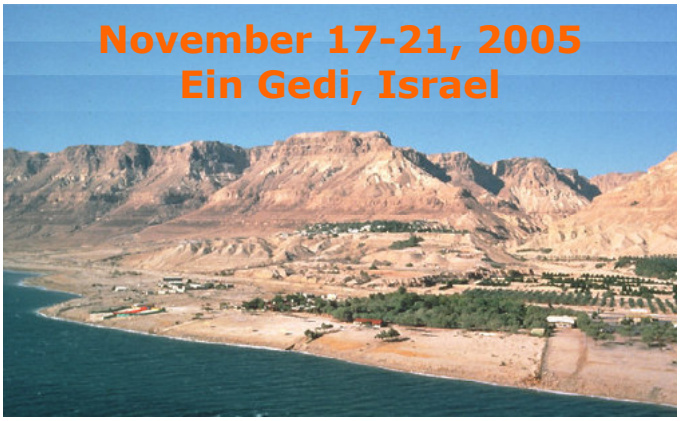
2nd International Workshop on Mutant p53:

The p53 Regulatory Network and Functional Consequences of p53 Mutations



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

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

ORGANIZING COMMITTEES

Meeting organizers

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Funding bodies

International Agency for Research on Cancer (IARC)
European community (FP6)

MEETING AIMS AND SCOPE

TP53 mutations are frequent in many cancers. Until recently, most studies have focused on the loss-of-suppressor function induced by mutation. However, it has been established for many years that several mutants induce a gain-of-function that may result in tumor promotion, although the molecular basis of this effect is not fully elucidated. In addition there are uncommon somatic and germline TP53 mutations that may relate to specific cancers or predispositions. These “special” mutants may provide interesting clues for approaching important aspects of cancer biology.

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 2nd Mutant P53 Workshop will focus on "The p53 regulatory network and functional consequences of p53 mutations". The spectrum of talks will range from distribution of mutations in human cancers, structural biology of mutant proteins, evaluation of their functional properties, to the clinical significance of mutations, with a strong emphasis on molecular and cell biology.

Programme and participants

The programme includes 29 presentations of 30 minutes’ duration each and a poster session (20 posters). 96 participants are registered.

HOUSEKEEPING DETAILS

Venue

The Ein Gedi Kibbutz Resort Hotel, overlooking the Dead Sea, Israel, is the p53 Marathon venue. Website - http://www.inisrael.com/eingedi

Registration, accommodation and tours

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Target Conferences is the official travel agent for the Marathon events. In addition to the Workshop Package, a pre- and post-tour is offered, specifically designed for participants.

Climate

In November the climate is warm during the day and relatively cold at night. Don't forget to bring your sun hat, sunglasses, bathing suit and comfortable walking shoes. Bring a jacket for the evenings.

Visas

Some participants may require a visa to enter Israel**.** We therefore suggest that you contact the Israeli Embassy/Consulate in your country or ask your travel agent for assistance. If you have difficulty obtaining a visa, kindly contact Target Conferences who will make every effort to assist.
Upon request, the Secretariat is willing to send a personal letter of invitation to the applicant to participate in the Workshop. It should be understood that such an invitation is only meant to help participants raise travel funds or obtain a visa, and does not represent a commitment on the part of the organizers to provide any financial support.

Flights and transportation

Direct flights exist from most major airports in northern America, Europe, India, China, Thailand and Africa by El Al Israel Airlines as well as by other International carriers.

Transfers from Ben Gurion international airport to Ein Gedi

Shared transfers from Ben Gurion Airport to Ein Gedi Kibbutz Hotel will be provided (with a maximum waiting time of up to one hour after flight arrival).

Important notes

Personal travel, luggage and health insurance are recommended. All tours are booked on the express condition that Target Conferences shall not be held responsible for and shall be exempt from all liability in respect of any loss, damage, injury or accident, to any person or his or her luggage or any other property, for any reason whatsoever.

Cancellation policy

Our refund policy is based upon receiving notification as follows: prior to Nov. 1, full refund less US$ 50 handling fee. Thereafter, no refund.

SCIENTIFIC PROGRAMME

Friday, November 18

From structure to regulation

08:45 a.m. **Alan FERSHT**, *Cambridge , UK*
Domain organization of p53 in solution

09:15 a.m. **Zippora SHAKKED**, *Rehovot, Israel*
The structural basis of differential DNA recognition by p53

09:45 a.m. **Emil PALECEK**, *Brno, Czech Republic*
Interfacial behavior of wild-type and mutant p53 proteins

10:15 a.m. Coffee break

10:45 a.m **Carol PRIVES**, *New York, USA*
Interactions of wild-type and mutant forms of p53 and p73 with DNA

11:15 a.m. **Itamar SIMON**, *Jerusalem, Israel*
Genome-wide approach for the identification of new p53 target genes

11:45 a.m. **Yosef SHAUL**, *Rehovot, Israel*
Degradation of mutant p53 via a ubiquitin-independent pathway

12:15 a.m. **Aart JOCHEMSEN**, *Leiden, The Netherlands*
Phosphorylation-dependent inhibition of HAUSP-mediated deubiquitination contributes to DNA damage-induced destabilization of Hdmx and Hdm2

12:45 p.m. Lunch

Afternoon Ein Gedi Spa

06:00 p.m. Dinner

p53 mutation and cancer

07:30 p.m. **Curt C. HARRIS**, *Bethesda, USA*
p53 response network: from cellular stress to cancer

08:00 p.m. **Jiri BARTEK**, *Copenhagen, Denmark*
DNA damage checkpoints and mutant p53: the cancer connection

08:30 p.m. **Pierre HAINAUT**, *Lyon, France*
p53 mutations as biomarkers for cancer detection and prognosis

09:00 p.m. **Mike RESNICK**, *Research Triangle Park, USA*
A SNP in the promoter of the VEGF Receptor 1 (FLT-1) reveals new “Rules of the game” for p53 target response elements

Saturday, November 19

From regulation to function

09:00 a.m. **Jean-Christophe MARINE**, *Bruxelles, Belgium*
Study of key upstream modulators and downstream effectors of the p53 pathway using mouse models

09:30 a.m. **Gianni DELSAL**, *Trieste, Italy*
The Role of the Prolyl-isomerase Pin1 in regulating the p53 protein family network: a fine tuner or a dangerous amplifier?

10:00 a.m. **Geoffray WAHL**, *La Jolla, USA*
Mouse bites dogma: mouse models provide new insights into the mechanisms that control the p53 stress response pathway

10:30 a.m. Coffee break

11:00 a.m. **Jo MILNER**, *York, UK*
Death without stress

11:30 a.m. **Ygal HAUPT**, *Jerusalem, Israel*
A role for PML in the regulation of p53 by CK1 in response to DNA damage

12:00 a.m. **Varda ROTTER**, *Rehovot, Israel*
Identification and characterization of p53 regulated “signature genes” in cells transforming in vitro

12:30 a.m. **Wolfgang DEPPERT**, *Hamburg, Germany*
Mutant p53: Oncogenic by perturbing chromatin structure and function?

01:00 p.m. Lunch

02:30 p.m. **Poster session** + Coffee break

05:45 p.m. **Dmitry VEPRINTSEV**, *Cambridge, UK*
Activation of the NF-kB pathway by mutant p53

06:00 p.m. **Xin LU**, *London,UK*
Regulating the anti-apoptotic function of mutant p53 by phosphorylation

06:30 p.m. **Moshe OREN**, *Rehovot,Israel*
Regulation of apoptosis by mutant p53

07:15 p.m. Dinner

Sunday, November 20

From function to therapy

08:45 a.m. **Antonio COSTANZO**, *Rome, Italy*
The p63-IKKalpha pathway in the control of keratinocyte proliferation

09:15 a.m. **Massimo LEVRERO**, *Rome, Italy*
To be announced

09:45 a.m. **Giovanni BLANDINO**, *Rome, Italy*
Mutant p53: an oncogenic transcription factor

10:15 a.m. Coffee break

10:45 a.m. **Tomoo IWAKUMA**, *Houston, USA*
Dominant-negative and gain-of-function phenotypes of Mutant p53 in vivo

11:15 a.m. **Matthias THEOBALD**, *Mainz, Germany*
Targeting p53 by therapeutic T cell receptors

11:45 a.m. **Galina SELIVANOVA**, *Stockholm,Sweden*
Reactivation of p53 by small molecule RITA

12:15 a.m. **Klas WIMAN**, *Stockholm,Sweden*
Novel small molecules for targeting mutant p53 in human tumors

01:00 p.m Lunch

02:00 p.m. Tour & Desert Fantasy Dinner

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PRESENTATION ABSTRACTS

PRESENTATION # 1

DOMAIN ORGANIZATION OF p53 IN SOLUTION

Alan R. Fersht

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p53 consists of four chains of 393 residues. Each chain has only two well-structured domains, with the N- and C- terminal domains being natively unfolded. The full-length protein has not been crystallised and perhaps the presence of so much unstructured protein will never allow the possibility of its being crystallised, especially in a form representative of its solution structure. The protein is also too large for conventional NMR studies. We are attempting to solve its structure and the effects of mutations by solving the structures of individual domains and their mutants and by biophysical methods in solution. In particular we are studying the structure of multi domain fragments of p53 and full-length protein by modern NMR methods to understand its structure and organization in solution.

PRESENTATION # 2

STRUCTURAL BASIS OF DIFFERENTIAL DNA RECOGNITION BY p53

Zippora Shakked, Malka Kitayner, Haim Rozenberg, Naama Kessler and Dov Rabinovich

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p53 acts as a transcription factor by binding as a tetramer to DNA targets containing two decameric motifs or half-sites of the general form: RRRCWWGYYY (R=A,G W=A,T Y=T,C). We obtained high-resolution crystal structures of functional sequence-specific complexes between the core domain of human p53 and different DNA half-sites. In all complexes, four p53 molecules assemble on two DNA half-sites to form a tetramer which is a dimer of dimers, whose symmetry coincides with the dyad symmetry of the DNA. The tetramer is stabilized by protein-protein interactions and by base-stacking interactions between half-sites. The protein-DNA interface varies as a function of the base sequence, thus providing a structural framework for understanding the origins of differential DNA recognition by p53.

