of mitotic arrest versus clinically more desirable apoptosis, we took advantage of two MDM2 antagonists, Nutlin-3 and RITA, that respectively produce these two outcomes. We show that, along with p53 reactivation, the proapoptotic p53-activator HIPK2 is degraded by MDM2 in Nutlin-3 treated cells, but activated by reduced MDM2 levels in RITA treated ones. Gain- and loss-of-function experiments revealed the functional significance of MDM2-mediated HIPK2 regulation in cell decision between mitotic arrest and apoptosis in both types of p53 reactivation. These data indicate that strategies of p53 reactivation by MDM2 inhibition should also take into consideration MDM2 targets other than p53, such as the apoptosis activator HIPK2.

Lecture 17

TARGETING p53 BY SMALL MOLECULES TO COMBAT CANCER

Martin Enge, Vera Grinkevich, Wenjie Bao, Elisabeth Hedstrom, Fedor Nikulenkov, Yao Shi, and ***Galina Selivanova***

Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 17177, Stockholm, Sweden

Reconstitution of p53 function might open new therapeutic avenues against cancer. We have previously identified the p53-reactivating compound RITA in a cell-based screen (1). We undertook an unbiased examination of the molecular mechanism of the activity of RITA by analyzing the gene expression profiles induced by RITA using DNA microarrays (2). Our study revealed high specificity of RITA in targeting p53. We detected major changes in gene expression in wild-type p53 expressing cells upon RITA treatment including the differential regulation of known p53 target genes. In contrast, no changes in gene expression in p53-negative HCT116 *TP53-/-* cells were detected. Pathway analysis revealed preferential induction of p53 apoptosis pathway, in line with apoptosis being the major response to RITA in various cell lines. We uncovered a previously unrecognized role of MDM2 by showing that MDM2 released from p53 by RITA promotes degradation of p21 and the p53 cofactor hnRNPK, required for p21 transcription. Functional studies revealed MDM2-dependent inhibition of p21 as a key switch regulating cell fate decisions upon p53 reactivation (2). Further, we found a potent inhibition of crucial oncogenes by p53 upon reactivation with small molecule RITA *in vitro* and *in vivo*. p53 unleashes transcriptional repression of anti-apoptotic proteins Mcl-1 Bcl-2, MAP4, and survivin, blocks Akt pathway on several levels and downregulates c-Myc, cyclin E and β-catenin (3). p53 ablates c-Myc expression via several mechanisms at transcriptional and posttranscriptional level. Inhibition of oncogenes by p53 reduces the cell's ability to buffer pro-apoptotic signals and elicits robust apoptosis (3). Our results emphasize the utility of targeting wild type p53 protein itself as a promising approach for anticancer therapy. The major efforts for identification of MDM-2 inhibitors are focused towards MDM-2-binding molecules. We show that targeting p53 could be a valuable strategy for prevention of its degradation by HDM-2 and for re-activation of p53 in tumors. Unexpected mechanism of p53 activation by RITA might help to open novel avenues for further research aimed at p53 reactivation.

1. Issaeva, N., Bozko, P., Enge, M., Protopopova, M., Verhoef, L. G., Masucci, M., Pramanik, A., and Selivanova, G. (2004). Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nature Medicine 10*, 1321-1328.
2. Enge M, Bao W, Hedström E, Jackson SP, Moumen A, and Selivanova G.et al., MDM2-Dependent downregulation of p21 and hnRNP K provides a switch between apoptosis and growth arrest induced by pharmacologically activated p53. (2009) *Cancer Cell*, March 3.
3. Grinkevich V, Nikulenkov F, Shi Y, Bao W, Enge M, Maljukova A, Sangfelt O, Gluch A, Kel A, and Selivanova G, *Cancer Cell, in press.* Ablation of key oncogenic pathways by RITA-reactivated p53 is required for efficient apoptosis of tumor cells

Lecture 18

TARGETING MUTANT p53 AS A STRATEGY FOR CANCER TREATMENT: FROM HIT COMPOUND TO CLINICAL TRIAL

Klas G. Wiman

Dept. of Oncology-Pathology, Cancer Center Karolinska (CCK), Karolinska Institutet, SE-171 76 Stockholm, Sweden

Restoration of wild type p53 expression triggers cell death and rapid elimination of tumors in vivo. Several mutant p53-targeting small molecules have been identified, raising possibilities for the development of more efficient anticancer drugs. PRIMA-1 restores wild type conformation to mutant p53, induces apoptosis in human tumor cells, and inhibits xenograft tumor growth in vivo. We also showed that PRIMA-1 synergizes with chemotherapeutic drugs in cultured tumor cells and in vivo in mice. PRIMA-1 induces the proapoptotic p53 target genes Bax, PUMA and Noxa, 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 limited number of genes in mutant p53-expressing cells, including genes that regulate cell cycle progression and apoptosis. PRIMA-1 binds to mutant p53 in vitro and in living cells. A better understanding of the cellular events triggered by PRIMA-1 as well as the molecular mechanisms of mutant p53 reactivation should facilitate the design of more potent and specific mutant p53-targeting drugs for cancer therapy.

Lecture 19

ORIGINS AND CONSEQUENCES OF P53 MUTATIONS: LESSONS FROM A MOUSE MODEL WITH HUMANISED p53.

M. Hollstein

Leeds Institute of Genetics, Health & Therapeutics, University of Leeds, UK

Explanted murine embryonic fibroblasts senesce after several cell divisions when grown under normal cell culture conditions, If the ARF/p53 pathway is disabled, however, the cells are able to replicate indefinitely in vitro. Replicative senescence thus exerts an efficient selection pressure for outgrowth of cells with a mutated p53 gene. We have used senesence bypass of embryonic fibroblasts from mice with a humanised p53 gene (Hupki, for human p53 knock-in) to generate mutation spectra in the polyproline and DNA-binding domains of the human p53 gene. Exposure of pre-senescent Hupki fibroblasts with cancer causing agents, followed by DNA sequencing of immortalised clones derived from the treated cultures generates mutation patterns that reflect the mutagenic properties of the carcinogens. The experimentally generated mutations can be compared directly with the mutations found in human tumours of patients exposed to the agent in question. We have used this approach recently to show that the p53 mutation signature in Hupki cells exposed to aristolochic acid supports the claim that this plant carcinogen has a direct role in the aetiology of Balkan endemic nephropathy-associated urothelial cancers.

Over 200 immortalised p53 mutant Hupki cell lines have been isolated to date. Comparison of these mutations with those recorded in human tumours shows there is high concordance between mutations that allow senescence bypass in vitro and mutations that arise in vivo during human tumourigenesis.

Lecture 20

MUTANT p53 ACTIVITIES

Guillermina Lozano, Shunbin Xiong, Tamara Terzian, and Young-Ah Suh.

Department of Genetics, The University of Texas M. D. Anderson Cancer Center. 1515 Holcombe Blvd., Houston, TX 77025

p53 mutants exhibit gain-of-function activities *in vitro* and *in vivo*. We have generated mice with the p53R172H mutation equivalent to the human p53R175H hotspot mutation. Studies of heterozygous mutant mice revealed a metastatic phenotype in contrast to $p53^{+/-}$ mice. Loss of *Mdm2* (which encodes a negative inhibitor of p53) or *p16* (which encodes a cell cycle inhibitor) led to stabilization of mutant p53, a shorter survival, and an enhanced metastatic phenotype. Expression array analyses between osteosarcomas from *p53*-null mice (non-metastatic) and *p53*-mutant (metastatic) mice have revealed differences. *In vitro* assays measuring motility and invasion show functional consequences of down modulating some of these genes.

