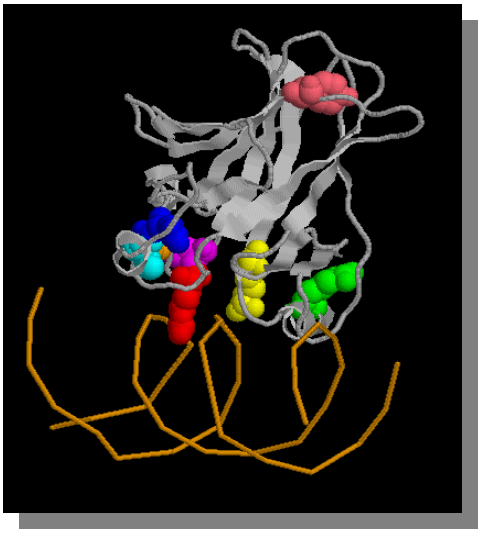
Functional consequences of TP53 mutations:

Characterization of common and rare p53 mutants and relevance to human cancer

IARC, Lyon

June 30 – July 3, 2003



An **international meeting jointly organized by the International Agency for Research on Cancer (IARC/WHO) and the National Institute of Environmental Health Sciences (NIEHS)**

http://www.iarc.fr/p53

ORGANIZING COMMITTEES

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Funding bodies

International Agency for Research on Cancer (IARC)
National Institute of Environmental Health Sciences (NIEHS/NIH)
Office of Rare Diseases, NIH

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 tumour 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. Furthermore, this information should be made available through an integrated database that uses standardised annotations and allows easy data retrieval and analysis. This database could be used as a "virtual centre of resources" for information on TP53 mutations and cancers.

The objective of this workshop was to review and discuss the state of the art, the experimental methods available for such global approaches, and to establish guidelines for the annotation of mutations into TP53 databases. Given the large mutation dataset currently available, the TP53 gene may serve as model for such approaches, which may be extrapolated to other disease-associated mutant genes.

Specific aims

- Review the current scientific data available on the specific properties of p53 mutants
- Examine approaches for the identification of changes in functional activities of p53
- Discuss the relationship between specific mutations, prognosis and therapies
- Initiate the development of a human p53 mutant functionality database that will be modelled after the TP53 mutation database that is maintained at IARC

Programme and participants

The programme included 20 presentations of 40 minutes' duration each, plus 6 short presentations of 25 minutes' duration each, 1 poster session (35 posters) and 2 workshop sessions. 160 participants attended the meeting.

Workshop sessions

During the meeting, two workshop sessions addressed p53 functional assays and the development of a p53 functional mutant database. Strategies and guidelines for TP53 mutant function testing and TP53 mutation annotations into databases have been discussed.

Meeting reports

From the workshop sessions and the various talks, a summary of the meeting, an overview of TP53 mutant functional assays and guidelines for the annotation of mutations into TP53 databases will be published in Oncogene.

SCIENTIFIC PROGRAMME

Monday, June 30

03:00 p.m. Registration and coffee

04:30 p.m. **Welcome by Paul KLEIHUES**, IARC Director, Lyon

Introductory Lectures, chaired by Pierre Hainaut & Mike Resnick

04:45 p.m. **Monica HOLLSTEIN**, *Heidelberg, Germany*
TP53 mutation fingerprints

05:25 p.m. **Carol PRIVES**, *New York, USA*
TP53 family members

06:05 p.m. **End of day 1**

Tuesday, July 1

Heterogeneity of p53 mutations, chaired by Janet Hall & Moshe Oren

08:45 a.m. **Pierre HAINAUT**, *Lyon, France*
IARC TP53 mutation databases

09:25 a.m. **Michael RESNICK**, *Research Triangle Park, USA*
Functionality of p53 mutants

10:05 a.m. **S. Perwez HUSSAIN**, *Bethesda, USA*
Endogenous and exogenous carcinogen fingerprints on p53

10:45 a.m. Coffee break

Modelling of interaction with DNA, chaired by Janet Hall & Moshe Oren

11:15 a.m **Richard IGGO**, *Epalinges, Switzerland*
DNA-binding activities of p53

11:55 a.m. **Ella KIM**, *Hamburg, Germany*
DNA binding of p53 is regulated by DNA topology

12:15 a.m. **Wolfgang DEPPERT**, *Hamburg, Germany*
Interaction of p53 mutants with DNA structures

1:00 p.m. Lunch break

Structure-function of p53, chaired by Ada Sacchi & Carl Anderson

2:15 p.m. **Karen S. VOUSDEN**, *Glasgow, UK*
P53/mdm2 interaction

2:55 p.m. **Thanos D. HALAZONETIS**, *Philadelphia, USA*
Biochemistry and biophysics of p53 mutants

3:35 p.m. Coffee break

04:05 p.m. **Assaf FRIEDLER**, *Cambridge, UK*
Structure of p53 mutants

04:45 p.m. **Rainer K. BRACHMANN**, *Irvine, USA*
Suppressor mutations of p53 mutants

05:25 p.m. **Klas WIMAN**, *Stockholm, Sweden*
Drug targeting of p53 mutants

06:05 p.m. **End of day 2**

Wednesday, July 2

Biochemistry and physiology of mutant p53, chaired by Carol Prives & A.-L. Borresen-Dale

08:45 a.m. **Moshe OREN**, *Rehovot, Israel*
Gene transcription by p53 mutants

09:25 a.m. **Varda ROTTER**, *Rehovot, Israel*
P53 mutants and apoptosis

10:05 a.m. **Carl W. ANDERSON**, *Upton, USA*
Post-translational modifications of p53 mutants

10:45 a.m. Coffee break

11:15 a.m. **Giovanni BLANDINO**, *Rome, Italy*
Interaction of p53 mutants with p63/p73 proteins

Short presentations

11:55 a.m. **Galina SELIVANOVA**, *Stockholm, Sweden*
Mutant p53 rescue by designed peptides

12:20 a.m. **Penka NIKOLOVA**, *London, UK*
Restoring function of p53 mutants

12:45 a.m. **Matthias DOBBELSTEIN**, *Marburg, Germany*
Reactivation of mutant p53

1:15 p.m. Lunch break

2:15 p.m. ***Discussion workshop I, led by*** **Alberto INGA**
Functional assays and reporter systems for mutant p53

3:45 p.m. Coffee break

4:15 p.m. Poster session

6:15 p.m. **End of day 3**

Thursday, July 3

Pathological aspects of p53 mutations (1), chaired by Sean Tavtigian

08:45 a.m. **Anne-Lise BØRRESEN-DALE**, *Oslo, Norway*
TP53 mutations in breast and ovarian cancers

09:25 a.m. **Gerard P. ZAMBETTI**, *Memphis, USA*
New mechanisms of tumorigenesis involving germline p53 mutants

10:05 a.m. Coffee break

10:35 a.m. ***Discussion workshop II, led by Magali OLIVIER***
TP53 database: quality control and integration of structural and clinical data

1:00 p.m. Lunch break

Pathological aspects of p53 mutations (2), chaired by Wolfgang Deppert

02:15 p.m. **Guillermina LOZANO**, *Houston, USA*
Transgenic mouse model of mutant p53

Short presentations

02:55 p.m. **Kent SØE**, *Jena, Germany*
p53 and the topoisomerase I damage response

03:20 p.m. **Kanaga SABAPATHY**, *Singapore*
P53 codon 72 polymorphism and breast cancer

Keynote lecture

03:50 p.m. **Arnold J. LEVINE**, *Princeton, USA*
Perspectives of mutant p53 biology

04:30 p.m. **Close of meeting**

PRESENTATION SUMMARIES

Presentation summary #01

Hupki (human p53 knock-in) mice model

Monica Hollstein

Hupki (human p53 knock-in) mice harbouring the human polyproline and DNA binding domain-encoding DNA sequences in place of the homologous murine sequence produce chimeric p53 protein that retains properties and functions characteristic of wild-type p53. The mice are not tumour-prone. The Hupki model is a unique experimental model that can be used to explore questions pertaining to molecular epidemiology and p53 biology.

Presentation summary #02

DNA binding properties of p53

Carol Prives

P53 has two DNA binding domains . The C-terminally located non-specific DNA binding domain was previously proposed to negatively regulate the core sequence- -specific DNA binding domain . However, Prives showed that while the C-terminus negatively regulates p53 binding to a linear DNA fragment this domain is required for the ability of p53 to bind with high affinity to circular DNA of the same sequence and length as the linear fragment. Furthermore, the C-terminus allows p53 to passively diffuse along DNA presumably to facilitate scanning for specific sites in genomic DNA. A second finding was described in which an L1 loop p53 mutant (H115N) was identified that possesses both reduced exonuclease activity but enhanced DNA binding ability. H115N p53 is more effective than wild type p53 at low intracellular concentrations in inducing down-stream target genes and producing cell cycle arrest. Nevertheless, H115N p53 is impaired in mediating DNA damage facilitated apoptosis in H1299 cells.

Presentation summary #03

IARC TP53 mutation database

Pierre Hainaut

The IARC TP53 mutation database compiles over 18000 somatic mutations and 223 families with germline TP53 mutations. Tumor specific mutation patterns can be identified. In several, well-documented instances, these patterns can be explained by site-specific mutagenesis (i.e. aflatoxin in liver cancer or tobacco smoke in lung cancers). However, there is also evidence for functional selection of mutations. Despite their importance for p53 regulation, phosphorylation, acetylation or ubiquitinylation sites in p53 are never targeted by mutations. Studies in Li-Fraumeni kindreds suggest that some mutation clusters may be associated with specific cancer types (i.e mutations in loops 2-3 and brain tumors; mutations in β-sheet scaffold and adrenocortical cancers). A pilot study has been developed in a cohort of 2000 european breast cancer patients to correlate mutation types with disease outcome. Preliminary results show that the clinical impact of the mutation may differ according to its effect on p53 protein structure.