PRESENTATION # 3

INTERFACIAL BEHAVIOR OF WILD TYPE AND MUTANT p53 PROTEINS

Emil Paleček, Luděk Havran, Vlastimil Dorčák, Sabina Billová, Marie Brázdová, Michal Masařík, Jan Vacek, Miroslav Fojta

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Peptides and proteins are electroactive substances, which can be sensitively analyzed by modern electrochemical methods [1]. To our knowledge we present the first report about the interfacial behavior of wild type and mutant p53 proteins. Wild type full length (fl) p53 protein as well as its isolated core domain (p53CD) and their mutants were analyzed using voltammetric and chronopotentiometric methods with carbon and/or mercury electrodes. p53CD produced a single anodic peak due to oxidation of tyrosine and tryptophan residues. With mercury electrodes a wealth of electrochemical signals were measured, including the Brdicka's catalytic signals (involving cysteine residues), capacitive signals due the protein adsorption/desorption behavior, signals due to reduction of the Hg-S bond and sensitive chronopotentiometric peak H due to the ability of proteins to catalyze the hydrogen evolution at highly negative potentials. Large differences in the intensity of this peak were observed between wt fl p53 and p53CD. Differences between wt and mutant proteins [fl p53 R273H (DNA contact) and G245S (DNA-binding region)] as well as p53CD mutants R248W and R282W (both DNA binding region) in all electrochemical signals were observed. We find it interesting that a single amino acid exchange in the proteins substantially changed the electrochemical behavior of the p53 proteins. More detailed studies will be necessary to elucidate the interfacial behavior of p53. The proposed methods of electrochemical analysis of p53 proteins appear as promising tools in p53 research. These methods make it possible to analyze microliter volumes of p53 at nanomolar concentrations.

Acknowledgement. The authors are grateful to Prof. Z. Shakked for purified samples of wt and mutant p53CD proteins. This work was supported by a Grant IAA 500040513 of the Grant Agency of the Academy of Sciences of the Czech Republic and by an EC Integrated Project No. 502983.

References

[1] E. Palecek, in E. Palecek, F. Scheller, J. Wang (Eds.) Electrochemistry of nucleic acids and proteins. Toward sensors for genomics and proteomics, Elsevier, Amsterdam, 2005, in press

PRESENTATION # 4

Carol Prives

PRESENTATION # 5

GENOME-WIDE APPROACH FOR THE IDENTIFICATION OF NEW P53 TARGET GENES

Itamar Simon, Helena Medvadovsky, Idit Shif and Zahava Kluger

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The genome-wide location analysis (GWLA) approach allows the identification of most the promoters a transcription factors is bound to. The advantage of this unbiased approach will be demonstrate by a study that use a modification of the GWLA approach for study DNA hypermethylation in cancer cells. Genome-wide analysis by this technique revealed that tumor-specific methylated genes belong to distinct functional categories, have common sequence motifs in their promoters and are chromosomally distributed in a clustered manner. In addition, many are already repressed in normal cells. These results are consistent with the hypothesis that cancer-related de novo methylation may come about through an instructive mechanism. Applying this technique for the identification of p53 target genes allowed us to compare its activity in various cell types and growth conditions.

PRESENTATION # 6

PROTEASOMAL DEGRADATION OF WT AND MUTANT P53 VIA THE UBIQUITIN INDEPENDENT PATHWAY IS REGULATED BY NQO1

Gad Asher, Peter Tsvetkov, Julia Adler and **Yosef Shaul**

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According to the current concept proteins are stable unless they are marked by poly-ubiquitination for degradation by the 26S proteasomes. A new twist in the concept became evident while studying the degradation of the tumor suppressor p53, a protein that appeared to satisfy this principle. We have discovered that native p53 is also prone to ubiquitin-independent 20S proteasomal degradation, suggesting that certain proteins are inherently unstable.

This process of degradation is mediated by 20S proteasomes and inhibited by NADH quinone oxidoreductase 1 (NQO1). NQO1 binds and stabilizes p53. Binding of NQO1 to p53 is augmented in the presence of NADH and inhibited by dicoumarol, an inhibitor of NQO1, which competes with NADH. Dicoumarol and several other inhibitors of NQO1 activity induce proteasomal degradation of p53 and inhibit p53-dependnet apoptosis. NQO1 regulates p53 stability in living cells under normal conditions. In accordance, knockdown of NQO1 with specific NQO1 siRNA reduces basal p53 levels.

Furthermore, NQO1 null mice exhibit reduced p53 protein levels and decreased apoptosis in the bone marrow. Analysis of the susceptibility of wild type and different p53 mutants revealed that the most frequent “hot spot” p53 mutants in human cancer, R175H, R248H and R273H, are resistant to dicoumarol-induced degradation.

These mutants remain sensitive to Mdm2-ubiquitin mediated proteasomal degradation. Remarkably, these “hot spot” p53 mutants show increased binding to NQO1 compared to wt p53. Dicoumarol does not disrupt the binding of these “hot spot” p53 mutants to NQO1 and therefore fails to induce degradation of these mutants. The ability of these p53 mutants to efficiently resist NQO1 regulated 20S proteasomal degradation may explain the relatively high steady state expression levels of these mutants in the cells.

PRESENTATION # 7

PHOSPHORYLATION-DEPENDENT INHIBITION OF HAUSP-MEDIATED DEUBIQUITINATION CONTRIBUTES TO DNA DAMAGE-INDUCED DESTABILIZATION OF Hdmx AND Hdm2

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The p53 tumor suppressor protein has a major role in protecting the integrity of the genome. In unstressed cells p53 is maintained at low levels by the ubiquitin/proteasome pathway. A balance between ubiquitin ligase activity (Hdm2, COP1, Pirh2) and the ubiquitin protease activity of HAUSP determines the half-life of p53. HAUSP also modulates p53 stability indirectly by deubiquitination and stabilization of Hdm2.

Upon DNA damage, activity and abundance of p53 is increased. For correct activation of p53 a temporal destabilisation of Hdm2[1] and degradation of Hdmx[2] appears to be essential. We have recently found that multiple phosphorylations, at least partly ATM-dependent, are needed for efficient DNA damage-induced increased ubiquitination and degradation of Hdmx[3]. However, via which mechanism the Hdmx ubiquitination is increased upon DNA damage remained unknown.

We now report that HAUSP also interacts with Hdmx, resulting in its direct deubiquitination and stabilization. HAUSP activity is required to maintain normal Hdmx protein levels. Upon DNA damage, the interactions between Hdmx/HAUSP and Hdm2/HAUSP are disrupted, resulting in impaired deubiquitination of Hdmx/Hdm2. This effect can explain the DNA damage-induced degradation of Hdmx and transient instability of Hdm2. We further show that phosphorylation of Hdmx mediates its dissociation from HAUSP, and that treatment of cells with caffeine prevents the dissociation of HAUSP from Hdmx and Hdm2[4]. These results implicate HAUSP in the activation of p53 after DNA damage by controlling two important p53-regulators, Hdmx and Hdm2.

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[2] Kawai et al., J. Biol. Chem. 278, 45946-45953, 2003
[3] Pereg et al., Proc. Natl. Acad. Sci. USA, 102, 5056-5061, 2005.
[4] Meulmeester et al., Mol Cell 18, 565-576, 2005.

PRESENTATION # 8

Curtis Harris

PRESENTATION # 9

DNA DAMAGE RESPONSE AS AN ANTI-CANCER BARRIER THAT SELECTS FOR p53 MUTATIONS

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The fact that p53 is one of the most commonly mutated genes in human tumours is well established. On the other hand, the molecular basis of the stimuli that initially lead to p53 activation in early stages of tumorigenesis in vivo is poorly understood. Our results show that the cellular machinery that responds to DNA damage is intimately involved in the process of tumorigenesis of major types of human cancer, and that constitutive activation of the DNA damage-activated cell cycle checkpoints creates an environment that provides a major selection pressure to favour survival and growth of tumour cells that have lost, mutated or otherwise inactivated the p53 tumour suppressor. The presentation will summarize our recent studies supporting a concept of the DNA damage response network as an inducible barrier against progression of early stages of human tumours in vivo, and in response to various oncogenes in cell culture models. We will present evidence for the feasibility of monitoring the activation of diverse components of the DNA damage response machinery directly on clinical tumour specimens and screening for such responses in a large spectrum of normal human tissues using optimized immunohistochemical approaches. This is complemented by a combination of genome-wide genetic analyses, as well as biochemical, genetic and cell biology approaches on both cell culture models of inducible oncogenes such as cyclin E, and analyses of clinical material from diverse stages of development of human carcinomas of the urinary bladder, breast, lung and colon, and testicular germ-cell tumours. The data show that the maximal constitutive activation of the DNA damage response precedes the occurrence of various defects within the ATM-Chk2-p53 pathway, including missense mutations and LOH in p53. The consistent patterns of constitutive activation of the DNA damage response network in early lesions of the epithelial tumours contrast with the less apparent activation seen in carcinoma in situ as well as advanced lesions among the testicular germ-cell tumours, a tumour type in which p53 mutations occur only rarely. These distinct patterns of DNA damage response activation seen in diverse types of human malignancies will be discussed in terms of their biological significance as well as the potential cellular and molecular basis. A model of how oncogene activation and/or loss of tumour suppressors may result in activation of the DNA damage checkpoints with the subsequent cell cycle arrest or cell death, and implications of this concept for oncology including the concept of multistep tumour development and relevance for cancer treatment, will conclude the presentation.

Selected references:
Bartkova J. et al., *Nature* 434: 864-870, 2005; Kastan M. and Bartek J. *Nature* 432: 316-323, 2004.