Lecture 21

OLIGONUCLEOTIDE-DIRECTED GENE MODIFICATION TO STUDY p53 MUTANTS

Marleen Dekker, Sandra de Vries, Camiel Wielders, ***Hein te Riele***

The Netherlands Cancer Institute, Division of Molecular Biology, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Email: h.t.riele@nki.nl

Gene targeting by single-stranded oligodeoxyribonucleotides (ssODN) is emerging as a powerful tool for introducting subtle gene modifications in mouse embryonic stem cells (ESCs) and the generation of mutant mice. The method (briefly referred to as "oligo targeting") entails the introduction into ESCs cells of short (±38 nucleotides) ssODN that are identical to a sequence in the target gene except for the few nucleotides to be modified. The mechanism of oligo targeting is not clear yet. However, we have found that the efficacy is strongly suppressed by the cell's DNA mismatch repair (MMR) system. This is due to the formation of mismatches that arise when the oligonucleotide matches to its complementary sequence in the genomic target. MMR removes such mismatches thereby aborting gene modification. For effective oligo targeting, we therefore have to disable the MMR pathway, which we achieve by transiently expressing a short-hairpin RNA molecule that reduces the level of the central MMR protein MSH2 for a brief period. This provides sufficient time for the oligonucleotide to perform its job after which full MMR capacity is restored. Suppressing MMR activity enhances the efficacy of oligo targeting up to 500-fold, reaching frequencies of $\pm 10^{-5}$-10^{-4} in mouse ESCs.
We have used oligo targeting to generate six mutant *p53* alleles in mouse ESCs: V154F, C173F, H176Y, R245Q, R245W and C272F (codon numbering of mouse *p53*). These cells have been injected into blastocysts to generate chimeric mice and to set up p53 mutant mouse lines.
Furthermore, in all mutant ESC lines, we have disrupted the *p53* wild-type allele by using a *p53* gene targeting vector containing a floxed STOP cassette. We will report here a preliminary analysis of $p53^{Mut/+}$ and $p53^{Mut/-}$ ES cell lines, in particular their response to DNA damage.

Lecture 22

SERINE SUBSTITUION OF PROLINE AT CODON 151 OF TP53 LEADS TO ANOIKIS RESISTANCE, SOFT AGAR GROWTH, AND ENHANCED IN VIVO TUMORIGENICITY OF HEAD AND NECK CANCER CELLS AND A PUTATIVE CONFORMATIONAL CHANGE WITHIN A HYDROPHOBIC SUBDOMAIN OF THE P53 PROTEIN

Tong-Xin Xie, Ge Zhou, Mei Zhao, Guillermina Lozano, Shunbing Xiong, Richard Brennan, ***Jeffrey N. Myers****.*

The University of Texas M.D. Anderson Cancer Center

Mutation of the Tumor suppressor TP53 occurs in over half the cases of squamous cell carcinoma of the head and neck (SCCHN) and is associated with a decreased survival for those patients treated surgically for this disease. During our studies of anoikis resistance of SCCHN cells, we identified a cell line, Tu138, that is highly resistant to detachment induced cell death or anoikis, and found that this cell line has a serine substitution of proline at codon 151 of TP53. Subcloning this mutation and expressing it in anoikis-sensitive cells has demonstrated that TP53 P151S, can lead to loss of transcription of Puma and Noxa and mediate anoikis-resistance, soft agar growth and enhance tumorigenicity in an orthotopic nude mouse model of oral cancer. These data have been corroborated with studies using siP53 to decrease the expression of TP53 P151S in the Tu138 cell line. Using an *in silico* approach based on the crystal structure of the p53 protein, we have hypothesized that the substitution of serine for proline creates a cavity in a hydrophobic pocket, that leads to loss of van der Waals contacts and is thermodynamically unfavorable due to the placement of a polar atom, the hydroxyl group of Ser151 within a hydrophobic region. Although this hydroxyl group could hydrogen bond to the hydroxyl group of nearby residue Thr150, it is predicted that the P151S substitution would result in a conformational change that would be deleterious to the function of the protein and its thermal stability. Structure function studies of this and related mutants are ongoing to test this hypothesis.

Lecture 23

CROSS-TALKS BETWEEN p53 AND ESTROGEN RECEPTORα PATHWAYS IN BREAST CANCER CELLS AND THEIR IMPACT ON ANTI-HORMONE AND CHEMOTHERAPY TREATMENTS

Lynnette Fernandez-Cuesta, Suresh Anaganti, Pierre Hainaut, ***Magali Olivier***

Molecular Carcinogenesis and Biomarkers Group, International Agency for Research on Cancer, Lyon, France

TP53 gene mutations are associated with poor prognosis in breast cancer. In a previous study including close to 1800 breast cancer cases, we found that *TP53* status was an independent prognostic factor and we observed an interaction between progesterone receptor (PR) status and *TP53* status where PR status lost its prognostic value in TP53 mutated tumors. These observations suggested that *TP53* status may influence anti-hormonal treatment or that p53 may interfere with the estrogen receptor (ER) pathway.
While it is well established that p53 participates in the anti-proliferative and apoptotic activities of several anti-cancer drugs that damage DNA, which may explain its prognostic and predictive values in breast cancer, its possible role in the response to anti-hormonal treatment is not known. Several lines of evidence from molecular studies suggest that p53 and ERα can influence each other activities through mechanisms involving protein-protein interactions. However the exact nature and biological impacts of these interactions remain to be determined.
To address these questions, we have investigated how cross-talks between p53 and ERa pathways may affect cells responses to anti-hormone and chemotherapy treatments. Estrogen-dependent breast cancer cell lines with different *TP53* status were subjected to anti-hormone and chemotherapy treatments and p53-dependent and estrogen-dependent biological and molecular responses were monitored. We show that p53 and ERα affect each other expression in unstressed cells and that estrogen deprivation compromises DNA-damage induced p53 responses by reducing p53 protein steady-state levels. Interestingly, p53 status influenced cell response to the SERM tamoxifen (TAM) but not to the pure anti-estrogen fulvestrant (ICI). Although the exact molecular mechanisms involved in these effects remain to be elucidated, these results provide some new insights for understanding the prognostic value of *TP53* mutations in breast cancer.

Lecture 24

TP53 MUTATIONS IN LUNG CANCER: CLINICAL EVIDENCE FOR GAIN-OF-FUNCTION.