A new isoform of p53 has been identified, which lacks the N-terminus containing the transactivation domain This isoform is produced by alternative splicing (retaining intron 2) and use of an internal translation codon in exon 3. This isoform is readily detected by thePAb1801 antibody and is relatively abundant in normal, unstresses tissue. Functionally, it appears to be homologous to deltaN isoforms of p63 and p73, with dominant-negative effects over transactivation by wild-type p53. This isoform does not bind Mdm2 and does not accumulate in response to stress but can switch-off the p53 cell-cycle checkpoint induced by unprogrammed growth stimuli through the p14arf pathway. This isoform may therefore carry a specific, p53-dependent function in cell-cycle regulation.

Presentation summary #04

Functionality of p53 mutants

Mike Resnick

Mutations of p53 are commonly with associated cancer. Given that most result in full length protein and many have partial function, it is likely that retained function can contribute to tumor progression. We have developed a system in yeast utilizing highly regulatable expression (rheostatable) of p53 to address the consequences of human p53 mutations on transactivation at over twenty p53 response elements upstream of a reporter. Using this "in vivo" test tube, we establish that there is a 1000-fold range in p53 transactivation capacity towards response elements derived from human p53 target genes. Surprisingly, there was wide variation in transactivation by the mutant p53's towards the various RE's. Mutants could result in a change of spectrum as well as changes in strength of transactivation. This leads us to suggest that the altered spectrum could influence the impact of functional p53 mutations on cancer progression. These results support a newly developed master gene hypothesis for phenotypic diversity where the master gene is a single transcriptional activator (or repressor) that regulates many genes (Resnick and Inga, PNAS, in press). Mutations of the master gene can cause a variety of simultaneous changes in both the selection of targets and the extent of transcriptional modulation at the individual targets.

Presentation summary #05

Endogenous and exogenous carcinogen fingerprints on TP53

S. Perwez Hussain and Curtis C. Harris

One of the interesting observations is the association between the presence of a specific hotspot mutation and exposure to a particular carcinogen in a specific cancer. Some of the prominent examples are AFB1 exposure and codon 249ser mutations in liver cancer, smoking and prevalence of G to T transversions at codons 248, 157 and 273 in lung cancer, and sunlight exposure and CC to TT tandem double mutations in skin cancer. The p53 mutational load or the frequency of p53 mutant cells with hot spot mutations in nontumorous tissue can provide molecular linkage between exposure to an exogenous or endogenous etiological agent and cancer and can also identify individuals at increased cancer risk. Using a highly sensitive mutation assay, we have analyzed p53 mutational load in nontumorous tissue in people with cancer-prone chronic inflammatory diseases and also in the nontumorous peripheral lung tissue in lung cancer patients. The data are consistent with the hypotheses: 1) reactive oxygen and nitrogen species and aldehydes can mutate p53 2) p53 mutation load is increased in a number of cancer-prone chronic inflammatory diseases. 3) Nontumorous lung tissues carry a higher specific p53 mutation load in smokers with lung cancer.

Presentation summary #06

Richard Iggo

1. The p53 yeast functional assay is being used to test the p53 status of patients enrolled in a taxone versus anthracycline phase III clinical trial organized by EORTC (study 10994.

2. Chromatin immunoprecipitation shows that DNA binding of p53 is not regulated by DNA damage in cell lines. Quantitative analysis shows that the p21, MDM2 and PUMA promoters have the highest p53 binding in vivo.

Presentation summary #07

1. Binding of mutant p53 to non-B DNA
2. deltap53, a novel isoform of p53 generated by alternative exon-exon splicing

Wolfgang Deppert

Heinrich-Pette-Institut, Hamburg, Germany

1. Binding of mutant p53 to non-B DNA
In contrast to wild-type p53 (wtp53), which binds to linear DNA with high affinity both in a specific and in a non-specific fashion, linear DNA is not a target for mutant p53 (mutp53). Mutp53 however binds to non-B DNA in a structure-selective fashion. Structure-selective binding of mutp53 is derived from the structure- and sequence-specific binding of wtp53 to non-B DNA. The p53 core domain and the p53 C-terminus coordinately mediate binding to structured DNA. High affinity structure-selective DNA binding of mutp53, which is observed for all p53 core mutants, may form the basis for the interaction of mutp53 with MAR/SAR DNA elements and mediate transcriptional regulation of mutp53 target genes. (with E.Kim, T. Goehler, K. Walter, M. Brazdova, H. Koga).

2. deltap53, a novel isoform of p53 generated by alternative exon-exon splicing
We have identified a novel splice variant of p53, Δp53, that is generated by alternative splicing between exons 7 and 9, creating an in frame deletion of amino acids 257-322. Δp53 is an independent p53 isoform that does not hetero-oligomerize with full-length (fl) p53. The splice elimates all mutations occuring in codons 257-322, generating two new classes of mutp53 p53, class A mutants, expressing a mutΔp53, and class B mutants, expressing a wtΔp53. Due to the unique property of wt?p53 to selectively upregulate transcription of the survival factors p21 and 14-3-3σ, class B mutant p53 cells (expressing a wtΔp53) are impaired in p53-independent apoptosis upon severe genotoxic stress. Instead, such cells undergo endoreplication that leads to polyploidy of the cells. Class B mutant p53 expressing tumors therefore more easily may respond to severe genotoxic stress with the generation of resistent cells than class A mutant expressing tumors. (with I. Dornreiter, G. Rohaly, D. Speidel, J. Chemnitz, S. Dehde).

Presentation summary #08

p53/mdm2 interaction

Karen Vousden

pending

Presentation summary #09

Structure of C. elegans p53 DNA binding domain

Yentram Hyen, Elena Stavridi, Philip Jeffrey, Nikola Pavletich*, Brent Derry and Thanos Halazonetis*

*Wistar Institute, Philaldephia, PA 19104-4268 and *Memorial Sloan-Kettering Cancer Center, New York, NY*

C. elegans p53 represents the evolutionary ancestral form of human p53 family members. We have solved the three-dimensional structure of its sequence-specific DNA binding domain. This domain adopts an overall fold that is very similar to the previously solved fold of the human p53 DNA binding domain. The only exception was the part of loops L1, L2 and L3 that in human p53 lack ordered secondary structure contain in C. elegans p53 short alpha-helices. The alpha-helix in loop L1 results in this loop being recessed in the structure, as compared to the position of loop L1 in human p53, where L1 is adjacent to helix H2 (the helix that contacts the major groove of DNA). As a result, in C. elegans p53, the lysine that is equivalent to Lys120 of human p53, cannot make a sequence-specific contact. The alpha-helix in loop L3 results in the arginine that is equivalent to Arg249 of human p53 facing towards DNA, unlike human p53. Thus, this arginine may contact DNA in C. elegans p53, unlike human p53. Other than these differences, C. elegans p53 appears capable of making the similar DNA contacts as human p53.

Presentation summary #10

Structure of p53 mutants

Assaf Friedler

Structural mutants of p53 are thermodynamically destabilized, and significantly unfolded. Such mutants could be rescued by a small molecule that binds the native but not the denatured state and shifts the equilibrium to the native state, resulting in refolding of the mutant protein and restoration of activity. P53 mutants are also kinetically unstable, with half life of only a few minutes at body temp. Hence, molecules that rescue the conformation of such mutant should act immediately upon biosynthesis, preventing the mutants from denaturing and allowing them time to transfer to their target DNA. We describe a peptide that can rescue p53 mutations as the above chaperone mechanism. The peptide, CDB3, is derived from the p53-binding protein 53BP2 and binds p53 core in loop 1 and helix 2. CDB3 is able to stabilize mutant p53 thermodynamically and kinetically and to restore its DNA-binding activity. NMR studies show that CDB3 is able to shift the conformation of the distorted mutant R249S towards the wild type conformation.

Presentation summary #11

Suppressor mutations of p53 mutants

Rainer Brachmann

Using genetic strategies in the yeast Sacharomyces cerevisiae, we have identified intragenic second-site suppressor mutations that rescue function of 16 out of 30 of the most common cancer mutations tested. The suppressor motif involves one or more amino acid changes in codons 235, 239 and 240. The suppressor motif rescues function in yeast and mammalian assays. Reporter gene assays in mammalian cells show restoration of function to 50 to 200% of wild-type p53. The structural basis for the rescue mechanism is being determined and will be exploited for the identification of small compounds able to rescue p53 cancer mutants in a similar fashion.

We have constructed yeast reporter strains for 67 p53 DNA binding sites representing 57 confirmed and putative target genes. Only one third of the isolated DNA binding sites (studied under isogenic conditions) was utilized by wild-type p53. p53 DNA binding sites of genes related to cell cycle arrest, DNA repair and receptor pathway of apoptosis had positive p53 DBS while other apoptosis-related genes had negative DBS. These results were identical in mammalian assays, suggesting additional requirements for wild-type p53 to utilize the DBS of target genes central to p53-mediated apoptosis.

The same p53 DNA binding sites were evaluated with 77 p53 cancer mutants. A small portion of p53 cancer mutants showed transcriptional activity with p53 DBS that were positive with wild-type p53. Experiments to evaluate the extent to which the cancer mutants interfere with wild-type p53 are ongoing.

Presentation summary #12

Oncogenic signalling to p53 and mutant p53 reactivation by small molecules

Klas Wiman

Karolinska Institute, Dept. of Oncology/Pathology, Cancer Center Karolinska (CCK), Stockholm, Sweden

Oncogene activation triggers p53 accumulation and p53/dependent apoptosis. Studies mainly in mouse fibroblasts have shown that activated oncogenes such as E1A, Myc and Ras induces the p19ARF protein that inhibits the p53 antagonist MDM2, thus inducing p53. We asked if p14ARF is important for the p53 response to Myc or E2F1 activation in human fibroblasts carrying Myc or E2F1 estrogen receptor (ER) fusion proteins. We found that both Myc and E2F1 induced p53, p21 and MDM2, and cell cycle arrest and/or apoptosis. E2F1 but not Myc induced p14ARF. We designed an siRNA that efficiently inhibited p14ARF expression in human cells. Transfection of Myc-Er or E2F1-ER-carrying human fibroblasts with this siRNA did not prevent the induction of p53 in response to Myc or E2F1 activation. Myc and E2F1 induced p53 Ser-15 and Ser-37 phosphorylation, which was inhibited by the ATM/ATR inhibitors caffeine and wortmannin. We conclude that p14ARF is not required for the p53 response to activated Myc or E2F1 in human fibroblasts. Instead, p53 phosphorylation, possibly by the ATM and/or ATR kinases, appears critical.