PRESENTATION # 10

TP53 MUTATIONS AS BIOMARKERS FOR CANCER DETECTION AND PROGNOSIS

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Despite the accumulation of studies on TP53 mutations, their significance for cancer detection and prognosis remains elusive. One of the reasons for this is the functional heterogeneity of mutations. We have recently developed studies aimed at better understanding the lessons that can be learned from TP53 mutation detection. In breast cancer, a multi-cohort study on 1794 patients has confirmed that mutation was an independent predictor of overall survival, with a particularly strong effect in patients who retain ER/PR expression. In liver cancer, mutations can be found ahead of diagnosis, in free DNA fragments retrieved in the plasma, thus providing a possible mean for early cancer detection. In Western Africa, a region where chronic infection by HBV and dietary contamination by aflatoxin is widespread, the so-called « aflatoxin-signature » mutation at codon 249 is found in the plasma DNA of chronic HBV carriers, with seasonal variations reflecting variations of exposure to aflatoxin. Thus, in this case, plasma TP53 mutation appears to be a biomarker of exposure to a mutagen, rather than of early cancer development.

Another factor of complexity is the possible impact of deltaN isoforms of p53 family members. In keratinocytes infected by skin-type HPVs such as HPV38, expression of deltaNp73 contributes to inhibit p53 activity and to protect cells against p53-dependent apoptosis during virus-induced immortalization. Thus, expression of isoforms may, in specific contexts, provide alternative mechanisms to down-regulate p53 function during progression towards cancer.

PRESENTATION # 11

A SNP IN THE PROMOTER OF THE VEGF RECEPTOR 1 (FLT-1) REVEALS NEW "RULES OF THE GAME" FOR P53 TARGET RESPONSE ELEMENTS

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The VEGF system is essential for angiogenesis. VEGF overexpression frequently correlates with increased microvascularity, metastasis and with decreased spontaneous apoptosis. Although a precise mechanism has not been established, studies suggest that VEGF expression is negatively regulated by p53, a master regulator and tumor suppressor. There are no reports of additional components of the VEGF signal transduction pathway being part of the p53 transcriptional network.

A target of VEGF, the VEGF receptor-1/flt-1 can regulate growth and migration of endothelial cells and modulate angiogenesis. VEGF appears to be up-regulated in various cancers where flt-1 may have a role in tumor progression and metastasis. We identified a C to T SNP upstream of the transcriptional start site in ~6% of the people examined. The SNP is located within a putative p53 response element. Only the promoter with the T SNP (FLT1-T) was responsive to p53 when examined with reporter assays or by endogenous gene expression analysis in cell lines with different SNP status. In response to doxorubicin-induced DNA damage there was clear allele discrimination based on p53 binding at the FLT1-T but not FLT1-C promoters as well as p53-dependent induction of flt-1 mRNA, which required the presence of FLT1-T.

Our results establish that p53 can differentially stimulate transcription at a polymorphic variant of the flt-1 promoter and directly places the VEGF system in the p53 stress-response network via flt-1 in a significant fraction of the human population. We suggest that the p53-VEGF-flt-1 interaction is relevant to risks in angiogenesis-associated diseases including cancer.

Interestingly, the functional p53 responsive FLT1-T SNP in the FLT1 promoter was not identified in our previous search for SNPs in p53 target response elements [PNAS 102(2005) 6431] utilizing computational approaches and functionality rules developed with yeast and human cells. With the FLT1-T SNP we establish the concept of synergy between a p53 half site and other target response elements.

PRESENTATION # 12

MOUSE BITES DOGMA: MOUSE MODELS PROVIDE NEW INSIGHTS INTO THE MECHANISMS THAT CONTROL THE P53 STRESS RESPONSE PATHWAY

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p53 is a short-lived transcription factor that has evolved to respond to a variety of stimuli to initiate cell cycle arrest or apoptotic programs, or to induce genes that participate in some forms of DNA repair. As p53 participates in life and death decisions, it is critical that its output be stringently regulated. The importance of the p53 pathway for tumor suppression is demonstrated by mutation of the p53 gene or alterations in its negative regulators in almost all cancers. We first used homologous recombination in mouse ES cells to critically test whether highly conserved C-terminal lysine residues are essential for degradation control and for activation. The surprising data to be shown demonstrate that the C-terminal modifications hypothesized to be critical for proper p53 regulation are not essential, but rather contribute to a fine-tuning mechanism of homeostatic control *in vivo*. The second model explores the p53 negative regulators MDM2 and MDMX. We made a series of mutations that either remove the proline-rich domain (i.e., p53ΔP), substitute alanine for proline, or mutate two Pin1 sites thought to be essential for binding of Pin1 to generate a conformational change that affects p53 binding by Mdm2. The data support the notion that the proline rich domain is an important determinant of Mdm2 binding and p53 stability control, but they show that it is more likely a spacer than a conformational switch. Interestingly, in vivo, p53 deleted for the proline rich domain is able to induce apoptosis, but not cell cycle arrest, which is the opposite of the phenotype described *in vitro*. Most importantly, we were able to rescue the lethality caused by MdmX deficiency, but not Mdm2 deficiency, with p53ΔP. This provided us with an opportunity to study the contributions of Mdm2 and MdmX to controlling p53, as well as to regulating each other. The data to be shown indicate that MdmX plays a significant role in controlling p53 transcriptional output, but it has no effect on controlling Mdm2 stability *in vivo*. Together, these mouse models suggest the need to revise our current concepts of how p53 is regulated by Mdm2 and MdmX. One new model consistent with the *in vivo* data will be discussed.

PRESENTATION # 13

STUDY OF KEY UPSTREAM MODULATORS AND DOWNSTREAM EFFECTORS OF THE P53 PATHWAY USING MOUSE MODELS

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The two structurally related proteins Mdm2 and Mdm4 function in a non-redundant manner to restraint the growth suppressive activity of p53 during embryonic development. To further understand whether Mdm2 and Mdm4 regulates p53 function in a tissue specific manner *in vivo* and to gain mechanistic insights on the Mdm4-Mdm2-p53 interplay, we generated mouse models allowing us to control the spatiotemporal expression of p53 in cells in vitro and tissues in vivo lacking either *mdm2*, *mdm4* or both. Using this strategy, we show that Mdm2 and Mdm4 are essential, in a non-redundant manner, for preventing p53 activity in the same cell type, irrespective of the proliferation/differentiation status of the cells. While Mdm2 prevents accumulation of the p53 protein, Mdm4 contributes to the overall inhibition of p53 activity independent of Mdm2. We propose a model in which Mdm2 is critical for the regulation of p53 levels and Mdm4 for the fine-tuning of p53 transcriptional activity, both proteins acting synergistically to keep p53 in check.

PRESENTATION # 14

THE ROLE OF THE PROLYL-ISOMERASE PIN1 IN REGULATING THE P53 PROTEIN FAMILY NETWORK: A FINE TUNER OR A DANGEROUS AMPLIFIER?

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Phosphorylation-directed prolyl isomerization is emerging as an important mechanism for fine tuning of complex signaling networks. This is based on the action of the prolyl isomerase Pin1, which recognizes and isomerizes phosphorylated Ser/Thr-Pro motifs on target proteins thereby modulating their activities. Pin1 has been implicated in the regulation of major cellular events, such as cell cycle progression, transcription, proliferation and apoptosis. We, and others, have previously shown that Pin1 regulates the checkpoint functions of wt p53. However, the majority of tumor cells bear mutant p53 while Pin1 expression becomes often deregulated. Importantly, mutant p53 forms are also phosphorylated on S/T-P sites and are recognized by Pin1 in tumor cells. Therefore, Pin1 may also regulate the pro-oncogenic activity of mut p53, adding a further level of complexity to the oncogenic process.

p53 contains six Pin1-consensus sites at Ser33, Ser46, Thr81, Ser127, Thr150 and Ser315. We are aimed at better characterizing the role of these phosphorylation events in regulating p53 functions, as well as examining how Pin1 becomes involved in these processes both in normal cells under different stress conditions as well as in tumor cells. We have constructed a panel of p53 phosphorylation mutants (Ser/Thr to Ala), and generated inducible cell lines expressing these proteins at physiological levels. We will present evidences that Pin1 regulates the timing of wt p53 activation upon phosphorylation events triggered by diverse stimuli, by rendering it suitable for subsequent modifications, in particular acetylation of C-terminal residues, and modulating its interaction with DNA and cofactors.

Mutant p53 forms R175H, R248W and R273H were also analyzed by this approach, revealing that phosphorylation of residues S33 and S46 and the polymorphism on codon 72 affect Pin1 binding. In contrast to wt p53, Pin1 did not appear to play a role in stimulating the transactivation of mutant p53 target genes (e.g, EGR1). The interaction between p73 and mtp53 was also studied, and data will be presented on the different behaviour of mtp53 proteins devoid of Pin1 binding sites in this assay.

PRESENTATION # 15

DEATH WITHOUT STRESS

Jo Milner

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The functioning of pro- and anti-apoptotic genes has largely been studied under conditions of applied stress. In order to investigate the balance of apoptotic forces under basal conditions, we have employed RNA interference induced by siRNAs. The p53 protein was used as a stress senor and monitored by stabilisation of p53 protein, phosphorylation at serine 15, and up-regulation of p53 target genes such as p21 and HDM2. No activation of p53 was observed under the conditions of the experiments. Controls for selective RNAi knock-down included (i) at least two distinct siRNAs for the target mRNA; (ii) demonstration that RNAi *per se* does not induce apoptosis in the cell models; (iii) quantitative PCR for mRNAa know-down plus immunoblotting for protein encoded by each targeted mRNA; and (iv) phenotypic monitoring of the siRNA-treated cells. The effects of a single dose of siRNA, were monitored up to 96 hours.

Clear-cut differences between epithelial cells of cancerous and non-cancerous origin were observed. For example, silencing SIRT1 (an NAD-dependent deacetylase) induced massive apoptosis in cancer cells but was without affect in non-cancer cells. Co-silencing experiments revealed that SIRT1 and Bcl-2 suppress separate apoptotic pathways in the same cancer cells: the “Bcl-2 pathway” being dependent upon p53, Bax and caspase 2, whilst the “SIRT1 pathway” was independent of these three pro-apototic mediators. However, both pathways converge and require Foxo4 (a member of the Forkhead family of transcription factors) for apoptosis.