Pierre Hainaut

International Agency for Research on Cancer, Lyon, France

The frequency, temporal occurrence, functional impact and clinical significance of TP53 mutation differ from one cancer type to another. In breast cancer, studies by us and others have demonstrated that mutations were independent and highly significant markers of prognosis. Studies on Non-Small Cell Lung Cancers (NSCLC) demonstrate a completely different pattern of clinical associations. TP53 mutations are common in NSCLC. They occur at very high frequency in lung cancers from smokers but are also common in those of never-smoker. In the latter group, TP53 mutation often occur in association with activating mutation in EGFR, while tumors with EGFR but without TP53 mutation systematically show loss of p14ARF expression. This observation suggests that p53 is involved as a rate-limiting factor in the control of proliferation and survival of cells with mutant EGFR through activation of p14ARF (1).
We have recently investigated the prognostic and predictive value of TP53 mutations in NSCLC in a large randomized therapeutic clinical trial, IALT (International Adjuvant Lung Cancer Trial, n=783), aimed at assessing the benefits of cisplatin-based adjuvant therapy in completely resected NSCLC patients (2). Results after 8 years of follow-up showed no prognostic value for TP53. However, mutations were significant predictors of response to therapy, in particular in Squamous Cell Carcinoma (SCC). Strinkingly, wild-type TP53 did not predict a better response to therapy, whereas mutant TP53 predicted a significantly decreased survival and disease-free interval as compared to patients who did not receive chemotherapy. When matched with p53 protein expression, the negative effect of TP53 mutations appeared to be restricted to tumors that accumulate mutant p53 protein. Overall, these results demonstrate that presence of mutant p53, although having no significant effect on the course of the disease in the absence of adjuvant therapy, significantly reduced the survival of patients upon treatment. This effect indicates a clinical gain-of-function (GOF) of mutant TP53 in NSCLC. To our knowledge, this is the first evidence for GOF in a clinical setting.

(1) Mounawar et al. Cancer Res 2007.
(2) Ma et al., abstracts presented at ESMO and EACR meetings, 2008.

POSTERS

Poster 1

PML REGULATES WT p53 AND GAIN OF FUNCTION OF MUTANT p53

*[1]Sue Haupt, [2]Silvia Di Agostino, [1]Inbal Mizrachi, [1]**Osnat Alsheich-Bartok**, [2]Giovanni Blandino and [1]Ygal Haupt*

[1]The Lautenberg Center, Hebrew University, Jerusalem Israel.
[2]Experimental Oncology Department, Institute Regina Elena, Rome Italy.

Wild type (wt) p53 plays a key role in the prevention of tumour development. In response to stress conditions, wt p53 promotes growth inhibition or cell death. In contrast, cells expressing common p53 mutants are released from these constraints. Further, mutant p53 may acquire distinct properties from its wild type counterpart, referred to as a "Gain of function" phenomena. An important modulator of wt p53 is the promeylocytic leukemia (PML) tumour suppressor. PML and the PML nuclear bodies (NBs) have been implicated in the regulation of the cellular response to certain stress conditions. It has been shown that PML is important for the proper accumulation and activation of wt p53 in response to DNA damage. PML protects p53 from negative regulation by Mdm2. This protection is associated with N-terminal phosphorylation of p53. Since mutant p53 is also subjected to post-translational modifications, it raised the question of whether PML also regulates mutant forms of p53. Here we addressed this question and found that PML interacts with and co-localizes with mutant p53. Surprisingly we found that PML activates mutant p53 transcriptional activity and is important for its gain of function in cultured human cancer cells. Our results support the notion that as in the case of wt p53, PML is a key regulator of mutant p53. The implications to anti-cancer treatment are discussed.

Poster 2

A MUTANT-p53/SMAD COMPLEX OPPOSES p63 TO EMPOWER TGF-BETA INDUCED METASTASIS.

***Michelangelo Cordenonsi*[1],** *Maddalena Adorno[1], Marco Montagner[1], Allan Balmain[2] and* Stefano Piccolo[1]

[1]Department of Medical Biotechnologies, Section of Histology and Embryology, University of Padua, viale Colombo 3, 35126 Padua, Italy
[2]Cancer Research Institute, University of California San Francisco, 2340 Sutter Street, San Francisco, CA 94115, USA

TGFβ ligands act as tumor suppressors in early stage tumors but are paradoxically diverted into potent prometastatic factors in advanced cancers. The molecular nature of this switch remains enigmatic. Here we show the workings of a previously undescribed pathway by which TGFβ fosters malignant progression. Mutant-p53 is required to empower TGFβ-dependent cell migration, invasion and metastasis. Mechanistically, TGFβ acts in concert with oncogenic Ras and mutant-p53 to induce the assembly of a mutant-p53/p63 protein complex in which Smads serve as essential platforms. Within this ternary complex, the anti-metastatic properties of p63 are antagonized. Two novel metastasis suppressor genes downstream of this pathway are associated with metastasis risk in a large cohort of breast cancer patients. Thus, two common oncogenic lesions, mutant-p53 and Ras, selected in early neoplasms to promote growth and survival, also prefigure a cellular set-up with particular metastasis proclivity by TGFβ-dependent inhibition of p63 function.

Poster 3

CHE-1 DEPLETION INHIBITS MUTANT p53 EXPRESSION AND INDUCES p73-DEPENDENT APOPTOSIS IN HUMAN BREAST CANCER

Tiziana Bruno, Agata Desantis, Gianluca Bossi, Silvia D'Agostino, Cristina Sorino, Francesca De Nicola, Simona Iezzi, Aristide Floridi, Ada Sacchi, Claudio Passananti Giovanni Blandino and ***Maurizio Fanciulli***

Regina Elena Cancer Institute, via E. Chianesi 53, 00144 Rome Italy

Che-1 is a human RNA polymerase II binding protein highly conserved from yeast to man and involved in the regulation of gene transcription. Recently, we showed that the checkpoint kinases ATM/ATR and Chk2 physically and functionally interact with Che-1 and promote its phosphorylation and accumulation in response to DNA damage. These Che-1 modifications induce transcription of p53 and Che-1 depletion strongly sensitizes tumor cells to anticancer drugs.

p53 plays critical roles in tumor suppression and the loss of its function is required for cancer progression. In this context, the p53 gene is the most commonly mutated gene in human cancers. Indeed, in addition to the loss of tumor suppression activity, accumulating evidence indicates that p53 cancer mutants gain new oncogenic activities in promoting tumorigenesis and drug resistance.

In this study, we demonstrate that Che-1 depletion by siRNA produces a strong decrease of mutant p53 expression in several breast cancer cell lines, inducing apoptosis selectively in these cells but not in breast cancer cells carrying wt p53. Knockdown of Che-1 induces the proapototic bcl-2 family members Puma and Noxa, and both their induction and subsequent cell death require transactivation of p73. These data define a new therapeutic strategy in cancer treatment by ablating the expression of mutant p53 protein and enhancing p73 activity.