We have previously screened a library of low molecular weight compounds from the National Cancer Institute (NCI) and identified a small molecule, PRIMA-1 (for p53 Reactivation and Induction of Massive Apoptosis), that reactivates mutant p53, induces apoptosis in human tumor cells, and inhibits human tumor xenograft growth in mice. NCI has tested more than 70,000 molecules in their chemical library for growth inhibition of a panel of 60 human tumor cell lines of different origin. Our statistical analysis of information in the NCI database revealed a statistically significant positive correlation between sensitivity to PRIMA-1 and mutant p53 expression levels, but not to proliferation rate. Moreover, PRIMA-1 showed a statistically significant preference for growth inhibition of mutant p53-carrying cell lines. In contrast, none of several known anticancer drugs showed such preference. This distinguishes PRIMA-1 from known anticancer drugs.

Structural analogs of PRIMA-1 will be screened in order to identify even more potent analogs. The best analog will be selected for large scale synthesis, toxicity test, and clinical trials in patients with various forms of cancer. In parallel, the possible interaction of PRIMA-1 with p53 will be examined by NMR. The effect of PRIMA-1 on cells will be studied by DNA microarray and proteomics analyses.

Our screening of the NCI library using a cellular assay also led to the identification of another mutant p53-reactivating small molecule, designated MIRA-1 (for Mutant p53-dependent Rapid Induction of Apoptosis). MIRA-1 is structurally unrelated to PRIMA-1 but has similar activity. MIRA-1 will be tested for antitumor activity in vivo. Our identification of mutant p53-reactivating small molecules will hopefully provide a basis for the development of novel and efficient anticancer drugs for the treatment of a wide range of tumors carrying mutant p53.

Acknowledgments:
Mikael Lindstrom (Myc/E2F1/p14ARF/p53)
Galina Selivanova, Vladimir Bykov, Natalia Issaeva, Alexandr Shilov, Jan Bergman (Mutant p53 reactivation by PRIMA-1 and MIRA-1)

Presentation summary #13

Gene transcription by p53 mutants

Moshe Oren

Overexpression of mutant p53 isoforms in p53-null tumor cell lines, such as H1299 or PC3, leads to increased resistance to killing by a variety of DNA damaging agents. In light of reports that killing by such agents may rely, to some extent, on the activity of the CD95/Fas death receptor pathway, we investigated whether mutant p53 overexpression can affect CD95 expression and activity. Stable transfection of PC3 cells with p53R175H was found to confer upon the cells partial resistance to killing by CD95 ligand. This was correlated with a reduction in CD95 mRNA, as well as CD95 surface receptor protein. Analysis of the underlying mechanism revealed that overexpressed mutant p53 represses several fold the activity of the CD95 promoter in luciferase reporter assays. Efficient repression required the integrity of the mutant p53 transactivation domain, as was not mediated by inhibition of endogenous p63 or p73 proteins. DNA binding assays, as well as chromatic IP, confirmed that mutant p53 could associate with CD95 promoter DNA in vitro and with CD95 promoter chromatin in vivo. The sequence requirements for this interaction, as well as the mechanism whereby mutant p53 binding leads to transcriptional repression, are presently under investigation. Repression of proapoptotic genes such as CD95 may contribute to enhanced chemotherapy resistance of tumor cells harboring mutant p53.

Presentation summary #14

P53 mutants and apoptosis

Varda Rotter

pending

Presentation summary #15

Posttranslational Modifications of Mutant p53

Carl W. Anderson

Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA
Shin'ichi Saito and Ettore Appella, Laboratory of Cell Biology, NCI, NIH, Bethesda, MD, USA

We prepared a panel of modification site-specific, affinity-purified polyclonal antibodies that individual recognize each of 14 of the approximately 20 know sites at which wildtype human p53 is posttranslational modified in response to DNA damage. These include antibodies that recognize include ser6, ser9, ser15, thr18, ser20, ser33, ser37, ser46, ser315, or ser392 when phosphorylated, and antibodies that recognize lys320 or lys382 when acetylated. We also have prepared antibodies that recognize Ser376 or ser378 when phosphorylated, but we have never observed a positive reaction with these antibodies with p53 immunoprecipitated from human cell extracts. Using these antibodies, we have characterized the modifications to wildtype p53 in A549 cells to three DNA damage-inducing agents, ionizing radiation, UV light, and adriamycin. While there are qualitative and quantitqative differences between agents, modification at each site is induced by DNA damage.

To examine site interdependence, we prepared vectors that express wildtype p53 in which each p53 modificaiton site was individually changed to alanine (for serine or threonine phosphorylation sites) or arginine (for lysine acetylation sites). The expression vectors where then transfected into H1299 cells, extracts were prepared after exposure to 8 Gy IR, immunoprecipitated, and then analyzed for phosphorylation or acetylation. Surprisingly, changing Ser6 to alanine blocked phosphorylation of ser9 and vice versa. Likewise, changing Ser15 to alanine blocked phosphorylation at Ser9, Thr18, and Ser20, but not at other sites, while changing Ser20 to alanine blocked phosphoryaltion at Thr18 but not at Ser9, Ser15 or other sites. Finally, Changing Ser33 to alanine blocked phosphorylation of Ser37 but not vice versa. Interdependencies between N-terminal phosphoryaltion sites and C-terminal phosphorylation or acetylation sites was not observed except that phosphoryaltion of Ser15 was required for acetylation of Lys382. While we cannot exclude all trivial explainations, these data sugest that the N-terminal phosphoryaltion sites may be arranged in a higharchy such that phosphoryaltion of a few critical sites (e.g. Ser15) is required for modification of other sites. We suggest that this arrangement could serve as a "checkpoint" mechanism to prevent inadvertant phosphoryaltion of p53 sites in response to inappropriate signals.

We have begun to analyze the posttranslational modifications of mutant p53s in response to DNA damage. Surprisingly, phosphorylation of conformational mutants, C135F, C176F, V143A, and E286K was abrogated at all N-terminal sites and only modest "constitutive" phosphorylation was observed at the C-terminal Ser315 and Ser392 sites; Lys 382 was not acetylated in these mutants. In contrast, DNA phosphorylation and acetylation of DNA contact mutants R248W and R273H in response to UV, IR or ADR was near normal, as was phosphorylation of the transcriptionally dead mutant L22F/W23S. Treatment of cells with gledamycin, a drug that binds hsdp90 and restores ubiquitin-mediated degradation of mutant p53 (as shown by others) also restored phosphorylation of p53 in response to DNA damage, suggesting that phosphorylation may be prevented by occlusion of p53 by chaperones, including hsp90, which bind mutant p53s.

Presentation summary #16

p53 family member interactions in cancer cells

Giovanni Blandino

The p53 tumor suppressor gene is the most frequent target for genetic alterations in human cancers, whereas the recently discovered homologues p73 and p63 are rarely mutated. We and others have previously reported that human tumor-derived p53 mutants can engage in a physical association with different isoforms of p73 inhibiting their transcriptional activity. Here, we report that human tumor-derived p53 mutants can associate in vitro and in vivo with p63 through their respective core domains. We show that the interaction with mutant p53 impairs in vitro and in vivo sequence specific DNA binding of p63 and consequently affects its transcriptional activity. We also report that in cells carrying endogenous mutant p53, such as T47D cells, p63 is unable to recruit some of its target gene promoters. Unlike wt-p53, the binding to specific p53 mutants markedly counteracts p63-induced growth inhibition. This effect is, at least partially, mediated by the core domain of mutant p53. Thus, inactivation of p53 family members may contribute to the biological properties of specific p53 mutants in promoting tumorigenesis and in conferring selective survival advantage to cancer cells.

Presentation summary #17

TP53 mutations in breast and ovarian cancers

Anne-Lise Borresen-Dale

Mutations in the TP53 gene is found in 30-50% of breast tumors depending on stage of the disease. Patients with mutations, specifically those that disrupting the L2 and L3 loop of the protein have shorter survival, and have lack of response to doxorubicin treatment, as well as FUMI (5FU and Mitomycin C). Mutations are more often found to reside on the codon 72 Arginine allele than the Pro allele, suggesting that mutations in combination with the Arg72 are selected for and enhance the oncogenic potential of the cell. Genome wide expression profiling of breast tumors has lead to identification of 5 different subclasses, two luminal epithelial derived oestrogen receptor-positive tumour subtypes, a basal epithelial-like, an ERBB2+ group, and a normal breast-like group. Survival analyses showed significantly different outcome for patients belonging to the various subtypes, including a poor prognosis for the basal-like and a significant difference in outcome for the two luminal /ER+ subtypes. Differences in *TP53* mutation frequency between the subtypes indicated an important role for this gene in determining the gene expression pattern in the various tumors.
Early stage ovarian tumors have mutations in the TP53 gene in 30% of the cases. No correlation to long-term survival (>10y) was seen. In a series of advanced ovarian tumors TP53 mutations were found in 78% of the cases, nonmissense mutations counting for more than 30%. No correlation to survival and TP53 mutation status was found. A preferential loss of the Arg72 allele were seen, and when patients were stratified according to the tumor genotype of the codon 72 Arg/Pro7. Patients with Pro genotype in their tumor and with mutation disrupting the L2 and L3 loop of the protein had significantly shorter survival.

Presentation summary #18

New mechanisms of tumorigenesis involving germline p53 mutants

Gerard Zambetti

We have identified a novel, germline p53 mutation (R337H) that selectively predisposes carriers to adrenal cortical carcinoma. In vitro overexpression assays (EMSA, promoter-reporters, apoptosis, colony reduction) fail to reveal a defect. Tumors undergo LOH and express high levels of missense protein in nucleus. Structural studies show a pH dependant defect in the stability of the 337H mutant. This mutation arose multiple times and no single founder exists, eliminating a linked gene. Penetrance is 10%, which indicates a 300,000 fold increased risk in developing this disease. Future studies will need to rely on physiological approaches to understand the biological consequence of this mutation on p53 tumor suppressor activity.