Jiang and Milner (2003) *Genes and Development* 17: 832-837
Ford, Jiang and Milner (2005) *Cancer Research* (in press).

PRESENTATION # 16

A ROLE FOR PML IN THE REGULATION OF P53 BY CK1

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Several mechanisms have been demonstrated to explain the protection of p53 from Mdm2 upon exposure of cell to stress, such as DNA damage. A dominant mechanism involves post-translational modifications of both p53 and Mdm2. Of special importance are modifications that modulate the p53/Mdm2 interaction. We have previously described the effect of Ser20 phosphorylation on the protection of p53 from Mdm2. This modification is enhanced by the action of PML, which recruits both p53 and Chk2 into the PML nuclear bodies in response to DNA damage. We found that PML is critical for the accumulation of p53 in response to DNA damage under physiological conditions. PML protects p53 from Mdm2-mediated ubiquitination and degradation and from inhibition of apoptosis. Phosphorylation of p53 on a nearby site, Thr18, has been proposed to be even more critical in the modulation of the p53/Mdm2 interaction. We therefore examined whether PML regulates this modification in response to stress. We report here that PML enhances the phosphorylation of p53 by CK1, and this phosphorylation is important for the protection of p53 by PML in cells exposed to DNA damage. We will describe the interplay between PML and CK1 in the regulation of p53 in response to stress.

PRESENTATION # 17

cancelled

PRESENTATION # 18

MUTANT P53: ONCOGENIC BY PERTURBING CHROMATIN STRUCTURE AND FUNCTION?

Marie Brázdová (1,2), Korden Walter (1), Genrich Tolstonong (1), and **Wolfgang Deppert (1)**

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Mutp53 proteins have lost wild type p53 sequence-specific DNA binding, but have retained the ability to interact in a structure-selective manner with non-B DNA elements (MAR/SAR elements, supercoiled DNA and stem-loop structures). We previously suggested that the high affinity binding of mutp53 proteins to MAR/SAR-elements, which constitute higher order regulatory elements of the chromatin, relates to the oncogenic functions of mutp53. Using chromatin immunoprecipitation, we established a library of genomic sequences bound by mutp53 in human glioblastoma cells *in vivo*. Sequence analysis revealed that such sequences display characteristics of MAR/SAR elements. About 50-60 % of the sequences were derived from regulatory intronic regions of genes associated with tumorigenesis. Knock-down experiments using siRNA led to altered expression (up- or down-regulation) of genes associated with mutp53 binding. We propose a model that attributes the oncogenic functions of mutp53 to its ability to interact with MAR/SAR elements, thereby increasing epigenetic and genetic instability of tumor cells. Such instability may provide an evolutionary advantage to tumor cells expressing mutp53, resulting in advanced tumor progression.

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PRESENTATION # 19

AMPLIFICATION[1] OF p53 INDUCED "SIGNATURE GENES" IN CELLS TRANSFORMED IN VITRO

Varda Rotter[1] , Michael Milyavsky[1], Yuval Tabach[1,2], Igor Shats[1], Neta Erez[1], Yehudit Cohen[1], Xiaohu Tang[1], Marina Kalis[1], Yossi Buganim[1], Naomi Goldfinger[1], Doron Ginsberg[1], Eytan Domany[2] and Yitzhak Pilpel[3]

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It is well accepted that inactivation of p53 plays a pivotal role in malignant transformation of cells. However, the networks of transcription programs that are modulated following p53 inactivation are still unknown. To decipher these, we subjected hTERT immortalized cells that were gradually transformed *in vitro* by p53 inactivation in conjunction with other oncogenic stress signals such as loss of the INK4A locus and *ras* over expression, to an extensive bioinformatics analysis. A genome wide expression profiling identified distinct genetic signatures corresponding to the genetic alterations listed above. Most importantly, unique cellular phenotypes, such as differentiation block, aberrant mitotic progression, increased angiogenesis and invasiveness were identified and coupled with genetic signatures assigned for the genetic alterations in the p53, INK4A locus, and H-Ras respectively. Furthermore, a transcriptional program that defines the cellular response to p53 inactivation was an excellent predictor of metastasis development and bad prognosis in breast cancer patients. Deciphering universal transcriptional programs, which are affected by the most common oncogenic mutations, provides considerable insight into regulatory circuits controlling malignant transformation and will, hopefully, open new avenues for rational therapeutic decisions.

PRESENTATION # 20

Xin Lu

PRESENTATION # 21

REGULATION OF APOPTOSIS BY MUTANT P53

Alexander Damalas[1,2], Lilach Weisz[1], Amir Zalcenstein[1], Perry Stambolsky[1], Massimo Levrero[2], Sabrina Strano[2], Vassilis G. Gorgoulis[3], Giovanni Blandino[2], Varda Rotter[1] and **Moshe Oren[1]**

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[3]*Molecular Carcinogenesis Group, Dept. of Histology and Embryology, School of Medicine, University of Athens, Greece*

Besides abrogating the tumor suppressor activity of the wt p53 protein, some of the frequently encountered tumor-associated mutant p53 isoforms often also elicit a pro-oncogenic gain of function. One of the manifestations of this gain of function is increased resistance to killing by anti-cancer agents.

We provide evidence that at least some of the anti-apoptotic gain of function of mutant p53 is due to its ability to modulate the transcription of apoptosis-related genes. One such example is CD95/Fas, a death receptor whose gene is subject to transcriptional repression by mutant p53. Another mutant p53-respressed gene is MSP (macrophage stimulating protein). While the literature suggests that MSP has anti-apoptotic effects, we show that, in cultured tumor cells, it actually contributes to apoptosis. Analysis of MSP gene expression in tumors supports the notion that its downregulation may benefit at least some human tumors.

Most notably, mutant p53 contributes to activation of NF-kB in cancer cells, resulting in enhanced induction of anti-apoptotic genes and increased resistance to apoptosis. The underlying mechanism may involve recruitment of mutant p53 protein to genomic NF-kB binding sites, which may be facilitated by a physical interaction between mutant p53 and NF-kB. NF-kB is a well-documented contributor to cancer development and therapy resistance. Its hyperactivation by mutant p53 may thus provide substantial benefits to tumors.

Thus, mutant p53 may constitute an important target for future anti-cancer therapies.

PRESENTATION # 22

Cancelled

PRESENTATION # 23

THE P63-IKKALPHA PATHWAY IN THE CONTROL OF KERATINOCYTE PROLIFERATION

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The P63 gene belongs to the p53 gene family of tumor suppressor genes and encodes for sequence specific transcription factors. The DNp63 protein, lacking the canonical transactivation domain, has been proposed to identify the epidermal stem cells and to regulate their survival and self renewal. In an attempt to identify new potential p63 targets, we have found that the epidermal morphogenesis modulator IKKalpha is tightly regulated at the transcriptional level by different isoforms of p63. We have observed that IKKa mRNA expression increases at the onset of the stratification program. The selective downregulation of DNp63 by siRNA in primary keratinocytes abolishes IKKa activation and the onset of terminal differentiation and increases keratinocyte proliferation rate. This is consistent with transcriptional assay data performed with an IKKa-promoter reporter gene showing a strong activation of IKKa transcription by DNp63 isoforms and a repressory activity of TAp63 isoforms. On the other hand, the downregulation of IKKa in primary keratinocytes hampers the ability of differentiating cells to exit the cell cycle inducing the formation of a K1 expressing proliferative layer that is reminiscent of the embryonic intermediate layer and of squamous cell carcinoma. Our results indicate IKKa as an important p63 target potentially involved in the control of epidermal stem cells proliferation in skin development and in cell transformation.

PRESENTATION # 24

PCAF-hSirt1 INTERACTION REGULATES P1p73 PROMOTER ACTIVITY AND APOPTOSIS IN RESPONSE TO DNA DAMAGE.

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 # Dept. of Internal Medicine - University of Rome "La Sapienza", Rome, Italy

The p53 paralog p73 is now recognized as a major player in DNA damaging drugs induced apoptosis and tumor chemosensitivity (Flores et al., 2002; Bergamaschi et al., 2003; Irwin et al., 2003, Costanzo et al., 2002). We previously reported that the P1p73 promoter is activated following doxorubicin treatment in a E2F1-dependent manner (Pediconi et al., 2003). By chromatin immunoprecipitation (ChIP) assays we showed that, in response to DNA damage, E2F1 and the PCAF acetyltransferase are selectively recruited on the P1p73 promoter, but not on the E2F-dependent and cell cycle regulated Tk and DHFR promoters. We also found that, in response to DNA damage, E2F1 is acetylated in vivo, that acetylation on lysines 117, 121, 125 is required for the selective recruitment of E2F1 on the P1p73 promoter and that abrogation of pCAF protein by PCAF-specific siRNA abolishes P1p73 activation. Here we show that, in addition to E2F4 and HDAC1, both E2F1 and PCAF are bound in vivo to the P1p73 promoter in proliferating cells the treatment with PCAF-specific siRNAs results in a reproducible 2-3 fold increase in the basal activity of the P1p73 promoter, suggesting that PCAF might be involved both in positive and negative regulatory events. We found that hSirt1, the human homologue of the Sir2 class III deacetylase, forms a trimolecular complex with PCAF and E2F1 and is recruited in vivo onto the P1p73 promoter. We show that hSirt1 represses E2F1-dependent transcription of the P1p73 promoter whereas the Sir2-inhibitor nicotinamide (NAM) potentiates E2F1-dependent activation of the P1p73 promoter and its activation in response to DNA damaging drugs. Interestingly, neither the E2F1-dependent activation nor the basal activity of the DHFR promoter are affected by hSirt1. Accordingly, inhibition of hSirt1 activity by NAM activates selectively transcription from the endogenous P1p73 vs the DHFR promoter. We next investigated whether hSirt1 might affects PCAF activity in vivo. P/CAF is both autoacetylated and a target for p300 acetylation in vivo, resulting in increased protein and enhanced histone acetyltransferase activity. We found that overexpression of Sir2 and the treatment with resveratrol (RES), a compound that activates Sir2/hSirt1, abrogate PCAF basal acetylation and NAM increases P/CAF acetylation in cells exposed to DNA damage. Finally, we show that doxorubicin-induced p53-independent apoptosis of SAOS2 cells is potentiated by nicotinamide and abrogated by RES. Our data identify the functional interaction between PCAF and hSirt1 as an important determinant for E2F1-dependent p73-mediated apoptotic responses.