Poster 4

PIN1 PROMOTES THE ACQUISITION OF AN AGGRESSIVE PHENOTYPE BY ENHANCING MUTANT p53 GAIN OF FUNCTION

***Javier Girardini**, Marco Napoli, Carolina Marotta and Giannino Del Sal*

Molecular Oncology Unit. Laboratorio Nazionale CIB (LNCIB). Padriciano 99,Trieste, Italy.

p53 is frequently mutated in tumor tissues and the majority of these mutations are missense, thus leading to the expression of full-length mutant proteins. Several lines of evidence have established that a subset of these p53 mutants gain new oncogenic functions and concur to the development of invasive and metastatic phenotypes (gain of function, GOF). However in spite of having been the object of extensive studies, mutant p53 still retains several aspects that need to be unveiled. We have shown that the prolyl isomerase Pin1 is able to interact with p53 point mutants, indicating that Pin1 may regulate mutant p53 GOF. Deregulated action of Pin1 can cooperate with tumorigenesis by amplifying signaling through several oncogenic pathways. Remarkably, we and others have shown that Pin1 is over-expressed in breast cancers (Rustighi et al 2009). In order to determine if there is a functional interaction between Pin1 and mutant p53 we used a model system of oncogene-induced transformation in primary mouse fibroblasts. We found that mutant p53 cooperates with oncogenic Ras in cell transformation and that Pin1 is necessary for this cooperation. Moreover, lack of Pin1 impairs the ability of mutant p53 to enhance the proliferation of tumor xenografts in nude mice. Mutant p53 GOF was associated with the development of metastasis in mouse models, therefore, we analyzed the involvement of mutant p53 in cell migration, since the ability to migrate is essential for tumor cells to develop metastasis. This is a critical aspect in the progression of the disease since metastasis remains the cause of death in 90% of patients with solid tumors. We observed that mutant p53 as well as Pin1 are necessary for cell migration *in vitro*. Moreover, our results suggest that Pin1 may regulate cell migration by enhancing the interaction between mutant p53 and p63. Previous work from our laboratory unveiled a key role for Pin1, acting in a phosphorylation-dependent manner, to produce conformational changes required for the full transcriptional activation of both wt p53 and p73 (Zacchi et al. 2002 Mantovani et al. 2004, Mantovani et al. 2007). Therefore, our results are consistent with the idea that Pin1, under physiological conditions, may act as a fine tuner of the tumor suppressor function of wt p53, however, during the transformation process, it can change its role becoming a dangerous amplifier of mutant p53 oncogenic activity.

Zacchi et al., Nature, 2002.
Mantovani et al., Mol Cell, 2004.
Mantovani et al., Nat Struct Mol Biol, 2007.
Rustighi et al., Nat Cell Biol, 2009.

Poster 5

MUTANT p53 AS AN EPIGENETIC FACTOR IN THE MODULATION OF GENE EXPRESSION IN TUMOR CELLS

Timo Quante, *Marie Brázdová, Lars Tögel, Wolfgang Deppert, and Genrich V. Tolstonog*

Heinrich-Pette-Institut, Abteilung Tumorvirologie, Hamburg, Germany

Missense point mutations in the *TP53* gene are frequent genetic alterations in human tumor tissue and cell lines derived thereof. Expression of mutant p53 (mutp53) is advantageous for tumor cells, however the molecular mechanism of mutp53 action is still not known. We used tumor cell lines expressing endogenous and inducible mutp53 proteins as models to study the role of mutp53 in transcriptional regulation. Mutp53 has lost sequence specificity in DNA binding activity, but retained the property to recognize DNA secondary structures, and, based on our ChIP-Seq data, is prone to interact preferentially with repetitive sequences possessing conformational flexibility. Stable and transient down-regulation of mutp53 expression strongly suggested that mutp53 binding to genomic DNA is functional. We hypothesized that mutp53 operates as an epigenetic factor on the level of chromatin organization rather than on modulating the expression of individual genes. In support, we found that differentially regulated genes frequently map to the same chromosomal locations or even are organized as physically-linked gene clusters. To test this hypothesis, physical DNA contacts encompassing co-regulated gene clusters were profiled using the chromosome conformation capture (3C) method. Preliminary results strongly support the proposed function of mutp53 in modulating gene expression at a higher level of chromatin organization.

Poster 6

CONFORMATIONAL STABILE p53 MUTANTS RETAIN SPECIFIC DNA BINDING CAPACITY BUT LOST TRANSACTIVATION FUNCTION

***Václav Brázda**, Eva Brázdová Jagelská, Miroslav Fojta*

Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Most of the human tumors contain inactivated p53 protein by mutations and/or functional deactivation, for example by overexpressed MDM2 protein. Restoration of wild-type p53 function could be one of the key tools in anticancer therapy. Using an electromobility shift assay we investigated the effect of temperature on the DNA binding of p53 wild-type and mutant proteins. Our results show that p53 mutants R280K , E285K and L194F from human cancer cell lines were capable bind to p53 target sequences *in vitro* at 32℃ or 4℃ and the p53 binding ability is strongly improved by UV and Roscovitine, but also by cultivation at 32℃. Mutant E285K was moreover capable of reactivating the transcription of the reporter gene at 32℃ *in vivo* while R285K and L194F were not. We observed changes in conformation of the mutant by MAbs. We conclude that conformation of p53 protein is only one of the preconditions in complex p53/DNA binding *in vivo*.

1. Jagelska, E.B., et al., *DNA topology influences p53 sequence-specific DNA binding through structural transitions within the target sites.* Biochem J, 2008. **412**(1): p. 57-63.
2. Brazda, V., et al., *Restoring wild-type conformation and DNA-binding activity of mutant p53 is insufficient for restoration of transcriptional activity.* Biochem Biophys Res Commun, 2006. **351**(2): p. 499-506.
3. Brazda, V., et al., *Searching for target sequences by p53 protein is influenced by DNA length.* Biochem Biophys Res Commun, 2006. **341**(2): p. 470-7.
4. Pospisilova, S., et al., *Activation of the DNA-binding ability of latent p53 protein by protein kinase C is abolished by protein kinase CK2.* Biochem J, 2004. **378**(Pt 3): p. 939-47.
5. Palecek, E., et al., *Enhancement of p53 sequence-specific binding by DNA supercoiling.* Oncogene, 2004. **23**(12): p. 2119-27.
6. Jagelska, E., et al., *New ELISA technique for analysis of p53 protein/DNA binding properties.* J Immunol Methods, 2002. **267**(2): p. 227-35.
7. Palecek, E., et al., *Binding of p53 and its core domain to supercoiled DNA.* Eur J Biochem, 2001. **268**(3): p. 573-81.

Poster 7

ONCOGENIC FUNCTIONS AND POST-TRANSLATIONAL MODIFICATIONS OF HDMX.

***Kristiaan Lenos**, Amina Teunisse and Aart G. Jochemsen*

Leiden University Medical Center, Dept. Molecular Cell Biology, PO Box 9600, 2300 RC Leiden, The Netherlands.

Hdm2 and Hdmx are the most important regulators of the tumor suppressor p53. Both Hdm2 and Hdmx are able to inhibit p53 transactivation activity, while Hdm2 also functions as an E3 ubiquitin ligase to promote the degradation of p53. Several types of tumors show overexpression or amplification of Hdm2 or Hdmx, in most cases correlating with expression of wild-type p53. These results suggested that Hdmx overexpression relieves the pressure for p53 mutation, and might be sufficient to inactivate the p53 tumor suppressor pathway.