We have also generated the PUMA knockout mouse model. Loss of PUMA expression confers strong persistence to DNA damage-induced apoptosis and nearly accounts for all p53 apoptotic activity. This represents the first demonstration of a bona fide p53 regulated gene that is required for p53-mediated cell death in a physiological in vivo model.

Presentation summary #19

Transgenic mouse model of mutant p53

Guillermina Lozano

Some p53 missense mutations exhibit gain-of-function properties in cell lines. To analyse the in vivo significance of these mutations, we have generated three different knock-in mouse models inheriting mutations that correspond to R175 in human p53.

Two mouse models inherit the R172H mutation, one of which expresses the R172H protein at low levels. Tumors in mice heterozygous for this mutation (p53 R172HAg) metastasize to high frequency and rarely show LOH. Preliminary data on the second two models, one expressing R172H at appropriate levels and one expressing the R172P mutation were designed to address the dominant negative nature of mutations and the role of apoptosis, respectively.

Presentation summary #20

The role of mutant p53 proteins in the phenotypes of cancers.

Arnold Levine

Based upon the literature, there is a clear impact of missense mutant p53 proteins upon the phenotype of cancer cells. These phenotypes include growth potential of cells in vitro and in vivo, resistance to chemotherapy and patterns of gene expression. These effects are mediated by protein-protein interactions and the candidates for these interactions are p63, p73 and transcriptional co-activators. The co-activators are no longer bound to mutant p53 proteins when additional mutations are present (residues 22,23 of p53) and then the gain of function phenotype disappears.

When we understand the mechanism of action of mutant p53 protein in cells, the gain of function phenotype will become accepted in the field.

POSTER AND SHORT PRESENTATION ABSTRACTS

Abstract #: 1

Reactivation of mutant p53 by a one-hybrid adaptor protein

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The most frequent genetic alteration in cancer is a mutation of p53. In most cases, this leads to a sharp increase of the p53 protein levels, but abolishes p53's function as an activator of transcription. To correct this defect, wild type p53 is being re-introduced into tumor cells through gene therapy vectors, thereby inducing cell death. However, this effect is not necessarily specific for tumor cells. Further, mutant p53 in tumor cells transdominantly impairs the function of wild type p53. As an approach to overcome these obstacles, we have developed an adaptor protein that reactivates mutant p53, rather than stimulating transcription on its own. The DNA binding and tetramerizing portions of the p53-homologue p73 were fused to the oligomerization domain of p53. This chimera binds to the DNA of p53-responsive promoters through the p73-derived portions, and it binds to mutant p53 by the p53-derived oligomerization domain. Through this one-hybrid system, mutant p53 is tethered to p53-responsive promoters and re-enabled to activate transcription. When the adaptor was expressed in tumor cells that contain mutant p53, expression of p53-responsive genes was activated, and growth was inhibited. No such effects were observed in cells that contain wild type p53 or no p53 at all. When the adaptor was expressed through an adenovirus vector, tumor cells containing mutant p53 were specifically induced to undergo apoptosis. This strategy can turn mutant p53 into an inhibitor of tumor cell growth and might enable gene therapy to eliminate cancer cells with specificity.

Abstract #: 2

Rationally designed peptide rescues mutant p53 in cancer cells.

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A rationally designed 9-residue peptide, Fl-CDB3, rescues the conformation and the DNA binding of p53 oncogenic mutants, as shown by structural studies (Friedler et al., PNAS 2002, 99, 937-942). We have now found that Fl-CDB3 was taken up by tumor cells and localized primarily in the cytoplasm and partly in a nucleus. Fl-CDB3 induced upregulation of the level of mutant and wild type p53 proteins in cells. CDB3 labeled with biotin bound to p53 in a context of cellular environment. The wild type PAb1620 conformation of mutant p53 proteins R273H and R175H was restored in cells by FL-CDB3. Induction of PAb1620(+) conformation was followed by downregulation of unfolded PAb240(+) form. Endogenous p53 target genes p21 and Mdm-2 were induced by FL-CDB3 in a p53-dependent manner. Rescue of p53 conformation resulted in partial restoration of apoptosis in cells carrying p53 mutants R273H and R175H. Interestingly, restoration of wild type conformation to mutant p53 by another p53-binding peptide, Fl-poly-Glu was also observed. However, transcriptional transactivation function of p53 was not induced by Fl-poly-Glu. Control Fl-conjugated peptide which does not bind p53 according to NMR data, had similar localization in cells but did not restore the wt conformation of mutant p53 protein and did not change the levels of p53 or its target genes. Possible applications of peptides that bind p53 with different affinity for modulation of p53-mediated cell cycle regulation and apoptosis will be discussed.

Abstract #: 3

Different modes of DNA interactions of wild type p53: The key to understanding how mutant p53 proteins bind DNA.

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The acquisition of novel activities by mutant p53 proteins known as "gain-of-function" has a significant impact on tumor progression in human cancers. Whereas the loss of wild type p53 activities resulting from TP53 mutations is well characterized, the mechanisms underlying the "gain-of-function" of mutant p53 proteins are poorly understood. Sequence-specific DNA binding is one of the best characterized activities that is crucial for the ability of wild type p53 to function as transcription factor. That mutant p53 proteins do not bind p53 response elements recognized by wild type p53 led to the wide spread opinion that mutant p53 proteins are DNA binding inactive. However, numerous pieces of evidence, including those from our group (Will et al. 1998; Koga and Deppert, 2000), strongly indicate that mutant p53 proteins are in fact DNA binding active proteins that do bind DNA in a highly specific mode that is different from that of wild type p53. Our studies revealed that DNA binding of mutant p53 proteins derives from the inherent ability of the wild type p53 protein to bind DNA in a DNA structure-dependent fashion. We found that SSDB of wild type p53 is determined not only by the presence of a specific sequence, but also by specific structural determinants of the DNA. Importantly, our results demonstrate that the presence of both, the specific sequence and of a specific DNA structure, are required for binding of wild type p53 to its cognate sites. Such a dual- mode of DNA recognition could explain, how the high specificity of wild type p53 DNA interaction can be ensured with p53 response elements represented by heterogeneous sequences. Our results demonstrate that SSDB of wild type p53 is versatile and occurs in strikingly different modes depending on DNA topology: whereas wild type p53 binds to linear DNA in a mode corresponding to the "latent" p53 model (Hupp et al. 1992), "latent" p53 exhibits a high preference for specific binding sites in non-linear DNA (Kim et al. 1997; Göhler et al. 2002). Our finding that wild type p53 is a DNA structure-dependent DNA binding protein raised the possibility that DNA binding of mutant p53 proteins, which have lost the ability to bind DNA sequence-specifically, may be primarily determined by DNA topology .

Abstract #: 4

Molecular mechanism of restoring function of p53 cancer mutations

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The tumour suppressor protein p53 is implicated in half of all human cancers and presents a classic example of protein misfolding and disease. It is of particular interest to study the mutations that are closely associated with human cancers, and more specifically, substitutions which target amino acid residues that are in direct contact with DNA and/or that destabilize the overall structure of the DNA binding domain i.e. the so called hot spots. It is hoped that pharmaceutical agents could restore function to some p53 mutants and could be used in cancer therapy.

Abrogation of the p53 function is caused by structural destabilization and unfolding of the native global fold of the core domain, or by distortion of the conformation required for DNA binding, or both. In principle, improving the stability of the fold or re-establishing the proper conformation should restore biological function to p53 tumorigenic mutant proteins. I have tested this hypothesis by investigating the mechanism of rescue of common p53 cancer mutations by second-site suppressor mutations. The results have implications for possible drug therapy to restore the function of key tumorigenic p53 mutants: the function of mutants such as V143A and G245S can theoretically be restored by small molecules that simply bind to and hence stabilize the native structure, whereas mutants such as R249S will require alteration of the mutant native structure. For this class of mutants, restoring the stability will not be sufficient to rescue the function of p53. Drugs targeting this class of mutants must also restore the conformation for DNA binding.

Abstract #: 5

P53 codon 72 polymorphism : The Proline allele is preferentially expressed in germline heterozygotes whereas the Arginine allele predisposes to breast cancer.

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p53 is arguably the most critical tumor suppressor gene product that prevents malignant transformation. The p53 gene can exist as two polymorphic variants, resulting in either an Arginine or Proline residue at codon 72, whose functional significance in physiology and carcinogenesis is not well understood. Here, we have investigated the expression profile of the p53 polymorphic variants in the different ethnic Asian populations of Singapore, i.e. the Chinese, Indians and Malays, and found that the Chinese population have more Arginine homozygotes compared to the Indians and Malays. In contrast, many of the Indians and Malays are Proline homozygotes. Strikingly, all the Chinese germline heterozygotes express the proline allele at the RNA level, thus skewing the number of Arginine expressers in the Asian population to a smaller proportion compared to the Caucasians. However, there is an increase in the number of Arginine expressers in the Chinese breast cancer samples. Moreover, sequence analysis revealed a distinct absence of mutations in the p53 gene in most of the Arginine expressing samples. In those samples with a p53 mutation, most of the mutations were found outside the DNA-binding domain of p53, which is in contrast to the Proline expressing tumors that almost exclusively carry a mutation in this domain. Taken together, it appears that there is a selective pressure against the expression of the arginine allele in Asians, suggesting that its expression might predispose people to cancer. Furthermore, the arginine allele appears to be the "weaker" of the two, as there is less pressure to mutate it during tumor development.