(Supported in part by AIRC, MURST COFIN, COFIN PNR and EC grants)

PRESENTATION # 25

MUTANT P53: AN ONCOGENIC TRANSCRIPTION FACTOR

Silvia Di Agostino[1], Sabrina Strano[1], Velia Emiliozzi[1], Marcella Mottolese [2], Ada Sacchi[1], Giulia Piaggio[1,3], and **Giovanni Blandino**[1,3]

[1,2]Dept. of Experimental Oncology and Pathology, Regina Elena Cancer Institute, Rome; [5]Rome Oncogenomic Center, Rome

The p53 gene is the most frequent target of genetic alterations, being mutated in half of all human cancers. It has been shown that the resulting proteins do not represent the mere loss of wt-p53 mediated anti-tumoral effects, but gain new additional functions through which they contribute to the appearance, the maintenance and the spreading of malignant tumors. There is not yet exhaustive knowledge on how mutant p53 proteins exert gain of function activity. By using microarray analysis we have found that inducible expression of mutant p53His175 modulates positively or negatively sets of specific genes. The open question is whether these genes are direct targets of mutant p53. To investigate this issue we have thought about the possibility that mutant p53 protein could be gathered in transcriptional complexes. These could potentially include other transcription factors, specific co-activators, acetylases, deacetylases and scaffold proteins. We have recently found that endogenous mutant p53 can physically associate with the transcription factor NF-Y. The expression of cell cycle regulated genes, such as cyclin A, cyclin B, cdk1 and cdc25C, as well as cdk-1- associated kinase activities, that increase DNA synthesis, are up-regulated by the protein complex mutantp53/NF-Y. Mutant p53His175 binds NF-Y target promoters in vivo, and upon DNA damage, recruits p300 leading to histones acetylation. Consistent with this, the formation of the protein complex NF-YA and m-p53His175 allows the recruitment of p300, which results in the acetylation of both mutant p53 and NF-YA. The immunohystochemistry analysis of a selected group of colon cancer samples reveals that the protein expression of cyclinA and Cdk1 is increased in those bearing mutant p53 proteins. Our study suggests that aberrant regulation of transcription is one of the molecular mechanisms underlying gain of function activity of mutant p53.

The work at the Rome Oncogenomic Center is supported by AIRC

PRESENTATION # 26

DOMINANT-NEGATIVE AND GAIN-OF-FUNCTION PHENOTYPES OF MUTANT P53 IN VIVO

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We recently generated a mouse model for Li-Fraumeni syndrome by introducing an arginine to histidine mutation at codon 172 of the mouse *p53* gene. The heterozygous mutant $p53^{+/R172H}$ mouse showed a metastatic tumor phenotype, suggesting gain of function by the mutant $p53^{R172H}$. $p53^{+/R172H}$ derived tumors showed strong p53 staining by immunohistochemistry in about 60% of tumors, while p53 was not stable in normal tissues surrounding tumors from the $p53^{+/R172H}$ mice and from the $p53^{R172H/R172H}$ mice. A dominant-negative effect was also observed for p53R172H in embryos after □-radiation (IR)-induced DNA damage. However, the dominant-negative effect by the mutant $p53^{R172H}$ in embryo development in crosses with the *mdm2* null allele was not observed. These phenotypes lead us to hypothesize that the mutant $p53^{R172H}$ needs to be stabilized by some signals to exhibit its gain-of-function or dominant-negative phenotypes. We therefore examined p53 levels in normal tissues from $mdm2^{-/-}p53^{R172H/R172H}$ mice. These stained positively for p53, suggesting that the mutant p53 can accumulate with loss of *mdm2*. $Mdm2^{-/-}p53^{R172H/R172H}$ mice also showed earlier onset of spontaneous tumorigenesis and subsequent death compared to $p53^{R172H/R172H}$ mice. We further examined if the mutant p53 is stabilized after IR. After IR, p53 was high in embryonic brains of $p53^{R172H/R172H}$ mice. Taken together, the data suggest that mutant p53 stabilization by loss of *mdm2* and/or irradiation uncovers the gain-of-function and/or dominant-negative phenotypes.

PRESENTATION # 27

TARGETING p53 BY THERAPEUTIC T CELL RECEPTORS

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Circumventing antigen-specific self-tolerance in HLA-A*0201 (A2.1) transgenic mice allows the generation of A2.1-restricted T cell antigen receptors (TCR) with high affnity for universal human tumor- and leukemia-associated peptide epitopes (TAA), such as p53. Retroviral transduction of human T lympocytes with wild-type and partially humanized mouse-derived TCR leads to the equipment of the otherwise tolerant T cell repertoire with effective cytotoxic T lymphocytes (CTL) with high avidity for a wide range of A2.1-positive tumors and leukemias. These results emphasize the successful preclinical establishment of TCR gene transfer as a novel molecular concept for the immunotherapy of malignant disease.

However, implementing TCR-based gene therapy of malignant disease requires further molecular optimization and research. To prevent or impair potentially harmful recombination of transduced and endogenous TCR chains, we have modified the interface of TCR a and b chains. Designing various single chain TCR constructs met the same purpose.

Developing CD8-independent TCR allowed not only the reprograming of $CD4^+$ T helper cells (Th), but also turned human $CD8^+$ CTL into hyper-avidity effector cells.

As the death versus survival fate of TCR transduced T cells is coregulated by conserved TCR transmembrane sequences, specific alterations within these TCR domains could create effector CTL equipped with beneficial properties for cancer immunotherapy, such as an increased proliferative capacity and prolonged survival.

PRESENTATION # 28

REACTIVATION OF THE p53 TUMOR SUPPRESSOR FUNCTION BY RITA

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In contrast to *in vitro* and *in silico* screens for p53 activators which are aimed at inhibition of HDM-2, cell-based screens are unbiased as to the mechanism of p53 activation. Therefore cell-based screens might help to discover compounds that activate p53 via yet unknown pathways and provide information about novel molecular mechanisms regulating p53. We have identified a small molecule named RITA (reactivation of p53 and induction of tumor cell apoptosis) in our cell-based screen aimed to find compounds that re-activate p53 growth suppressor function in tumor cells *in vitro* and *in vivo*. We have shown that RITA binds the N-terminus of p53 and induces a conformational change which prevents the binding of several p53 inhibitors, including HDM-2 (Issaeva et al, Nature Medicine, 10, p. 1321, 2004).

RITA induces accumulation of p53 in various types of tumor cells, restores the transcriptional activity of p53 and induces apoptosis in tumor cells of different origin in a wtp53-dependent manner. The specificity of RITA-induced response was demonstrated using DNA microarray analysis comparing the gene expression profiles after RITA treatment in isogenic lines which differ only in their p53 status. The great majority of differentially expressed genes were observed in wtp53 cells, including a number of known p53 targets. In contrast, gene expression in p53-null cells was largely unaffected by RITA.

Notably, the ability of RITA to induce p53 and p53-dependent biological response depends on the presence of oncogenes. The growth of non-transformed cells is not suppressed by RITA, even at high concentrations, whereas the same cells ectopically expressing c-Myc become sensitive to RITA. This correlates with only transient p53 induction by RITA in the absence of oncogenes.

RITA was able to restore the growth suppression function to His-273 mutant p53 in several cancer cell lines. RITA induced wild type conformation of mutant p53 in cells, followed by induction of p53 target genes. Unexpected mechanism of p53 activation discovered with the help of RITA might help to open a completely novel avenues for further research aimed at p53 reactivation.

PRESENTATION # 29

NOVEL SMALL MOLECULES FOR TARGETING MUTANT p53 IN HUMAN TUMORS

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Mutant p53 is an appealing target for novel cancer therapy. Reactivation of mutant p53 in tumors should trigger massive apoptosis and thereby rapidly eliminate the tumor. Screening of chemical libraries have led to the discovery of several small molecules that target mutant p53. PRIMA-1 and its methylated form PRIMA-1MET can restore mutant p53 conformation and function, induce apoptosis through the mitochondrial pathway preferentially in mutant p53-expressing tumor cells, and inhibit human tumor xenograft growth in mice upon systemic administration. PRIMA-1MET acts synergistically with anticancer drugs to inhibit tumor cell growth, both in vitro and in vivo. Enhancement of mutant p53 expression levels by DNA-damaging chemotherapeutic drugs may increase sensitivity to PRIMA-1-induced apoptosis. Screening of a series of structural analogs of PRIMA-1 have allowed identification of analogs that are equally or even more potent than PRIMA-1 itself. Another small molecule, MIRA-1, is structurally different from PRIMA-1 but shows similar activity. MIRA-1 preserves native conformation and DNA binding of wild type p53, and stimulates DNA binding of certain mutant forms of p53. MIRA-1 also induces mutant p53-dependent cell death in human tumor cells, although with more rapid kinetics than PRIMA-1. The structural analog MIRA-3 has anti-tumor activity in vivo against human tumor xenografts carrying mutant p53. Thus, the MIRA scaffold is a novel lead for the development of mutant p53-targeting anticancer drugs. Reactivation of mutant p53 by small molecules alone or in combination with conventional chemotherapeutic drugs is a new strategy for cancer therapy that should allow efficient eradication of tumors that express mutant p53.