To show a direct function of Hdmx in the transformation process, we have set up in vitro transformation conditions. It had been shown before that expression of hTERT, H-RasV12, SV40 small t and knock-down of Rb or p16 and p53, leads to neoplastic transformation of human fibroblasts. By overexpressing Hdmx or Hdmx splice variants instead of knocking down p53, the oncogenic role of Hdmx has been studied. Several cell lines have been made, with combinations of pRb knock down, H-RasV12 overexpression, p53 knock down or Hdmx overexpression. In this way the influence of the individual factors can be assessed using several growth assays. The role of different functional domains in Hdmx was studied with the use of alternative splice variants Hdmx-A, -G or -S, of which the latter was shown to be more effective in the inhibition of p53-mediated transcriptional activation and induction of apoptosis. In addition, the effects of anti-cancer drugs are being studied on these cell lines. The activity and levels of Hdmx and Hdm2 can be modified by several post-translational modifications, such as ubiquitination and ubiquitin-like molecules, such as SUMO-1/SUMO-2. Several phosphorylations on both Hdm2 and Hdmx play a yet not fully understood role in the regulation of the activity of these proteins. A part of our research is to investigate these post-translational modifications and their functions in regulating the activity of Hdmx and Hdm2. The influence of different phosphorylations on the activity of Hdmx has been studied by using phosphorylation-site mutants and/or phospho-specific antibodies. In collaboration with the group of Ygal Haupt, the effects of phosphorylation of Hdmx by c-Abl have been studied. Importantly, phosphorylation of Hdmx-Y99 by c-Abl prevents the interaction between Hdmx and p53 (Zuckerman et al 2008).

In addition, SUMOylation of Hdm2 and Hdmx and the possible role of this modification are being investigated. SUMOylation-dependent ubiquitination of these proteins might play a role in the regulation of p53.

Poster 8

TP53*PIN3* POLYMORPHISM INVOLVED IN *G-QUADRUPLEX* STRUCTURES: EFFECT ON ALTERNATIVE SPLICING OF INTRON 2.

V. Marcel[1], *J. Hall*[2], *EI. Palmero*[1], *MI. Achatz*[3], *G. Martel-Planche*[1], *P. Ashton-Prolla*[4], *H. Moine*[5], *P. Hainaut*[1] *and E. Van Dyck*[1]

[1]Molecular Carcinogenesis and Biomarkers Group, International Agency for Research on Cancer, Lyon Cedex 08, France; [2]INSERM U612: Institut Curie-Recherche, Orsay, France ; [3]Department of Oncogenetics, Hospital A.C. Camargo, São Paulo, Brazil ; [4]Department of Genetics, Federal University of Rio Grande do Sul and Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Brazil ; [5]Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS (UMR7104)/INSERM (U596)/ULP/College de France, 67404 Illkirch.

The tumour suppressor gene *TP53* is expressed as several isoforms. The canonical p53 protein corresponds to a full-length product encoded by the fully-spliced p53 mRNA (FSp53). Its suppressor activities are counteracted by a N-terminal truncated isoform, Δ40p53, generated by an alternatively spliced mRNA retaining intron 2 (p53I2).

In this study, we investigated the mechanisms of regulation for alternative splicing of intron 2. We demonstrated, using reverse transcriptase elongation assay, that *G-quadruplex* structures are formed in intron 3. By site-directed mutagenesis and treatment with TMPyP4, a cationic porphyrin modulating *G-quadruplex* formation, we showed that these structures regulate the exclusion of intron 2 and thus the production of FSp53 mRNA.

In addition, we observed that these *G-quadruplexes* overlap a common polymorphic motif, TP53*PIN3*, a 16bp duplication in intron 3 (A1: non-duplicated allele; A2: duplicated allele), which is associated with an altered cancer risk and a reduced level of p53 mRNA. We showed that *G-quadruplex* structures are also formed in TP53*PIN3* A2 allele, leading to modulation of their topologies. In A2 cells, site-directed mutagenesis and treatment with TMPyP4 showed that *G-quadruplexes* are involved in p53 mRNA expression, but other mechanisms of mRNA processing are also involved, suggesting a complex pattern of p53 mRNAs expression depending on TP53*PIN3* status. Analysis of both FSp53 and p53I2 transcripts in lymphoblastoid cells, carrying either A1 or A2 allele, revealed a large decrease of these two transcripts in A2 cells compared to A1 cells. This reduction may be explained in part by the influence of *G-quadruplexes* on alternative splicing of p53.

The polymorphic nature of the *G-quadruplexes* provides a mechanism for both the regulation of the splicing of intron 2 leading to p53 expression and the decrease of p53 expression in A2 TP53*PIN3* allele.

Poster 9

THE STRUCTURE AND INTERACTIONS OF THE PROLINE RICH DOMAIN OF ASPP2

***Shahar Rotem**, Chen Katz, Hadar Benyamini and Assaf Friedler*

Institute of Chemistry, The Hebrew University of Jerusalem, Israel

ASPP2 is a pro-apoptotic protein that stimulates the p53-mediated apoptotic response. The C-terminus of ASPP2 contains ankyrin repeats (Ank) and a SH3 domain, which mediate its interactions with numerous partner proteins such as p53, NFkB and Bcl-2. It also contains a proline-rich domain (Pro), whose structure and function are unclear. Here we used biophysical and biochemical methods to study the structure and the interactions of ASPP2 Pro, in order to gain insight into its biological role. We show, using biophysical and computational methods, that the ASPP2 Pro domain is natively unfolded. We found that the ASPP2 Pro domain interacts with the ASPP2 Ank-SH3 domains, and mapped the interaction sites in both domains. Using a combination of peptide array screening, biophysical and biochemical techniques, we found that ASPP2 Ank-SH3, but not ASPP2 Pro, mediates interactions of ASPP2 with peptides derived from its partner proteins. ASPP2 Pro-Ank-SH3 bound a peptide derived from its partner protein NFkB weaker than ASPP2 Ank-SH3 bound this peptide. This suggested that the presence of the proline-rich domain inhibited the interactions mediated by the Ank-SH3 domains. Furthermore, a peptide from ASPP2 Pro competed with a peptide derived from NFkB on binding to ASPP2 Ank-SH3. Based on our results, we propose a model in which the interaction between the ASPP2 domains regulates the intermolecular interactions of ASPP2 with its partner proteins.

1. Rotem S. , Katz C. and Friedler A. (2007): Biochem. Soc. Transac., 35, 966-69
2. Rotem S. , Katz C. , Benyamini H. , Lebendiker M. , Veprintsev D. , Rudiger S. , Danieli T. , and Friedler A. (2008): J Biol Chem 283(27):18990-9

Poster 10

COMMON CHEMICAL FUNCTIONALITY AMONG MUTANT p53 REACTIVATING COMPOUNDS

Jeremy M.R. Lambert[1,2], Petr Gorzov[1], Dimitry B. Veprintsev[3], Nicole Zache[1], Jinfeng Shen[1], Nina Rökaeus[1], Maja Söderqvist[1], Dan Segerbäck[4], Jan Bergman[4], Alan R. Fersht[3], Pierre Hainaut[2], Klas G. Wiman[1] and ***Vladimir J.N. Bykov[1]***

[1]Dept. of Oncology-Pathology, Cancer Center Karolinska (CCK), Karolinska Institutet, SE-171 76 Stockholm, Sweden. [2]International Agency for Research on Cancer (IARC), 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France. [3]Centre for Protein Engineering, Medical Research Council, Cambridge CB2 2QH, UK. [4]Dept. of Biosciences and Nutrition, Karolinska Institutet, Novum, SE-141 57, Huddinge, Sweden

Given the frequent mutations of p53 in almost all types of human tumors, mutant p53-targeting drugs should have a wide clinical applicability. Moreover, since mutant p53-carrying tumors are usually more resistant to currently used chemotherapy, there is an urgent need for more effective treatment of such tumors. The identification of mutant p53-reactivating small molecules such as PRIMA-1, CP-31398, MIRA-1 and STIMA-1 opens new opportunities for curative cancer treatment. We have investigated stability and chemical reactivity of several mutant p53 targeting molecules. Surprisingly, they all share one chemical trait, namely potential reactivity with thiol groups. We linked this activity to double carbon bonds located in the proximity of an electron withdrawing group, creating the Michael acceptor functionality. Our experiments revealed that lack of such structural feature renders the tested molecules biologically inactive. We show that PRIMA-1via its conversion products binds to mutant p53 in cells and that the binding is p53 conformation-specific, i.e. that binding occurs preferentially to unfolded p53. Our findings identify chemical groups responsible for mutant p53 reactivation by novel compounds and may guide the design of new more efficient mutant p53-rescuing molecules.