Abstract #: 6

p53 and the topoisomerase I damage response

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In the mid 90's it was reported that wildtype and mutant p53 bound to human topoisomerase I (htopoI) in vitro as well as in vivo. Through this interaction p53 was found to directly stimulate the activity of htopoI. Soon a connection to a DNA damage response was made as it was reported that this activation only took place after treatment with DNA damaging agents in vivo. HtopoI was only stimulated by wt p53 for a short time after DNA damage whereas cells expressing mutant p53 constantly activated htopoI. Recently, it was found that UV-irradiation of cells lead to formation of stable covalent htopoI-DNA complexes (the so called cleavage complexes) which were found to be dependent on p53. However, it was not clear what the importance of a p53 mediated htopoI damage response was. Through our recent research we have gained new insights on this issue. According to our results the htopoI damage response seems to be involved in an apoptotic pathway. Apoptotic cells contain high levels of stable cleavage complexes whereas other cells within the same population seem to escape this apoptotic signal possibly by repairing the htopoI cleavage complexes. We have identified a p53-stimulated repair reaction for such complexes called TIRR (topoisomerase I induced recombination repair). TIRR is based on the recognition of a htopoI cleavage complex by a second htopoI molecule which in turn causes the release of the cleavage complex attached to a short piece of DNA (~13 nts). The remaining htopoI ligates a foreign DNA strand in a recombination-like event and repairs the damage. We believe that this repair pathway may be involved in both the induction of apoptosis as well as the possible repair of htopoI cleavage complexes. Both reactions would be expected to occur in a p53 dependent manner. In the case of apoptosis unresolved recombination structures could trigger apoptosis if too many repair events take place. We suggest that there may exist a threshold under which repair of the htopoI cleavage complexes take place and above which apoptosis is triggered. Investigations are ongoing in order to find out if TIRR is involved in the apoptotic htopoI damage response in vivo. Since mutant p53 has been reported to activate htopoI in the absence of damage we believe that a p53 stimulated TIRR pathway could play an important role during genomic instability observed in cells expressing "gain of function" p53 mutants.

Abstract #: 7

Interaction of mutant p53 with DNA in the chromatin context: the way to understand gain of function

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The gene of the tumor suppressor p53 is mutated in about 50-60 % of all human tumors. Functional analyses indicate that in addition to a loss of activities normally exhibited by wild-type p53 (wtp53), mutant p53 proteins (mutp53) often obtain new properties providing tumor cells with the enhanced oncogenic potential. But the molecular basis underlying activities of mutant p53 proteins are not well understood. Wild-type p53 is a sequence-specific DNA binding protein. Mutant p53 proteins have lost this ability. Therefore, it is widely believed that mutant p53 proteins are inactive for DNA binding. Nevertheless, earlier results from our laboratory with isolated mutp53 (R175H, R273P and R273H) have shown that mutant core domain and C-terminus bind with high affinity to nuclear matrix attachment regions of DNA (MARs) and nuclear scaffold attachment regions (SARs) in vitro [1, 2, 3, 4]. These elements are characterized by a high content of repetitive elements and their ability to adopt a non-B DNA conformation under superhelical stress.
We demonstrated that DNA topology is an important parameter for regulating the selective and highly specific interaction of wtp53 with its target binding sites [5, 6, 7]. The C-terminal domain plays the key role for wtp53 selective binding to its target sites in a non-linear DNA conformation (stem-loop structure) [7]. Also for structure selective binding of wtp53 to supercoiled DNA is the C-terminus essential [8].
This DNA conformational contex of p53 binding also seems to be important for mutp53. By EMSA we have shown that some p53 mutants have sustained the ability to interact with non-B elements (stem-loop structures) and with supercoiled DNA specifically. To support these in vitro studies we analyzed the binding of mutp53 (G245S and R273H) to cellular DNA by chromatin immunoprecipitation in two glioma cell lines (Onda 11 and U251). Isolated genomic DNA fragments are different in sequence, some of them have repetitive character [9] and some contain the MAR "unwinding" motif.
We suppose that defining the DNA structure and sequence requirement for high affinity structure-selective DNA binding of mutp53 can contribute to understand the molecular basic of the "gain of function" activities of mutp53.

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Abstract #: 8

Recognition of Cisplatin-Modified DNA by Protein p53. Roles of the p53 Domains and its Posttranslational Modifications.

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Binding of latent and active protein p53 to DNA fragments modified by cisplatin was studied using electrophoretic mobility shift assay in agarose gels and immunoblotting analysis. Both latent (expressed in bacteria, posttranslationally unmodified) and active (expressed in insect cells, or in vitro-activated) p53 forms bound to cisplatin-modified DNA lacking the p53 consensus sequence (p53CON) with a higher affinity than to the same but unmodified DNA fragments. The latent form of p53 exhibited a more pronounced selectivity for the platinated DNA than the active p53. The preference of p53 for platinated DNA decreased as a consequence of the activation of the bacterially expressed p53 by phosphorylation at the PKC site within its C-terminus or by binding of the monoclonal antibody Bp53-10.1. Competition experiments involving a 20-mer oligonucleotides spanning the p53CON suggested that the p53 core domain was the primary binding site of the active p53 when it bound to the unmodified or platinated DNA fragments lacking the p53CON. The latent protein was found to selectively interact with DNA modified by cisplatin probably via its C-terminus. These suggestions were further supported by protein deletion studies showing that isolated p53 C-terminal domain [p53(320-393)] but not p53 constructs lacking the basic C-terminal DNA binding site [e.g., p53(1-363)] bound the platinated DNA with a high selectivity.

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Abstract #: 9

Binding of p53 protein to supercoiled DNA

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A key feature of the p53 tumor suppressor protein is its ability to bind specific DNA sequences (p53CON) via its core domain (CD). Activation of p53 itself is achieved trough posttranslational modifications. The protein C-terminal domain (CTD) involves a site that binds DNA sequence non-specifically. It has been shown that p53 can recognize certain DNA structural motifs via either its CD (hairpins, Holliday junctions) or CTD (in general, entities related to DNA damage). A new type of p53-DNA interaction, the supercoil-selective (SCS) binding, has been recently discovered in our laboratory [1-9]. Wild type p53 selectively binds supercoiled (sc) DNA regardless of the presence or absence of the p53CON. Using deletion p53 constructs and by means of manipulating the protein with oxidation agents, transition metals and monoclonal antibodies, essential roles of the p53 CTD and of oligomerization of the protein in the SCS binding has been established [2]. Using a new competition assay, we investigated the effect of DNA negative supercoiling on the DNA sequence specific binding (SSDB) of human wild-type (wt) p53 protein. We found that supercoiled (sc) pBluescript DNAs with different inserted p53 target sequences were stronger competitors than a mixture of scDNA pBluescript with the given 20-mer target ODN. scDNAs were always better competitors than their linearized or relaxed forms. In contrast to the full length wt p53, the deletion mutant p53 CD30 and the p53 core domain (aa 93-312) showed no enhancement of p53 SSDB to scDNA suggesting that, in addition to the p53 core domain, the CTD was involved in this binding. We conclude that cruciforms and DNA bends contribute to the enhancement of p53 SSDB to scDNA and that the DNA supercoiling is an important determinant in the p53 sequence specific binding [10]. Supercoiling may thus play a significant role in the complex p53 regulatory network.

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Abstract #: 10

RECOGNITION OF DNA DAMAGE BY P53: THE ROLE OF THE CORE DOMAIN

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In addition to its ability to bind specifically to DNA, p53 recognizes DNA strand breaks and insertion/deletion mismatches (IDL's). Interaction with damaged DNA is mediated by C-terminal negative regulatory domain of p53. Taken together, these observations suggest that p53 can serve as a sensor of DNA damage in vivo. Interaction of p53 with damaged DNA can trigger a conformational change in p53 protein, resulting its activation and/or stabilization. It was demonstrated that the core domain of p53 could interact with IDL's. In this study we address the question which structural features in DNA the different domains of the p53 protein recognize. C-terminal tail interacts with unpaired nucleotides in double stranded DNA, either as a protruding end or as an internal gap. In contrast the core domain binds preferentially to the hairpin structures in single-stranded DNA. The affinity of p53 towards hairpin like structures is comparable with that for the specific consensus-binding site. Moreover the specific DNA binding site coincides with the site for hairpin recognition. Our results demonstrate that distinct p53 domains recognize different type of lesions. This might allow the recognition of a broad range of different types of DNA lesions by p53. The ability of p53 to sense different types of DNA damage could be important for the maintenance of the genomic integrity.

Abstract #: 11

Functional characterization of p53 mutations of non-small-cell lung cancers by molecular simulation.

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Recent studies have demonstrated that TP53 mutations result in an altered p53 function, rather than a simple loss of function. The functional properties of mutants are likely important for cancer progression and treatment. Our aim was the identification and structural characterization of three p53 mutations involved in non-small-cell lung cancers (NSCLCs) .
Patients with squamous cell-type (25%) NSCLCs were analyzed. Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) and sequencing was performed. The 50% of the tumors had p53 mutations involving amino acids in the DNA contact region (codons 275 and 281) and a mutation in the central domain of p53 (codon 181) which is close to the Zn coordination site.
Models were built with InsightII and were relaxed with Discover. Structural analysis showed that Arg181Leu mutation could change the affinity of Zn through structural and electrostatic potential alterations; while the double mutant Asp281His/Cys275Tyr was directly involved in the DNA-protein interactions. The Asp281His/Cys275Tyr mutations presented conformational changes in zones where the appropriate docking DNA-protein could be modified. The electrostatic potential analysis (Delphi) showed critical changes for all mutations, so the electrostatic interactions between Zn-protein and DNA-protein should be affected. Our results show that these mutations could change the affinity and the architecture of the DNA-p53 complex.

Abstract #: 12

Discrimination of single amino acid mutations of the p53 protein by means of deterministic singularities of recurrence quantification analysis

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p53 is mutated in roughly 50% of all human tumors, predominantly in the DNA-binding domain codons. Structural, biochemical, and functional studies have reported that the different p53 mutants possess a broad range of behaviors that include the elimination of the tumor-suppression function of wild-type protein, the acquisition of dominant-negative function over the wild-type form, and the establishment of gain-of-function activities. The contribution of each of these types of mutations to tumor progression, grade of malignancy, and response to anticancer treatments has been so far analyzed only for a few "hot-spots". In an attempt to identify new approaches to systematically characterize the complete spectrum of p53 mutations, we applied recurrence quantification analysis, a non-linear signal analysis technique, to p53 primary structure. Moving from the study of the p53 hydrophobicity pattern, which revealed a singular deterministic structuring, we could statistically discriminate, on a pure amino acid sequence basis, between experimentally characterized DNA-contact defective and conformational p53 mutants with a very high percentage of success. This result indicates that recurrence quantification analysis is a mathematical tool particularly advantageous for the development of a database of p53 mutations that integrates epidemiological data with structural and functional categorizations.