POSTER ABSTRACTS

POSTER # 1

CONTRIBUTION OF p53 DNA BINDING DOMAIN TO p53 STABILITY

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There are five regions – boxes I-V - in p53 protein which reveal strikingly high conservation between species from human to rainbow trout, suggesting they are functionally important. Box I comprises the binding site of mdm2, which supports the importance of negative regulation of p53 by mdm2. Boxes II-V, residing in DNA binding domain of p53 are the most frequent sites of mutations found in human tumours. p53 mutants with deletions of conserved boxes II-V behave like tumor derived point mutants in this region in that they fail to bind DNA and are therefore not transcriptionally active. In addition, these mutants adopt a conformation associated with tumor-derived p53 mutants.

p53 point mutants are stable in human tumors and expressed at higher levels in tumor cells compared to wild-type p53. However, recent studies of mutant p53 knock-in mice suggest that these p53 proteins are not stable in normal tissue, indicating that a failure to activate expression of Mdm2 is not the underlying cause of mutant p53 stability in tumors. Another possibility is that the stability of mutant p53 is related to the over-expression of ARF. However, out initial studies have suggested that inhibiting ARF expression does not reduce the stability of mutant p53 in tumor cell lines.

Although mutant p53 has been reported to be sensitive to Mdm2-mediated degradation, our previous studies showed that mutations in the DNA binding region could render p53 resistant to degradation by HPV E6. We therefore examined the stability of mutant p53 proteins more closely. Consistent with previous reports, we found that conserved box II-V deletion mutants are degraded by Mdm2 to the same extent as wild type p53 when over-expressed in cells. We also show that p53 deletion mutants are not defective in the ability to bind to YY1, which efficiently promotes mdm2-mediated degradation of p53 deletion mutants. Furthermore, although each of the p53 mutants showed an ability to interact with p73, as described for p53 point mutants, this interaction did not appear to contribute to the stabilization of the mutant p53 protein. However, in *in vitro* assays the p53 deletion mutants are less efficiently ubiquitinated by Mdm2 compared to wild type p53. These results suggest that the degradation of mutant p53s by Mdm2 may not be dependent on efficient ubiquitination. Interestingly, significantly higher levels of ubiquitination of each of the mutant p53s were found in Mdm2 null cells compared to wild type p53, suggesting that the mutants may acquire an increased sensitivity to other E3 ligases such as Cop1 or Pirh2.

POSTER # 2

A PROMISCUOUS, HIGHLY ELECTROSTATIC, PROTEIN-BINDING SITE IN p53 CORE DOMAIN

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Many protein-protein interfaces contain regions of residues that are crucial for the affinity to other proteins and are promiscuous. These regions are highly adaptable, and many proteins use them to bind numerous partners. Here we show that the DNA-binding interface in the core domain of the tumour suppressor p53 serves as such a multipurpose promiscuous protein-binding site. We performed peptide mapping of several proteins that were reported to bind p53 core domain, and used analytical ultracentrifugation and fluorescence and NMR spectroscopy to study binding of the peptides to p53 core domain. We have found that peptides

corresponding to residues 490-498 of 53BP2 [1] and to residues 179-190 of Rad51 [2] bind to the p53 core domain in a site that overlaps with its specific DNA binding site. The p53 site is promiscuous as it also binds proteins such as 53BP1, Hif-1α and BCL-X_L in overlapping regions. Studies of the ionic-strength dependence of peptide binding by this site revealed that it is mediated mainly by a strong, non-specific, electrostatic component, and is fine-tuned by specific interactions. Competition of the different proteins with each other and with specific DNA for a single site in p53 could be a key factor in regulation of its activity.

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POSTER # 3

STRUCTURES OF p53 CANCER MUTANTS AND MECHANISM OF RESCUE BY SECOND-SITE SUPPRESSOR MUTATIONS

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Most cancer-associated p53 mutations are located in the DNA-binding core domain of the protein. On the basis of their location in the structure and their effect on stability and DNA binding, these cancer mutations have been classified as “structural” and “contact” mutations. We have solved high-resolution crystal structures of a number of oncogenic mutants of the p53 core domain. The mutations were introduced into a stabilized variant containing four point mutations (M133L, V203A, N239Y, and N268D) that has an identical structure to wild type, apart from the mutated side chains. Our structures reveal that the cancer hot-spot mutation R273H, for instance, simply removes an arginine involved in DNA binding, leaving the overall architecture of the DNA-binding surface intact. In contrast, the “structural” oncogenic mutations H168R and R249S induce substantial structural perturbation around the mutation site in the L2 and L3 loops, respectively. The R249S mutation disrupts a number of interactions that stabilize the conformation of the L3 loop and induces partial misfolding of this loop. H168R is a specific intragenic suppressor mutation for R249S. When both cancer mutations are combined in the same molecule, Arg168 mimics the role of Arg249 in wild type, and wild-type conformation is largely restored, resulting in restoration of DNA binding to wild-type levels. Our structural and biophysical data elucidate the different mechanisms of rescue of mutant p53 by “global” and “specific” second-site suppressor mutations and reveal features by which proteins can adapt to deleterious mutations.

POSTER # 4

PREDICTING THE FUNCTIONAL IMPACT OF MISSENSE SUBSTITUTIONS IN THE DBD OF p53 USING AN APPROACH BASED ON DELAUNAY TESSELLATIONS

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Because single point mutations in the tumor suppressor protein p53 have a high incidence in cancer, further understanding their structural and functional impact is important. We present a novel approach, based on Delaunay tessellations, for predicting the functional impact of missense mutations in the DNA binding domain (DBD) of p53 and compare our predictions with experimental measurements of transactivation activity of the mutants. The transactivation activity was measured, using a yeast assay, for all possible mutants in the DBD of p53 across 8 p53 response elements. For each residue, Residual Scores (RS) were calculated and reflect the differences in the compositional preferences of four-nearest neighbor residues (defined by the Delaunay tessellation) between mutant and wild-type proteins. The RS were then combined into a Residual Score Profile (RSP), representing a vector of RS for all 194 DBD residues. Using decision tree models with the RSP as input to predict transactivation, we obtained an accuracy varying between 64.2 and 78.5%, depending on the p53 response element considered. Our computational approach provides a fast and reliable method for predicting the functional impact of p53 mutants associated with cancer, easily applicable to other mutant proteins with known tertiary structures.

POSTER # 5

MODULATION OF BINDING OF DNA TO THE C-TERMINAL DOMAIN OF P53 BY ACETYLATION

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The binding of non-specific DNA to the C-terminal negative regulatory domain (CTD) of p53 modulates its activity. The CTD is a natively unfolded region, which is subject to acetylation and phosphorylation at several residues as part of control. To measure the effect of covalent modification on binding to DNA, we synthesized a series of fluorescein-labelled CTD peptides with single and multiple acetylations at lysine residues that we had identified by NMR as making contact with DNA, and developed an analytical ultracentrifugation method to study their binding to DNA. Binding depended on ionic strength, indicating an electrostatic contribution. Mono-acetylation weakened DNA binding at physiological ionic strength 2-3-fold, di-acetylations resulted in further 2-3-fold decrease in the affinity, and tri- and tetra-acetylations rendered DNA binding undetectable. Phosphorylation at S392 did not affect DNA binding. NMR spectroscopy showed binding to DNA did not induce significant structure into CTD, apart possibly from local helix formation.

POSTER # 6

PML ENHANCES THE PHOSPHORYLATION OF p53 ON THREONINE 18 BY CK1 IN RESPONSE TO STRESS

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Mdm2 plays a key role in maintaining p53 labile in non-stressed cells, and during the recovery to the basal levels in cells exposed to stress. However, during the activation of p53 in response to stress Mdm2 inhibitory activities are neutralized. We have previously described a critical role for PML in the accumulation of p53 in response to DNA damage under physiological conditions. PML protects p53 from Mdm2-mediated ubiquitination and degradation and from inhibition of apoptosis. One mechanism by which PML protects p53 is by enhancing its phosphorylation on serine 20, a phosphorylation site residing within the Hdm2 binding domain of human p53 (residues 17-24). We showed that PML recruits the checkpoint kinase 2 (Chk2) and p53 into the PML nuclear bodies in response to DNA damage, thereby prolonging and extending this phosphorylation. Thermodynamic studies revealed that phosphorylation of a nearby site, threonin18 (Thr18), plays a critical role in the p53/Mdm2 interaction (Schon et al., 2002, JMB 25:491-501).We therefore asked whether the activation of p53 by PML involves the regulation of Thr18 phosphorylation. We present here evidence that PML enhances the phosphorylation of p53 on Thr18 in response to DNA damage, correlating with the formation of PML nuclear bodies. Since Casein kinase 1 (CK1) mediates this phosphorylation we examined the physical interaction between PML and CK1. We found that PML interacts with CK1. We propose that the recruitment of CK1 by PML may serve as an important arm through which PML protects p53 from Mdm2, and cooperates with p53 in tumor suppression.

POSTER # 7

TRANSCRIPTIONAL ACTIVATION OF CYCLOOXYGENASE 2 BY TUMOUR SUPPRESSOR p53 REQUIRES NUCLEAR FACTOR-KAPPAB

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Expression of Cox-2 is a critical factor in carcinogenesis. Enhanced Cox-2 enzymatic activity and synthesis of prostaglandins stimulates proliferation, angiogenesis, invasiveness and inhibits apoptosis. COX-2 overexpression has been detected in different tumor sites, including esophagus, colon and lung. Many signals that activate Cox-2 also induce p53. Furthermore, there is evidence that p53 can activate Cox-2, but the mechanism involved is still poorly understood. We have used gastrointestinal tumor cell lines (TE esophageal cells and HCT116) to investigate this mechanism. We show that p53 up-regulates Cox-2 in cancer cell lines by recruiting NF-kappaB to its response element in the *COX-2* promoter. Cooperation between p53 and NF-kappaB is required for activation of *COX-2* promoter in response to daunomycin, a

DNA-damaging agent. Several mutant p53 proteins retain the capacity to cooperate with NF-kappaB, albeit with lower efficiency than wild-type p53. Pharmacological inhibition of Cox-2 enhances apoptosis in response to daunomycin, in particular in cells containing active p53. In esophageal cancer tissues, there is a positive correlation between Cox-2 expression and wild-type *TP53* status in adenocarcinoma, but not in squamous cell carcinoma ($p<0.01$), two types of cancers that differ by the role of inflammation as causal factor. These results indicate that p53 and NF-kappaB may cooperate in up-regulating Cox-2 as part as an anti-apoptotic adaptative response in highly inflammatory cancers.