Poster 11

CRYSTAL STRUCTURES OF p53 DNA-CONTACT MUTANTS AND THEIR RESCUE BY SECOND-SITE SUPPRESSOR MUTATIONS

Amir Eldar, *Haim Rozenberg, Yael Diskin-Posner, Naama Kessler, Yaacov Halfon and Zippi Shakked*

Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel

The tumor suppressor protein p53 is inactivated by mutations in approximately 50% of human cancers. The majority of these mutations are missense mutations in the DNA binding domain region of the protein (the core domain). These mutations can be roughly divided into two groups; structural mutations that lead to distortion and/or destabilization of the protein structure, and DNA contact mutations that diminish the DNA binding affinity of p53 without affecting significantly the structure of the protein. A frequent DNA-contact mutation is that of Arginine at position 273 (R273) to Histidine or Cysteine. The crystal structures of tetrameric core domain bound to DNA show that the positively charged guanidinium group of R273 interact with the negatively charged DNA backbone thereby playing a pivotal role in docking p53 to the DNA at the central region of each half-site where no base-mediated contacts exist [1]. Substitution of R273 by Histidine (R273H) or Cysteine (R273C), leads to a dramatic reduction in the DNA binding affinity of the protein and its transactivation and tumor suppression functions [2]. It was found that such activities can be rescued by the introduction of a second mutation T284R [2], referred to as second-site suppressor mutation. To gain structural insight into the effects of such primary and second-site mutations, we elucidated the crystal structures of the human p53 core domain harboring the single mutations R273H or R273C and the double mutations R273H/T284R or R273C/T284R, as well as the rescued proteins bound to their DNA targets. The structural findings based on high-resolution X-ray analyses (1.7-1.9 Å) will be presented.

1. Kitayner M. Rozenberg H. Kessler N. Rabinovich D. Shaulov L. Haran T. E. and Shakked Z., *Structural Basis of DNA Recognition by p53 Tetramers.* Molecular Cell, 2006. **22**(6): p. 741-753.

2. Wieczorek A. M. Waterman J. L. F. Waterman M. J. F. and Halazonetis T. D., *Structure-based rescue of common tumor-derived p53 mutants.* Nature Medicine, 1996. **2**(10): p. 1143-6.

Poster 12

MAPPING THE INTERACTION NETWORK OF THE p53 TETRAMERIZATION DOMAIN: A BASIS FOR DRUG DESIGN

***Ronen Gabizon*[1],** *Michal Mor*[1], *Masha M. Rosenberg*[1,2], *Zvi Hayouka*[1], *Deborah E. Shalev*[2], *Assaf Friedler*[1]

[1]Institute of Chemistry

[2]Wolfson Centre for Applied Structural Biology, The Hebrew University of Jerusalem

p53 is known to be active in the nucleus as a tetramer, while its dimeric and monomeric forms are inactive. Oligomerization of p53 is mediated by a structurally independent tetramerization domain (p53Tet, residues 326-355). Stabilizing the p53 tetramer is an important goal in anti-cancer therapy, since it will result in activated p53 that causes apoptosis of cancer cells. In this study we searched for peptides that bind to the p53 tetramerization domain and activate p53. We quantitatively characterized the previously reported interaction between p53Tet and the HIV-1 Tat protein by peptide mapping, fluorescence anisotropy and NMR spectroscopy. We showed that p53Tet binds directly to residues 1-35 and 47-57 in Tat. All oligomerization states of p53(326-355) bind the Tat peptides without inhibiting p53 tetramerization. Also, the p53 residues that are mainly involved in binding to Tat(47-57) are E343 and E349, which face away from the dimer-dimer interface of the p53Tet tetramer. We conclude that p53 is able to bind Tat as a transcriptionally active tetramer.

In parallel, we screened the sequences of proteins known to bind the p53 tetramerization domain using peptide arrays. We identified several peptides derived from various cellular proteins which bind specifically to p53Tet. These peptides will serve as the basis for development of p53-activating peptides in the future.

Poster 13

TARGETED MUTANT p53 RESCUE

***Andreas C. Joerger**, Frank M. Boeckler, Gaurav Jaggi & Alan R. Fersht*

MRC Centre for Protein Engineering, Hills Road, Cambridge CB2 0QH, UK

Y220C is the ninth most frequent p53 cancer mutation and accounts for an estimated 75,000 new cancer cases per year worldwide. The mutation creates a surface cavity that destabilizes the core domain by 4 kcal/mol, at a site that is not functional. Accordingly, small molecules that stabilize the protein by selectively binding to this surface cavity could be effective anti-cancer drugs. Using virtual screening and rational drug design, we found a class of molecules based on the carbazole scaffold, the PhiKans, that bind to Y220C with 100-200 μM affinity, raise the melting temperature of the protein and increase its half-life. We have elucidated the exact binding mode of a representative member, Phikan083 (1-(9-ethyl-9H-carbazol-3-yl)-N-methylmethanamine), by solving the crystal structure of the Y220C mutant in complex with the small molecule at 1.5 Å. PhiKan083 binds to the mutation-induced surface cleft, next to Cys-220, and fills the cleft created by the mutation. The central carbazole moiety is largely buried in the cleft, with the 9-ethyl group occupying the deepest part of the hydrophobic pocket, whereas the methanamine moiety forms a hydrogen bond at the periphery of the surface cleft. The structure reveals key interactions between the protein and ligand, as well as small conformational changes that occur upon binding, which provides the basis for lead optimization. We are currently developing second and third generation ligands by structure-guided design. The Y220C mutant is an excellent “druggable” target and an ideal paradigm for developing and testing p53-stabilizing anti-cancer drugs in general.

Poster 14

BIOPHYSICAL ANALYSIS OF p53 SLOW SUBUNITS EXCHANGE FOR WT AND R273H MUTANT AND INVESTIGATION OF THE HETERO-TETRAMER WITH DNA RESPONSE ELEMENTS AS A MODEL FOR HETEROZYGOUS CELLS

***Eviatar Natan*[1]**, *Daniel Hirschberg*[2], *Carol V Robinson*[2], *Alan R Fersht*

Cambridge University, [1]MRC-CPE at the Addenbrooke's Hospital, [2]Department of Chemistry, Cambridge UK

The P53 tumour suppressor protein is active as a homo-tetramer, which consists of a dimer of primary dimers linked via a tetramerization domain in the C-terminus. P53 mutations can be divided into two classes: structural and contact mutations. Contact mutations, which represent the majority of cancer mutations, are located in the DNA-binding core domain and reduce its DNA binding potential. It is reasonable to believe that the initiation of cancer will be gradual and that only one of the alleles in a heterozygous cell will be mutated, as in the case of the Li-Syndrome. In such a cell the wild-type and mutant protein will form a mixed population with a variety of subunit ratios.