Abstract #: 13

Rationally designed FL-CDB3 peptide restores the conformation and transcriptional transactivation function to mutant p53 in tumor cells.

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A rationally designed 9-residue peptide, Fl-CDB3, rescues the conformation and the DNA binding of p53 oncogenic mutants, as shown by structural studies (Friedler et al., PNAS 2002, 99, 937-942). We have now found that Fl-CDB3 was taken up by cells and localized primarily in the cytoplasm and partly in a nucleus. It caused accumulation of wild type p53 and representative oncogenic mutants R273H and R175H in cells. CDB3 labeled with biotin bound to p53 in a context of cellular environment. The conformation of mutant p53 proteins R273H and R175H was restored in cells by FL-CDB3. Endogenous p53 target genes p21 and Mdm-2 were induced by FL-CDB3 in a p53-dependent manner. Interestingly, restoration of wild type conformation to mutant p53 by another p53-binding peptide, Fl-poly-Glu was also observed. However, transcriptional transactivation function of p53 was not induced by Fl-poly-Glu. Control Fl-conjugated peptide which does not bind p53 (according to fluorescence anisotropy data) had similar localization in cells but did not upregulate the p53 protein and p53 target genes Mdm2 and p21. Control peptide did not restore wt conformation of p53 protein as well. Possible applications of peptides that bind p53 with different affinity for modulation of p53-mediated cell cycle regulation and apoptosis will be discussed.

Abstract #: 14

DNA microarray analysis of molecular pathways of apoptosis induced in tumour cells by p53-reactivating compounds PRIMA-1 and RITA-1.

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Since its discovery as a tumour suppressor some fifteen years ago, the transcription factor p53 has attracted paramount attention for its role as "the guardian of the genome". TP53 mutations occur so frequently in cancer, regardless of patient age or tumour type, that they appear to be part of the life history of at least 50% of human tumours. In tumours that retain wild-type p53, its function is inactivated due to deregulated HDM2, a protein which binds to p53 and which can inhibit the transcriptional activity of p53 and induce its degradation.

PRIMA-1 and RITA-1 are two compounds which both reactivate p53, but do so in very different ways. PRIMA-1 was found in a screen of the NCI library of low molecular weight compounds, for molecules that selectively target cells with mutant p53. The compound has been shown to refold several p53 mutants - both structural, and contact - into an active conformation and induce apoptosis in vitroand tumour suppressionin vivo in a mutant p53 – dependent manner. RITA-1 was found in a different screen of the same library, which was aiming to identify compounds that selectively kill tumour cells carrying wt p53, and deregulated HDM2.

We are applying microarray analysis, using both Affymetrix GeneChip microarrays and cDNA-microarrays (the latter in collaboration with J. Lundeberg, KTH), to take a holistic view on the effects of the two compounds on the transcriptome. This will hopefully result in the elucidation of the precise transactivation properties of reactivated p53, but might also deepen our understanding of the transactivation function of both mutant and wt p53 in hyperproliferative disease.

Abstract #: 15

Restoration of apoptosis inducing function of p53 in human tumor cells by small molecules via disruption of the p53/mdm2 interaction.

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P53 is one of the key players in signaling apoptosis: a number of stress signals converge on p53, which responds to them by triggering cell cycle arrest and/or apoptosis. This function of p53 is crucial for the prevention of tumor development as well as for the response to anticancer therapy. Wild type p53 induces expression of Mdm-2 which in turn degrades p53. In addition, p53 down regulates Mdm-2 inhibitor p14ARF creating another auto regulatory loop. Due to activation of Mdm-2 and inhibition of p14ARF p53 levels are maintained low in normal cells.
Disruption of the interaction between p53 and MDM2 in human tumors by drugs should release transcriptionally active p53, which could suppress further tumor development or induce apoptosis in cancer cells.
We have screened the library of the low molecular weight compounds (NCI, USA) and identified two compounds that are capable of disruption of Mdm-2/p53 interaction, stabilization of p53 protein in cells, reactivation of p53 transactivation function and growth suppression of tumor cells containing wild type p53 without apparent toxic effects on normal cells. The results and further studies will be discussed.

Abstract #: 17

ACTIVATION OF P53 BY THE CYTOPROTECTIVE AMINOTHIOL WR1065: INSIGHTS ON THE MOLECULAR MECHANISMS INVOLVED

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WR1065 is an aminothiol with selective cytoprotective effects in normal compared to cancer cells, which is used to protect tissues against the damaging effect of radiation and chemotherapeutic drugs. WR1065 induces the accumulation and activation of the tumor suppressor protein p53, raising the possibility that p53 induction may play a role in cytoprotection. We show that WR1065 exerts a p53-dependent cytostatic effect by the transactivation of a selective subset of p53 target genes involved in cell cycle arrest but not in apoptosis. We show that WR1065 modulates p53 activity by several distinct mechanisms. (i) WR1065 can stimulate to a higher degree than DTT the p53 redox-dependent, DNA-binding activity in vitro. (ii) WR1065 acts on the p53 protein conformation by inducing part of p53 temperature sensitive mutant (p53V272M) to fold into wild-type form at the nonpermissive temperature of 37°C. (iii) WR1065 reduces the level of p53-dependent apoptosis induced by gamma-irradiation. Moreover, p53 activation is not accompagnied by p53 phosphorylations of Ser 15, 20 or 37, suggesting that p53 accumulation does not result from the formation of DNA-damage. Furthermore, WR1065 activates the c-Jun N-Terminal Kinase (JNK). This kinase exerts multiple regulatory functions in response to pro- and anti-oxidant stresses as well as many DNA damaging signals. WR1065 is apparently capable of selectively activating the JNK pathway, without inducing DNA damage. The stable transfection of a dominant negative form of JNK (JNK-APF) into MCF-7 cells reduced by over 50%, the activation of p53 by WR1065.

We propose that p53 activation by WR1065 through redox-mechanism, conformational modulation and JNK-dependent DNA-damage independent signaling pathway may represent an important step in the mechanism of action of WR1065. WR1065 has pleiotropic effects on many signaling pathways and although the contribution of p53 activation to cytoprotection remains to be determined, this pathway may prove useful for pharmacological modulation of p53 activity through non-genotoxic mechanisms.

Abstract #: 19

Characterization of the p53 mutants ability to inhibit p73b transactivation using a yeast-based functional assay.

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It was clearly shown that p73 transactivation from a p53 responsive promoter is inhibited by some tumor-derived p53 mutants in eukaryotic cells. In this study we adapted a yeast based p53 functional assay for the analysis of the influences of different p53 mutants on the activity of one of the p73 isoforms, namely p73b. We determined the ability of a panel of 61 p53 mutants to inhibit p73b activity following the net transcription of the ADE2 color (red/white) reporter gene driven by a p53-responsive promoter. By analyzing a large number of mutants we could conclude that interference: a) is a quite frequent phenomenon; b) is not confined to p53 mutations located in particular topological regions of the DNA binding domain; c) does not appear to be dependent on the kind of side chains introduced at a specific position; d) appears to significantly correlate with evolutionary conservation of the mutated p53 codon, frequency of occurrence of the mutation in tumors. Furtermore, two sets of polymorphic variants (R and P) for 14 mutants were constructed and analysed. In all cases, the R/P 72 polymorphism was phenotypically irrelevant. In conclusion, our results suggest that the interpretation of the biological effects of p53 mutants should take into consideration the possibility that p53 mutants show loss or gain of function also through the interference with p53 family members.

Abstract #: 20

CHARACTERIZATION OF THE FUNCTIONAL HETEROGENEITY OF P53 MUTANTS USING A YEAST-BASED ASSAY

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The transcription factor p53 is frequently altered in human tumors and nearly 1200 different amino acid changes have been reported. Mounting evidence indicates that p53 mutants may retain partial activity or even acquire new functions. Understanding the specific impact of any p53 mutant protein and particularly the potential to differentially modulate the expression of the various p53 target genes is a major issue in evaluating the role of p53 mutations in cancer and the possible correlation between p53 status and clinical outcome. Therefore, we initiated a systematic analysis of mutant p53 function with the aim of characterizing the transactivation capability of a panel of 200 mutations that ranges from commonly to rarely associated with tumors. 23 isogenic p53-reporter yeast strains each containing a p53 response element (RE) upstream of the ADE2 color (red/white) gene were used in this study. We selected the 23 different p53 REs from target genes involved in cell cycle control, apoptosis, DNA repair, and p53 stability. Our results indicate that nearly 30% of p53 mutants are transcriptionally active, without any clear correlation with functional groupings of the p53 target genes. Temperature sensitivity (both cold and heat) was observed in ~35% of the mutants suggesting that conformational flexibility and defects in folding stability are common features of p53 mutants associated with cancer.

Abstract #: 21

Impact in human cells of p53 mutants that exhibit altered transactivation activities in yeast.

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The status of p53 is clearly important to human health since it is the most frequently altered gene identified in human cancers. Interestingly about 1100 different p53 mutations have been reported in human tumors. However, relatively few of them have been characterized in terms of their functional impact. In a previous study, we had developed a simple, yeast-based screening system with variable (rheostatable) p53 expression that reveals the transactivation capacities of p53 mutants towards over 30 different Response Elements (RE) targeted by p53. While some mutants were more active than WT p53 with all the REs (supertrans), others exhibited altered sequence specificity resulting in enhanced, normal, reduced or lack of activity, depending on the RE. We anticipated that these mutants would help to understand p53 function in human cells as well as help to assess the contribution of p53 mutations to cancer. Six mutants were examined for their impact on downstream target genes as well as various biological endpoints when expressed ectopically in p53+ and p53- human cell lines. Similar to results in yeast, we found that mutants S121F and N288K were more active than WT p53 in inducing apoptosis and growth suppression when overexpressed in human cells, demonstrating that they retain biological function. Using a real time PCR approach, we found that the transactivation potential of the supertrans mutant (S121F) towards 13 p53 target genes after transfection, is altered relative to WT, similar to observations in yeast. There were differences in the magnitude and pattern of expression, as well as biological consequences, among the cell lines.