POSTER # 8

p53 MAY AFFECT THE ACCURACY OF DNA SYNTHESIS BY DNA POLYMERASE γ IN MITOCHONDRIA

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P53 protein, that is important in maintaining genetic integrity, exerts an intrinsic 3′→5′ exonuclease activity and acts as proofreader for errors produced by cellular and viral DNA polymerases. Mitochondrial p53 targeting occurs in a wide spectrum of cell types after a variety of stress signals. Mitochondrial DNA(mitDNA) mutations, resulting from replication errors, leads to genomic instability, increased cell survival and cancer in humans. DNA polymerase γ (pol γ) is the enzyme responsible for replication of mitDNA and implicated in all repair processes. Recent studies indicate that p53 may play a role in maintaining mitochondrial genetic stability through its ability to physically interact with mitDNA and pol γ. The intramitochondrial dNTP pool asymmetries contribute to mitochondrial mutagenesis. Although, DNA pol γ has an intrinsic 3′→5′ exonuclease activity, which is believed to perform a proofreading function, a misincorporated nucleotide is not efficiently removed by the 3′→5′ exonuclease activity of the enzyme. To analyze the contribution of external exonucleolytic proofreading to accuracy of mitDNA pol γ following the incorporation of incorrect base pairs, we examined the error correction in highly enriched mitochondrial fractions of H1299 cells (p53-null) in the presence of recombinant purified p53. A functional interaction between the exonuclease (p53) and DNA pol γ activities was observed; p53 exonuclease activity significantly reduces the number of mismatched nucleotides incorporated by into DNA by pol γ. Following the incorporation, various nucleoside analogs (NAs) are poor substrates for 3′→5′ exonuclease activity of DNA pol γ. However, the efficient excision of the incorporated NAs was observed in the presence of purified p53. The data demonstrate that p53 in mitochondria could play an important role of external proofreader in removing mispairs and NAs from the 3'-end of the DNA.

POSTER # 9

DIFFERENTIAL EFFECTS OF DNA MODIFICATION WITH CISPLATIN ON THE p53 PROTEIN BINDING TO DIFFERENT CONSENSUS SEQUENCES

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Sequence-specific recognition of response elements (consensus sequences, p53CON) in promoters of downstream genes (such as *p21, mdm2,* etc.) by the tumor suppressor protein p53 is crucial for the p53 functions in the cell cycle control [1, 2]. The typical p53CON consists of two copies of a motif RRRGWWCYYY separated by 0-13 nucleotides. Natural p53 response elements exhibit surprisingly high sequence variability and may contain one or several nucleotides not fitting exactly the above formula [3]. It has been shown previously that DNA

modification with an antitumor drug cisplatin inhibits the p53 sequence-specific DNA binding to an optimized artificial p53CON pPGM1 (AGACATGCCTAGACATGCCT) [4, 5]. Here we show that effects of DNA modification with cisplatin on p53 binding to different p53CON's significantly differ. Among the pPGM1 sequence and natural p53CON's p21 (GAACATGTCCcAACATGTTg) and mdm2 (GGtCAAGTTgGGACAcGTCC), the cisplatin inhibition effect increased remarkably in the order p21<pPGM1<mdm2. Relative sensitivities of p53-p53CON binding towards the cisplatin modification correlated with occurrence of sequence motifs known to form easily stable bifunctional adducts with the drug (namely, GG, AG and GNG in either of the two DNA strands [6, 7]). Binding of the protein to mutated p53CON's in which these motifs were eliminated exhibited very low sensitivity towards cisplatin modification, suggesting that formation of the bifunctional cisplatin adducts within the consensus sequences was primarily responsible for the inhibition of p53 sequence-specific binding. Distinct effects of cisplatin DNA modification on the recognition of different response elements by the p53-family proteins may have differential impacts on particular regulation pathways in cisplatin-treated cells.

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POSTER # 10

DISSECTING THE ONCOGENIC ACTIVITY OF MUTANT p53

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Expression of the oncogene mtp53 (mtp53) is associated with various hallmarks of cancer including genomic instability, drug resistance, invasion and metastasis. Since mtp53 can induce these neoplastic features in normal cells, it has been ascribed putative "gain of function" activities, although the mechanistic basis of these functions is not well understood. We have used siRNA to knockdown the expression of endogenous mtp53 and we have carried out gene chip analysis to identify the resulting changes in gene expression. Among the genes observed to be induced after mutant p53 knockdown is cyclin D1. We show that cyclin D1 induction occurs in cancer cells from different tissue origins and carrying different p53 mutations. Using a mutant E2F (dnE2F) and siRNA against E2Fs, we have determined that cyclin D1 induction after mtp53 knockdown is dependent on the transactivating E2Fs, thus establishing E2Fs as downstream mediators of mtp53 activity. We further show that mtp53 can repress the E2F3 promoter. This repression involves the direct interaction of mtp53 with the promoter. We hypothesize that mtp53 promotes chemotherapy resistance and genomic instability by blocking the expression of the "activating" E2Fs.

POSTER # 11

MUTANT p53 GAIN OF FUNCTION: REDUCTION OF TUMOR MALIGNANCY OF HUMAN CANCER CELL LINES THROUGH ABROGATION OF MUTANT p53 EXPRESSION

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The *TP53* tumor suppressor gene is the most frequent target for genetic alterations in human cancers. These alterations are mostly missense point mutations that cluster in the DNA binding domain and abolish the wild type transacting activity. Divergently from other tumor suppressor mutants, mutant-p53 proteins tend to gain new functions that increase oncogenic transformation. The purpose of this study was to investigate whether the abrogation of mutant-p53 proteins reduces the tumor malignancy of human cancer cell lines. We inhibited mutant-p53 expression by RNA interference in three different cancer cell lines endogenously expressing mutant-p53 proteins, and evaluated the effects on the biological activity through which mutant-p53 exerts gain of function. We found that, depletion of mutant-p53 reduces cell proliferation, *in vitro* and *in vivo* tumorigenicity, and resistance to anticancer drugs. These results demonstrate that knocking-down mutant-p53 weakens the aggressiveness of human cancer cells, at least under our experimental conditions, and contribute to the understanding of the gain of function of human tumor derived p53 mutants.

POSTER # 12

TRANSCRIPTIONAL ACTIVATION OF SPECIFIC TARGET GENES BY MUTANT p53R175H

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Conformational defective p53 mutants can increase resistance of tumor cells to anticancer treatment and promote genomic instability. The molecular mechanisms underlying such effects remain to be elucidated. To investigate how mutant p53 proteins exert gain of function activity a microarray analysis was performed using H1299 cells overexpressing inducible p53R175H (upon Ponasterone A addition). Affymetrix Human Genome U133A arrays (22,000 genes) were hybridized. In order to understand whether mutant p53 contributes to chemoresistance by actively modulating transcription, the expression profile of p53R175H inducible cells was also analyzed upon different amounts of Cisplatin treatment. We found that there is a clear effect on gene expression after induction of p53R175H. Interestingly, for many genes the difference of expression between non-induced and induced conditions increases in presence of high Cisplatin concentrations. Three genes, gamma-cystathionase, Polo-like kinase 2 (PLK2), and Inhibitor of Differentiation-4 (ID-4) were chosen among the modulated group and are currently under deeper investigation. Expression and function of these genes in cell lines carrying endogenous mutant p53 have been analyzed. Our study suggests that the transcriptional activation of specific target genes is one of the molecular mechanisms underlying gain of function activity of mutant p53.

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POSTER # 13

FUNCTIONAL EVALUATION OF p53 MUTANTS USING A QUANTITATIVE YEAST-BASED ASSAY

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p53 missense mutations are commonly found in human cancers. The resulting single amino acid changes can alter the p53 protein transactivation activity. The functional behaviour of different p53 mutants was previously studied using a semi-quantitative assay in yeast, based on the ADE2 color (red/white) reporter gene and a panel of p53 response elements (REs). The results showed that p53 mutants were functionally heterogeneous. To quantify these differences we have now begun using isogenic yeast strains containing the luciferase gene under the control of p53 REs (e.g. P21, BAX, MDM2, PUMA). The transactivation capability, the ability to interfere with a) wild-type p53 (dominance) or b) with p73 isoforms (interference) were evaluated. Preliminary results confirm the functional heterogeneity previously observed but appear to discriminate additional subgroups of mutants, particularly for their ability to inhibit wild type p53 and/or p73. While the visual ADE2-based assay provides an effective screening tool, the more sensitive quantitative assay should also be adopted to address the functional consequences of p53 mutations.

POSTER # 14

THE BIOLOGICAL IMPACT OF THE HUMAN MASTER REGULATOR P53 CAN BE ALTERED BY MUTATIONS THAT CHANGE THE SPECTRUM AND EXPRESSION OF ITS TARGET GENES

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Human tumor suppressor p53 is a sequence-specific master regulatory transcription factor that targets response elements (REs) in many genes. Missense mutations in the DNA binding domain are often cancer associated. As shown with yeast-based systems, p53 mutants can alter the spectrum and intensity of transactivation from individual REs. We address directly in human cells the relationship between changes in the p53 master regulatory network and biological outcomes. Expression of integrated, tightly regulated DNA binding domain mutants resulted in many patterns of apoptosis and survival following UV, ionizing radiation or spontaneously. These patterns reflected changes in the spectra and activity of target genes, as demonstrated for P21, MDM2, BAX and MSH2. Thus, as originally proposed for “master genes of diversity,” p53 mutations in human cells can differentially influence target gene transactivation resulting in a variety of biological consequences which, in turn, might be expected to influence tumor development and therapeutic efficacy.