The 'hot-spot' mutation R273H is considered to be a pure contact mutation with significantly lower affinity for the DNA promoters than the wild type. One would therefore assume that the affinity of the wild-type/mutant complex would also be affected. This effect has been previously reported *in-vivo* as the dominant negative effect.

An understanding of the composition of a mixed population and the nature of the hetero-tetramer complex would help to define a model of the initial step in the development of cancers in the heterozygous cell. We therefore explored the exchange rate of wild-type and R273H mutant constructs containing the Core and Tetramerization domain (94-360). We found that the exchange was unexpectedly slow at both roomtemperature ($20C^0$) and at physiological temperature ($37C^0$): $t_{½}$ = 1.6 h and 26 min, respectively. Incubation at physiological temperature resulted in an additional and even slower phase of monomer exchange.

We explored the affinity of DNA response elements for the different populations at equilibrium using anisotropy and ES-MS, and found dissociation constants for the complexes.

Poster 15

PRIMA-1^{MET} RESCUES PRO-APOPTOTIC ACTIVITY TO THE MUTANT SPECIES OF THE ENTIRE p53 FAMILY

Shen JF (1), *Vakifahmetoglu H (2), Rökaeus N(1), Stridh H(1), Wilhelm M(1), Bykov V(1), Zhivotovsky B(2), Wiman KG(1)*

(1)Dept of Oncology-Pathology, CCK, and (2) Division of Toxicology, Karolinska Institutet, SE-171 76 Stockholm, Sweden

Mutations in the tumor suppressor gene p53 are frequently found in human tumors. The low molecular weight compound PRIMA-1^{MET} restores tumor suppressor activity to mutant p53, and induces mutant p53-dependent apoptosis in tumor cells *in vitro* and *in vivo*.

Here we show that PRIMA-1^{MET} triggers mutant p53-dependent apoptosis via multiple parallel signalling circuits converging to mitochondria. Early activation of caspase-2 in mutant p53 cells triggers loss of mitochondrial membrane potential, release of apoptotic factors and finally cell death. Inhibition or silencing of caspase-2 can rescue 50% of cells from apoptosis induced by PRIMA-1^{MET}. Activation of Bax and induction of PUMA and NOXA in a mutant p53-dependent manner form additional independent apoptotic signalling pathways.

The p53 family has three members, p53, p63, and p73. p63 and p73 share 60% similarity with p53 in DNA binding domain. Interestingly, PRIMA-1^{MET} also rescued pro-apoptotic function to mutant p63 and p73, suggesting a common mechanism for reactivation of the entire p53 family.

Our findings suggest that mutant p53 reactivation by PRIMA-1^{MET} will also be effective against tumor cells deficient in some components of apoptotic signalling cascade downstream of p53. This opens more broad perspectives for the treatment of tumors with the complex blockade of apoptotic signalling.

LIST OF PARTICIPANTS

ADI-HAREL **Shelly**
Weizmann Institute of Science
Israel
@mail: shelly.adi-harel@weizmann.ac.il
Abstract links:

ALSHEICH-BARTOK **Osnat**
The Hebrew University of Jerusalem
Israel
@mail: shaybartok@gmail.com
Abstract links: P1, (L11)

ANTUNES FERREIR **Edite**
University Medical Center Utrecht
The Netherlands
@mail: e.antunes@umcutrecht.nl
Abstract links:

AYLON **Yael**
Weizmann Institute of Science
Israel
@mail: yael.aylon@weizmann.ac.il
Abstract links:

BIAGIONI **Francesca**
Regina Elena Cancer Institute
Italy
@mail: biagioni@ifo.it
Abstract links: (L12)

BISSO **Andrea**
Laboratorio Nazionale CIB
Italy
@mail: andrea.bisso@lncib.it
Abstract links: S5

BLANDINO **Giovanni**
Regina Elena Cancer Institute
Italy
@mail: blandino@ifo.it
Abstract links: L12, (L11), (P1), (P3)

BOSSI **Gianluca**
Regina Elena Cancer Institute
Italy
@mail: bossi@ifo.it
Abstract links: S2, (P3)

BRAZDA **Vaclav**
Insitute of Biophysics ASCR, v.v.i
Czech Republic
@mail: vaclav@ibp.cz
Abstract links: P6

BROSH **Ran**
Weizmann Institute of Science
Israel
@mail: ran.brosh@weizmann.ac.il
Abstract links: (L8)

BRUNO **Tiziana**
Regina Elena Cancer Institute
Italy
@mail: bruno@ifo.it
Abstract links: (P3)

BYKOV **Vladimir**
Karolinska Institute
Sweden
@mail: Vladimir.Bykov@mtc.ki.se
Abstract links: P10, (P15)

CORDENONSI **Michelangelo**
Universita di Padova
Italy
@mail: michelangelo.cordenonsi@unipd.it
Abstract links: S7, P2

COSTANZO **Antonio**
University of ROME
Italy
@mail: antonio.costanzo@uniroma2.it
Abstract links:

de LANGE **Job**
LUMC gebouw 2
The Netherlands
@mail: j.de_lange@lumc.nl
Abstract links: S3

DEL SAL **Giannino**
Laboratorio Nazionale CIB
Italy
@mail: delsal@lncib.it
Abstract links: L10, (S5), (P4)

DELL'ORSO **Stefania**
Regina Elena Cancer Institute
Italy
@mail: dellorso@ifo.it
Abstract links: (L12)

DEPPERT **Wolfgang**
Heinrich-Pette Institut
Germany
@mail: wolfgang.deppert@hpi.uni-hamburg.de
Abstract links: L3, (L4), (S6), (P5)

DI CAPUA **Emma Nora**

Israel
@mail:
Abstract links:

DISKIN-POSNER **Yael**
Weizmann Institute of Science
Israel
@mail: yael.diskin-posner@weizmann.ac.il
Abstract links: (P11)

DONZELLI **Sara**
Regina Elena Cancer Institute
Italy
@mail: donzelli@ifo.it
Abstract links: (L12)

ECHCHANNAOUI **Hakim**
Wilhelmina Children's Hospital
The Netherlands
@mail: h.echchannaoui@umcutrecht.nl
Abstract links:

ELDAR **Amir**
Weizmann Institute of Science
Israel
@mail: amir.eldar@weizmann.ac.il
Abstract links: P11

EZRA **Osnat**
Weizmann Institute of Science
Israel
@mail: osnat.ezra@weizmann.ac.il
Abstract links: (L8)

FANCIULLI **Maurizio**
Regina Elena Cancer Institute
Italy
@mail: fanciulli@ifo.it
Abstract links: P3

FREED-PASTOR **William**
Columbia University
USA
@mail: waf2102@columbia.edu
Abstract links:

FRIEDLER **Assaf**
The Hebrew University of Jerusalem
Israel
@mail: assaf@chem.ch.huji.ac.il
Abstract links: L15, (P9), (P12)

GABIZON **Ronen**
The Hebrew University of Jerusalem
Israel
@mail: ronen.gabizon@mail.huji.ac.il
Abstract links: P12

GIRARDINI BROVEL **Javier E**
Laboratorio Nazionale CIB
Italy
@mail: javier.girardini@lncib.it
Abstract links: P4, (L10), (S5)