Abstract #: 22

High frequency of temperature-sensitive mutations of p53 tumor suppressor in acute myeloid leukemia revealed by functional assay in yeast

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The tumor suppressor p53 is a transcription factor that participates in control of many cellular functions. All these activities are mediated by direct binding of the p53 tetramer to specific target sequences in promoters of directed genes. Lack of p53 function is often connected with development of cancer, but the frequency of p53 mutations is low in almost all types of leukemia. The aim of this study was to assess the frequency of mutations in the p53 gene in leukocytes of patients with acute myeloid leukemia (AML) using the FASAY functional analysis and to assess the relationship between the presence of p53 mutation and disease outcome. The following observations were made: (1) The presence of p53 mutations was detected in 13 of 62 tested AML cases (21%) and in 1 of 4 tested myelodysplastic syndrome (MDS) cases by FASAY. (2) The presence of p53 mutation was shown to be a poor prognostic/predictive factor in AML (P = 0.03/0.002). (3) Although there is a statistically significant relationship between the presence of p53 mutation and p53 protein accumulation (p = 0.05), not all samples having p53 mutation exhibited p53 protein accumulation. (4) Five of 13 p53 mutations detected in the leukocytes of AML patients (38.5%) and the mutation detected in the leukocytes of the MDS patient (altogether 6/14 - 42.9%) were partially inactivating temperature-sensitive mutations. (5) Different ts mutations differ in the level of their temperature sensitivity and in their responsiveness to the cytoprotective drug amifostine.

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Abstract #: 23

Functional characterization of rare p53 mutants observed in radiation-induced sarcomas developing after radiotherapy.

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Seven radiation-induced sarcomas occuring in the field of irradiation of predisposed patients treated for bilateral retinoblastoma were studied for TP53 gene mutations (1). In the seven cases, the two alleles of TP53 were inactivated, one by loss of the chromosome 17 short arm, the other by mutation. By comparaison with non radiation-induced tumors, the observed panel of mutations was uncommon. Four point mutations (C135F, V216E, M237I, E258K) were found at codons rarely mutated in spontaneous tumors. None of these mutations occurred at a CpG dinucleotide. The remaining three mutations were deletions larger than 1bp. This rate of deletion (3/7) is significantly higher than the values observed in spontaneous tumors.
We carried out the functional characterization of these rare mutants after transfection of their cDNAs in Saos2 cells. We determined by western blot that two of the p53 mutants with a deletion were unstable. Using a luciferase functional assay, we showed that the five other mutants had lost their functional ability to transactivate p21, Mdm2, Bax and PIG3. In addition, the four point mutations led to p53 mutants with a dominant-negative phenotype.
In these series of radiation-induced tumors, the wild type functions of p53 were lost by mutations whose uncommon caracteristics suggest the involvement of mechanisms induced by or associated with ionizing radiations.

(1)Lefevre SH et al., Oncogene 20, 8092-9, (2001). Genome instability in secondary solid tumors developing after radiotherapy of bilateral retinoblastoma.

Abstract #: 24

Role of Thioredoxin in the control of p53 protein response to genotoxic stress

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The transcription factor p53 controls proliferation and survival of cells exposed to many stresses. Its DNA-binding domain has a complex architecture stabilized by zinc, which makes the protein redox-sensitive. We have investigated the involvment of the redox regulator thioredoxin (Trx) in the regulation of p53 conformation and activity. First, we show that transient overexpression of Trx significally augmented the transcription of a p53-dependent reporter. Co-immunoprecipitations in MCF7 and HCT116 carcinoma cells showed that p53 and Trx co-precipitated in normal conditions, with increased complex formation upon activation by genotoxic treatments. Exposure to the zinc chelator TPEN, that transiently unfolds p53 structure, showed that Trx-binding to p53 depends on p53 conformation. Second, using MCF7 cells derivatives stably transfected with wild-type or dominant-negative Trx, we observed small differences in p53 accumulation between the different cells subtypes. However, phosphorylation of p53 on serine 15 in response to doxorubicin was more abundant in cells expressing WT than mutant inactive Trx, suggesting a role for Trx in this post-translational modification.
Overall, these results show that Trx may interact with p53 in a conformation-dependent manner and play a role in p53 activation.Thus, Trx may act as a regulator of p53 activity, in particular in response to DNA damage.

Abstract #: 25

The biologic function of p53 mutations common in breast cancer

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p53 mutation has been shown to be an independent adverse prognostic marker in breast cancer, which is one of the most common cancer types affecting females. A p53 mutation is present in about 25% of breast cancer cases, and it is associated with a more aggressive disease and a poor response to therapy. Hotspot mutations of p53, that disrupt DNA contact directly or destruct the normal protein conformation, have been thought to block the transactivation function of p53, but little is known about the specific effects that other mutations have on the biologic activity of the protein. No consistent results have been reported on the prognostic significance of mutations to single codons or functional domains of p53 in breast cancer. The function of twelve different p53 mutations common in breast cancer has been studied in several biological assays, including a bio-oligo based DNA binding pull -down assay, GFP transactivation assay and colony formation assay. Mutations of different subclasses were included, i.e. 248Trp and 273His for DNA contact mutants, 175His, 245Asp, 245Ser and 282Trp for structural, 238Phe and 179Arg for zinc binding and 220Cys and 280Thr for other mutants. In these studies three of the p53 mutants were found to retain some wild type like activity, namely, 245Asp, 245Ser and 280Thr were able to transactivate GFP expression through a p53 consensus response element in vitro. The preliminary results from the colony forming assay indicate that at least one (245Ser) of the four mutants tested (273His, 245Asp, 245Ser and 280Thr) may be able to inhibit colony formation of p53 null tumor cells. The implications of these findings in relation to p53 activities will be discussed.

Abstract #: 26

Systematic evaluation of naturally occurring p53 mutants in primary malignancies

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The tumor suppressor gene p53 is frequently mutated in hematological malignancies. Loss of p53 function not only promotes tumor formation, but also has been linked to chemoresistance in clinical trials as well as in preclinical models that harbor p53-deficient tumors. However, naturally occurring alterations at the p53 locus typically result in expressed, but structurally altered mutants which carry an amino acid exchange within the DNA-binding domain. Moreover, some p53 mutants are known to act in a dominant-negative fashion, as they can suppress proper p53 function even in the presence of retained p53 wild-type alleles.
Hence, we sought to systematically evaluate the biological role of a panel of lymphoma-related, naturally occurring p53 mutations in primary murine tumors in vitro and in vivo. Using oncogene-transformed mouse embryo fibroblasts and primary myc-transgenic hematopoietic stem cells, we introduced mutant p53 cDNAs by retroviral gene transfer into p53-proficient and –deficient backgrounds, and monitored growth characteristics of infected cells, analyzed the selective potential of individual mutants as well as their ability to protect from allelic loss of retained wild-type p53, and exploited novel properties that were not detected in p53 null controls.
Understanding the complexity of mutant p53 function in tumorigenesis and malignant growth control is expected to facilitate the development of small compounds that target tumors expressing mutant p53.

Abstract #: 27

Differential expression of TA and DNp63 in hepatocellular carcinoma cell lines

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The TP63 gene, a member of the p53 family, encodes several isoforms. They result either from the use of two promoters (TA and DN isoforms) or from alternative splicing in the C-terminus (a, b, g isoforms). The p63 protein is clearly involved in differentiation of several epithelial tissues, but its role in tumorigenesis is not well defined so far. Nevertheless, TAp63 isoforms are able to induce growth arrest and apoptosis, whereas transactivation-deficient DN isoforms are found amplified in squamous cell carcinoma, suggesting that p63 could have some tumor suppressor properties similar to those of p53.

We investigated the expression of the TA and DN p63 isoforms by RT-PCR in four hepatocellular carcinoma (HCC) cell lines, which differ in their p53 status: HepG2 (p53wt), Hep3B (p53null), Malhavu and PLC/PRF/5 (both p53R249S mutant). We observed the expression of TA isoforms in the cell lines expressing an inactive or none p53 protein, whereas DN isoforms are expressed only in the p53-null cell line.

We also studied the TP63 expression in HCC cell lines in response to various stress signals. Doxorubicin (DOX) and etoposide (ETO), two topoisomerase II inhibitors used in chemotherapy, were able to dramatically increase the expression of TA isoforms in all the cell lines, independently of their p53 status. The expression of DN isoforms was not modified by drug treatment.

We then investigated the expression of some p53-target genes in the HCC cell lines. All the target genes we tested were constitutively expressed in HepG2 cells, namely WAF1, 14-3-3s, GADD45, PIG3, BAX and increased upon DOX or ETO treatment, with the exception of BAX. In wild-type p53-deficient cells, only WAF1 and BAX expression showed a similar profile. On the contrary, 14-3-3s is undetectable in non-treated Malhavu cells, but is dramatically induced by DOX or ETO. Also, the constitutive expression of GADD45 is higher in Malhavu cells than in HepG2, but is not induced by DOX or ETO. We are currently performing ChIP assays, in order to determine if TAp63 isoforms play a direct role in the constitutive or inducible expression of such target genes in wild-type p53-deficient cells.

In conclusion, our results indicate that the expression of TA isoforms, which is not detected in cells expressing a wild-type p53, is induced upon stress in all cell lines tested, independently of the p53 status. On the contrary, the expression of the DN isoforms seems only linked to the presence of a p53 protein in the cell. Finally, the presence of HBV does not seem to influence TP63 expression, since Hep3B and PLC/PRF/5, both positive for HBV, show different profiles.