POSTER # 15

TP53 MUTATIONS IN SINO-NASAL CANCER

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Mechanisms by which exposure to wood dust increases the risk of sino-nasal cancer (SNC) are not clear. In an on-going project, we are currently studying *TP53* mutations in SNCs from cases with and without occupational exposure to wood dust. Paraffin-embedded tissue (PET) samples of SNC were collected for DNA extraction and analysis in collaboration with national cancer registries of Denmark, Finland and France. The study population includes all incident cases of the cancer of the nose and paranasal sinuses in Denmark (1992-2002), in Finland (1989-2002) and in France in three regions covered by a cancer registry (1990-2002). The preliminary results from Capillary Electrophoresis Single Strand Conformation Polymorphism (CE-SSCP) analysis and direct sequencing show that *p53* mutations are common in SNC, with as high mutation frequency as 75-85 %. Interestingly, our preliminary results indicate a gradient of mutation frequencies between the three populations studied, with the lowest frequency of mutations in cases from Finland, and the highest in those from France. Mutations have been observed in tumours of both squamous cell and adenocarcinoma histology, but it seems that adenocarcinomas carry mutations more frequently than squamous cell carcinomas. This difference may at least partially explain the difference observed between the different SNC populations. Sequencing of the mutations is underway. According to a preliminary immunohistochemistry (IHC) analysis of p53 protein expression among tumours from France and a subset of Finnish tumours, there is a fairly good correlation between *TP53* mutation analysis and IHC. The mutation data will be complemented with RAS analysis being carried out in Denmark. A pathology panel has reviewed tumour histologies for the whole tumour collection (more than 400 tumours). In addition, occupational exposure histories of the cases have been assessed or are presently under evaluation by industrial hygienists.

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POSTER # 16

P53 TRANSCRIPTION FACTOR FUNCTION IS ABROGATED IN P53 MUTATED AS WELL AS WILD-TYPE MELANOMA CELL LINES

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Background: Cutaneous malignant melanoma is an aggressive form of skin cancer, characterized by strong chemoresistance and poor patient prognosis. The molecular mechanisms underlying its resistance to chemotherapy remain unclear but are speculated to involve the dysregulation of apoptotic pathways. The tumor suppressor protein p53 promotes apoptosis in response to death stimuli by transactivation of target genes and by transcription-independent mechanisms. Several studies report that the p53 protein is accumulated in a high proportion of primary cutaneous melanomas and their metastases up to 100%. In contrast,

sequence analyses found that mutations in these tumors are rare (0-30%) thus suggesting other protein stabilizing mechanisms. However, the functional integrity of the accumulated p53 peptide in the remaining melanomas is unclear.
Methods: In a panel of 6 melanoma cell lines and primary melanocytes the p53 status was determined by transcript analysis of the entire coding region. Cells were either treated with UV-A or UV-B irradiation, or with cisplatin or dacarbazine, and induction of p53 was determined using Western Blot analysis. Macroarrays served to investigate expression patterns of p53-regulated genes, and real time RT-PCR was perfomed in order to confirm observed differences.
Results: Three cell lines carry p53 aberrations comprising two missense mutations (SK-MEL 1:R273W; SK-MEL 3:R267W) and one deletion (IgR37:C229DelTG). Whereas SK-MEL 1 and IgR37 express mutated transcripts only, SK-MEL 3 retains p53 wild type transcripts. The primary melanocytes and most cell lines showed high constitutive p53 expression levels in Western Blot analysis. Regardless of the mutation status, p53 levels rose after cisplatin treatment. In contrast, either UV irradiation or exposure to dacarbazine resulted in higher p53 peptide levels in some cell lines only. Cell line IgR37 showed no p53 peptides at all. The overall expression patterns of p53-regulated genes were very similar amongst tumor cell lines and melanocytes. However, the number and type of differentially expressed genes varied remarkably according to the cell line and applied treatment. The most frequently affected genes were APEX, CDC2, CDKN1A, GADD45A, HIF1alpha, HIPK2, MAP4, SP1, and TEAD1. Observed differences were verified by real time RT-PCR. None of the melanoma cell lines showed similar expression changes as primary melanocytes. Since the expression pattern of SK-MEL 1 resembles IgR37, the missense mutation R273W appears to exert the same effect on p53 function as the frameshifting deletion mutation.
Conclusion: Despite of p53 wild-type expression the regulation of p53-dependent genes is modified in melanoma cell lines suggesting aberrations downstream in its signalling pathway.

POSTER # 17

RESCUE OF INTEGRIN αV-DEPENDENT UNFOLDING OF WILD TYPE p53 SUPPRESSES MELANOMA TUMOR GROWTH

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p53 is rarely mutated in malignant melanoma and the functional status of p53 in melanoma remains unclear. Integrin $\alpha v\beta 3$ is induced in malignant melanoma progressions and promotes melanoma cell survival. To elucidate integrin αv-mediated melanoma cell survival mechanisms, we utilized a three-dimensional (3D) collagen gel model mimicking the pathophysiological microenvironment of malignant melanoma in the dermis. We found that integrin αv-mediated inactivation of p53 functionally controlled melanoma cell survival in 3D-collagen *in vitro* as well tumor growth *in vivo*. Interestingly, integrin αv induced an unfolded, inactive conformation of wt p53 *in lieu* of p53 mutations in melanoma cells, which might help to explain why p53 mutations occur rarely in malignant melanoma. Importantly, a small compound, PRIMA-1^{MET}, restored the active conformation of wt p53 in melanoma cells, promoted p53 target genes, triggered apoptosis and suppressed melanoma tumor growth *in vivo* in a wt p53-dependent manner. Refolding of wt p53 represents a new strategy for development of urgently needed therapy for malignant melanoma.

POSTER # 18

IMPACT OF P53 MUTATIONS ON RADIO- AND CISPLATIN-SENSITIVITY IN SQUAMOUS CELL CARCINOMAS OF THE HEAD AND NECK

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Background: Current treatment regimens for squamous cell carcinomas of the head and neck (SCCHN) comprise surgery as well as radio- and chemotherapy. As yet, the potential impact of mutated p53 on the outcome of multimedial therapy protocols is discussed controversially.
Methods: In a panel of 29 cell lines established from 22 tumors, the p53 status was determined by transcript analysis of the entire coding region. The colorimetric MTT-assay served to examine chemosensitivity towards cisplatin (IC50; 50% inhibitory concentration) and radiosensitivity was determined in a clonogenic assay (AUC; area under the survival curve).
Results: 18 of the 22 tumors (78%) expressed an aberrant p53 transcript comprising point missense mutations, splice mutations, deletions and insertions. Three of the mutations were detected outside the DNA-binding domain of exons 5 to 9. In addition, 3 tumors showed no p53 transcript at all (14%). IC50 varied by factor 20 between 0.5µM and 10µM cisplatin with a mean of 5.44µM $\pm$ 1.01. Radiosensitivity ranged almost by factor 2 between AUC 1.4 and 2.6 with a mean of 1.99 $\pm$ 0.32. We found no correlation between p53 aberration and radiosensitivity. A non-significant trend was observed for p53 mutated tumors to exhibit higher cisplatin-sensitivity. Furthermore, there was no relation between various types of p53 aberrations or the presence of an additional wild-type allele and radio- and/or cisplatin-sensitivity.
Conclusion: Mutations of p53 have to be considered a typical event in head and neck cancer, thus challenging the concept that the mere presence or absence of mutated p53 might be a relevant predictive marker. Accordingly, we were not able to correlate in vitro radio- and cisplatin-sensitivity to the p53 status in a panel of SCCHN lines.

POSTER # 19

20% OF CLASSICAL LI-FRAUMENI SYNDROME FAMILIES WITHOUT MUTATIONS IN THE *TP53* GENE DETECTABLE BY DIRECT SEQUENCING HAVE LARGE DELETIONS IN THE GENE IDENTIFIABLE BY MULTIPLEX LIGATION DEPENDENT PROBE AMPLIFICATION (MLPA)

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About 75% of classical LFS and 40% of LFL families have germline mutations in the *TP53* gene that are detectable by direct sequencing. We have used Multiplex Ligation dependent Probe Amplification (MLPA) to screen the gene for large alterations in 19 classical LFS families and 108 LFL families which had previously been found to have no mutations identifiable by direct sequencing of all exons. 20% of these Classical LFS but no LFL families were found to have large deletions. This suggests that a substantial fraction of classical LFS families have large deletions in the *TP53* gene and that this analysis should be included in a comprehensive screen for mutations in the gene in these families.

POSTER # 20

INDEPENDENT PROGNOSTIC VALUE OF SOMATIC *TP53* GENE MUTATIONS IN 1794 BREAST CANCER PATIENTS

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Retrospective series of breast cancer cases from 10 hospitals in 7 different European countries were combined to assemble a large dataset of clinical and molecular data on 1794 European women with primary breast cancer who were followed-up for 10 years, and whose tumor has been screened for *TP53* mutation by gene sequencing. The association between *TP53* gene mutation and breast-specific cancer death was examined in univariate and multivariate models including classical prognostic factors of survival.

TP53 gene mutations were more frequent in tumors of ductal and medullar types, aggressive phenotype (high grade, large size, node positive cases and low hormone receptor contents) and in women below 60 years old. An elevated risk of breast-specific cancer death within 10 years of follow-up was found in patients with a *TP53* mutation within exons 5-8 in their tumor compared with patients with no such mutation (relative risk, 2.27; $P<0.0001$). This association remained valid after adjustment for tumor size, nodes status and hormone receptor contents. An interaction between *TP53* gene mutation and PR content was found, patients with *TP53* mutation and negative PR status having a very bad prognosis independently of tumor size, node status and ER status. More importantly, in patients with PR positive status, *TP53* mutation was associated with a strong reduction in survival over 10 years. Among specific types of *TP53* mutations, non-missense mutations and missense mutations in the DNA-binding surface (L2/L3 and LSH motifs) had a worse prognosis than mutations outside the DNA-binding surface. Among missense mutations, those at codon 179 and the R248W mutant were associated with the highest mortality rates.

These results clearly show that *TP53* gene mutation is an independent factor of prognosis in breast cancer and advocate for its use in clinical practice to improve cancer management.

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