GOLDFINGER **Naomi**
Weizmann Institute of Science
Israel
@mail: n.goldfinger@weizmann.ac.il
Abstract links: (L8)

GOLDSHTEIN **Ido**
Weizmann Institute of Science
Israel
@mail: ido.goldshtein@weizmann.ac.il
Abstract links: (L8)

GOLOMB **Lior**
Weizmann Institute of Science
Israel
@mail: lior.golomb@weizmann.ac.il
Abstract links:

HAINAUT **Pierre**
International Agency for Research on Cancer
France
@mail: hainaut@iarc.fr
Abstract links: L24, (S4), (L23), (P8), (P10)

HARAN **Tali**
Technion
Israel
@mail: bitali@tx.technion.ac.il
Abstract links:

HARRIS **Curtis C**
National Cancer Institute, NIH
USA
@mail: Curtis_Harris@nih.gov
Abstract links: L1

HAUPT **Ygal**
The Hebrew University of Jerusalem
Israel
@mail: ygal.haupt@petermac.org
Abstract links: L11, (P1)

HOLLSTEIN **Monica**
LIGHT Laboratories
UK
@mail: m.hollstein@leeds.ac.uk
Abstract links: L19

IGGO **Richard**
University of St Andrews
UK
@mail: Richard.Iggo@st-andrews.ac.uk
Abstract links:

JOERGER **Andreas**
MRC Centre for Protein Engineering
UK
@mail: acj2@mrc-lmb.cam.ac.uk
Abstract links: P13

KAMER **Iris**
Weizmann Institute of Science
Israel
@mail:
Abstract links:

KAPITKOVSKY **Aviva**
Weizmann Institute of Science
Israel
@mail: aviva.kapitkovsky@weizmann.ac.il
Abstract links:

KITAYNER **Ester Malka**
Weizmann Institute of Science
Israel
@mail: malka.kitayner@weizmann.ac.il
Abstract links:

KRANZ **Dominique**
German Cancer Research Center
Germany
@mail: d.kranz@dkfz-heidelberg.de
Abstract links:

LENOS **Kristiaan**
LUMC gebouw 2
The Netherlands
@mail: k.lenos@lumc.nl
Abstract links: P7

LEVAV **Yaara**
Lautenberg Center for Immunology
Israel
@mail: shaybartok@gmail.com
Abstract links: (L11)

LILJEBRIS **Charlotta**
Aprea AB
Sweden
@mail: charlotta.liljebris@aprea.com
Abstract links:

LOZANO **Guillermina**
U.T. MD Anderson Cancer Center
USA
@mail: gglozano@mdanderson.org
Abstract links: L20, (L22)

MADAR **Shlomi**
Weizmann Institute of Science
Israel
@mail: shalom.madar@weizmann.ac.il
Abstract links:

MARCEL **Virginie**
International Agency for Research on Cancer
France
@mail: marcel@iarc.fr
Abstract links: S4, P8

MICHAEL **Dan**
Weizmann Institute of Science
Israel
@mail: d.michael@weizmann.ac.il
Abstract links:

MOHELL **Nina**
Aprea AB
Sweden
@mail: nina.mohell@aprea.com
Abstract links:

MOSKOVITS **Neta**
Weizmann Institute of Science
Israel
@mail: neta.moskovitz@weizmann.ac.il
Abstract links: (L2)

MYERS **Jeffrey**
U.T. M.D. Anderson Cancer Center
USA
@mail: jmyers@mdanderson.org
Abstract links: L22

NATAN **Eviatar**
Gonville & Caius College
UK
@mail: en243@cam.ac.uk
Abstract links: P14

OLIVIER **Magali**
International Agency for Research on Cancer
France
@mail: molivier@iarc.fr
Abstract links: L23, (S4)

OREN **Moshe**
Weizmann Institute of Science
Israel
@mail: moshe.Oren@weizmann.ac.il
Abstract links: L2

PRIVES **Carol**
Columbia University
USA
@mail: clp3@columbia.edu
Abstract links: L7

QUANTE **Timo**
Heinrich-Pette-Institut
Germany
@mail: timo.quante@hpi.uni-hamburg.de
Abstract links: P5, (S6)

RAKOVITSKY **Nadya**
Weizmann Institute of Science
Israel
@mail: nadya.rakovitsky@weizmann.ac.il
Abstract links:

RESNICK **Michael A.**
National Institute of Environmental Health Scienc
USA
@mail: resnick@niehs.nih.gov
Abstract links: L9

ROTEM **Shahar**
The Hebrew University of Jerusalem
Israel
@mail: shahar.rotem@mail.huji.ac.il
Abstract links: P9, (L15)

ROTTER **Varda**
Weizmann Institute of Science
Israel
@mail: varda.rotter@weizmann.ac.il
Abstract links: L8, (L2)

ROZENBERG **Haim**
Weizmann Institute of Science
Israel
@mail: haim.rozenberg@weizmann.ac.il
Abstract links: (P11)

SABAPATHY **Kanaga**
National Cancer Center Singapore
Singapore
@mail: cmrksb@nccs.com.sg
Abstract links: S1

SARIG **Rachel**
Weizmann Institute of Science
Israel
@mail: rachel.sarig@weizmann.ac.il
Abstract links: (L8)

SCHERZ-SHOUVAL **Ruth**
Weizmann Institute of Science
Israel
@mail: ruth.scherz@weizmann.ac.il
Abstract links:

SELIVANOVA **Galina**
Karolinska Institute
Sweden
@mail: Galina.Selivanova@mtc.ki.se
Abstract links: L17, (L16)

SHAKKED **Zippora**
Weizmann Institute of Science
Israel
@mail: zippi.shakked@weizmann.ac.il
Abstract links: L14, (P11)

SHALGI **Reut**
Weizmann Institute of Science
Israel
@mail: reut.shalgi@weizmann.ac.il
Abstract links:

SHEN **Jinfeng**
Karolinska Institute (CCK) R8:04
Sweden
@mail: jinfeng.shen@ki.se
Abstract links: P15, (P10)

SHIFF **Idit**

Israel
@mail: idit.shiff@mail.huji.ac.il
Abstract links:

SIMON **Itamar**
The Hebrew University of Jerusalem
Israel
@mail: itamarsi@ekmd.huji.ac.il
Abstract links:

SODDU **Silvia**
Regina Elena Cancer Institute
Italy
@mail: soddu@ifo.it
Abstract links: L16

SOLOMON **Hilla**
Weizmann Institute of Science
Israel
@mail: hilla.besserglick@weizmann.ac.il
Abstract links: (L8)

STAMBOLSKY **Perry**
Weizmann Institute of Science
Israel
@mail: perrystam@hotmail.com
Abstract links: (L8)

TE RIELE **Hein**
Netherlands Cancer Institute
The Netherlands
@mail: h.t.riele@nki.nl
Abstract links: L21

THEOBALD **Matthias**
University Medical Center Utrecht
The Netherlands
@mail: m.theobald@umcutrecht.nl
Abstract links:

TOLSTONOG **Genrich**
Heinrich-Pette-Institut
Germany
@mail: genrich.tolstonog@hpi.uni-hamburg.de
Abstract links: L4, (L3), (P5)

WIMAN **Klas**
Karolinska Institute
Sweden
@mail: Klas.Wiman@mtc.ki.se
Abstract links: L18, (P10), (P15)