Further characterisation of the molecular mechanisms involved in the regulation of TP63 expression in HCC cells is still in progress.

Abstract #: 28

Gain of function of p53 mutant C277Y in the transcriptional regulation of pig3 and hdm2 genes

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We report the gain of transcriptional activity of the p53 mutant C277Y isolated from Ewing's sarcoma (EFT) and subsequently described in osteosarcoma, leiomyosarcoma and carcinomas of the colon, breast, and liver. Reporter gene assays performed in the EFT cell line SK-N-MC revealed strong transcriptional activity on the pig3 and hdm2 promoters, but not on the rgc promoter. In case of pig3, which plays a role in the cellular response to oxidative stress and in the induction of apoptotic pathways, p53 C277Y was 5 times more active than wt p53. In case of the major p53 regulatory protein HDM2 that is strongly activated by wt p53 the mutant C277Y induced similar promoter activity to wt p53. In contrast, the p53 mutant R273C did not activate either of the two promoters. Among a series of EFT cell lines with variable p53 gene status, steady state pig3 mRNA levels were increased and constitutive expression of p53 inducible s-HDM2 transcripts was detectable only in the cell line from which the C277Y mutant was isolated. Upon irradiation, a further increase in hdm2 and pig3 gene expression was observed confirming the transcriptional activity of this mutant. In addition, after transient transfection of p53 C277Y into a p53 null cell line, induction of endogenous pig3 and mdm2 genes and residual apoptotic activity of p53 C277Y when compared to R273C and wt p53 was observed. Our results provide evidence for a gain of function of p53 mutant C277Y.

Abstract #: 29

Head and neck cancer cell lines with mutations in the p53 gene are sensitive to drug-induced apoptosis: Role of p73 and

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More than 50% of human cancers contain p53 mutations resulting in the loss of most if not all p53-assosiated functions. p53 protein has been shown to play an important role in apoptosis induced by DNA-damaging agents. Mutations in the p53 gene can therefore result in resistant to killing by anticancer drugs. Two recently identified p53 family members, p63 and p73 have been shown to be important for cellular response to DNA damage. p73 shares significant sequence homology with p53 but in contrast to p53 human p73 transcripts contain multiple C-terminal splice variants, p73a to p73F. p73 also has an alternative promoter in intron 3, which encodes a truncated p73 protein known as DNp73 which lacks the transactivation domain and acts as a dominant negative suppressor of p73. p73 function has been shown to be required for p53 dependent apoptosis in response to DNA damage.
We have analysed a panel of 85 primary head and neck squamous cell carcinomas (HNSCC) and have identified mutations in exons 5-8 of the p53 gene in 68% of tumours. Most of the detected mutations were in exon 5 indicating that this region may contain hotspots for etiological factors associated with HNSCC. There was no significant correlation between p53 mutations and patient response to chemotherapy.

To further investigate the role of p53, a panel of 9 cell lines derived from primary HNSCC and 3 matched metastatic cell lines were treated with cisplatin (cis), doxyrubicin (dox) and taxol. Drug treatment resulted in the induction of cell death measured by MTT and FACS analysis and induced activation of TAp73 but not DNp73 transcription detected by RT-PCR. Interestingly, three metastatic cell lines, expressing endogenous DNp73 were substantially more resistant to apoptosis induced by these drugs.
Expression of wild-type p53 caused inhibition of cis-induced apoptosis in HNSCC and resulted in an increased G1 arrest detected by FACS. However, expression of p73 in the absence of p53, increased sensitivity of HNSCC cells to cisplatin. Furthermore, p73 expression in p53 mutated cell lines activated transcription of p53 apoptotic target genes measured by luciferase reporter assays. Expression of DNp73 resulted in increased resistance to drug induced apoptosis.
In conclusion our results demonstrate that HNSCC cell lines despite having mutations in p53 can be efficiently killed by DNA damaging drugs. Expression of DNp73 was detected only in the metastatic cell lines and was associated with resistance to drug-induced apoptosis. Expression of TAp73 was shown to increase sensitivity to drug induced apoptosis by activation of p53 targets. Our data suggest that the expression of some mutated p53, present in HNSCC, although clearly lack DNA binding may still retain the ability to co-operation with p53 family members to activate apoptotic targets.

Abstract #: 31

Mutant p53 gain of function: repression of CD95(Fas/APO-1) gene expression by tumor-associated p53 mutants

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Tumor-associated mutant forms of p53 can exert an anti-apoptotic gain of function activity, which probably confers a selective advantage upon tumor cells harboring such mutations. We report that mutant p53 suppresses the expression of the CD95 (Fas/APO-1) gene, encoding a death receptor implicated in a variety of apoptotic responses. Moderate (40-50%) downregulation of CD95 mRNA and surface protein expression by mutant p53 correlates with partial protection against CD95-dependent cell death. Excess mutant p53 represses the transcriptional activity of the CD95 promoter, with the extent of repression varying among different tumor-associated p53 mutants. Furthermore, mutant p53 protein binds the CD95 promoter in vitro, in a region distinct from the one implicated in tight interactions of the CD95 gene with wild type p53. Hence, the CD95 promoter is likely to be a direct target for down-regulation by mutant p53. This activity of mutant p53 may contribute to its gain of function effects in oncogenesis.

Abstract #: 32

Description of TP53 mutation spectrum in oesophageal cancers from North-Western France, a European high-incidence area.

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One of the major European areas for the incidence of oesophageal cancer is located in North-Western France. We screened TP53 mutations (exons 5-9) in tumours coming from patients recruited in a case-control study related to cancers of the oesophagus in Lower-Normandy. We used first DGGE and sequencing which allowed us to validate a DHPLC procedure. The results confirmed that oesophageal adenocarcinomas (ADC) and squamous cell carcinomas (SCC) show distinct mutations profiles. Ninety seven % (33/34) of SCC presented at least one mutation or polymorphism, mainly G to A and G to T substitutions. The alteration frequency was 69% (11/16) with a majority of G to A transitions at CpG dinucleotides for adenocarcinomas of the esophagus and cardia. This work will describe in detail this spectrum combined with those already published and established in the same geographical area. We will particularly stress the assumptions linking each type of mutation with carcinogens and the potential repercussions of these alterations on the structure and function of the p53 protein. In the near future, our aim will be to use the yeast functional assay in order to test in vitro mutagens involved in oesophageal carcinogenesis. This approach could permit us to retrieve mutations characterized in human tumour samples.

Abstract #: 34

ETIOPATHOLOGY OF HCC IN THAILAND : IMPLICATION OF HBV, HCV AND P53 MUTATIONS

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With near to 500 000 new cases diagnosed annually, liver cancer is the fifth more common cancer worldwide and ranks fourth in mortality rate. Two distinct pathologies are involved in liver cancer: Hepatocellular Carcinoma (HCC) and Cholangiocarcinoma (CCA). The major risks factor identified to be related with HCC are HBV and HCV chronic infections. Many genetic deregulations were identified in HCC; they concern genetic functions or pathways involved in cell growth, proliferation, apoptosis or DNA repair control. However, the molecular basis of how HBV or HCV interfere with these genetic deregulations in the pathogenesis of HCC is still poorly understood. TP53 gene inactivation due to either gene mutation is frequently found in HCC. In this study we describe how different viral infection features correlate with TP53 gene mutations in a cohort of HCC patients from Thailand.
A total of 244 cases of primitive liver cancer were identified in a cohort of 24 000 residents of Thailand recruited between 1991 and 2001. About 50 liver resections from these patients were available. 35 HCC were identified among the 50 tumours. We determined the viral status (HBV, HCV) of each samples by PCR and RT-PCR respectively, identified TP53 gene mutations by PCR\RFLP and sequencing. We located the position of viral proteins (PreS1, S, C, E, X for HBV and C, NS3, NS5a for HCV) within tumoral tissue and surrounding area by immunohistochemistry.
HVB DNA was detected in 18 HCC cases (51%), HCV RNA was detected in 6 HCC cases (17%), including 4 (11%) co-infections HCV/HBV. TP53 mutation was detected in 14 (40%) samples. Specific TP53 mutation at codon 249 occurred in 8 cases (22%). This mutation is known as reflecting population exposure to aflatoxin. Finally, cases that do not exhibit P53 mutation neither HBV nor HCV infection represent 23% of the total 35 HCC cases.
The distribution of TP53 mutations and viral infections suggests that these two events may occur independently of each other during liver carcinogenesis.

Abstract #: 35

Screening of TP53 mutations by DHPLC and sequencing in central nervous system cancers with an occupational exposure to pesticide or solvents.

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Several epidemiological studies described inconsistently an association between pesticide exposure and brain cancers. TP53 mutations would be an initial step in brain carcinogenesis. We hypothesised that pesticide exposure could rise the frequency of TP53 mutations in the central nervous system, thus contributing to the brain carcinogenesis. We investigated TP53 mutations in exons 2 to 9 by DHPLC and sequencing and p53 accumulation by immunohistochemistry in cerebral tissue of 34 cases with occupational exposure to pesticides or solvents from a population based case-control study on occupational and environmental risk factors of brain cancers. At present we detected p53 accumulation in 19 tumours and mutations in 3 tumours. The mutations are apparently unrelated to the intensity of pesticide exposure. Taken into account the histology of the present cases of brain cancers, we expected 4 mutations. These preliminary results are not in favour of an association between pesticide exposure and TP53 mutations.

Abstract #: 36

SER-249 TP53 MUTATION IN HEPATOCELLULAR CARCINOMA FROM TWO POPULATIONS EXPOSED TO AFLATOXIN: CORRELATION WITH FINDINGS IN CIRCULATING FREE DNA

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Hepatocellular carcinoma (HCC) is frequent in areas of high aflatoxin exposure and HBV prevalence, as Western Africa and South-East China. A specific mutation in TP53 (Ser-249) is a hotspot in HCCs from these areas, reflecting DNA damage by aflatoxin. We have analysed Ser-249 in HCCs from The Gambia, West Africa (29 biopsies) and from Qidong, Eastern China (20 cases: 12 biopsies and 8 surgical specimens). We have also analysed free DNA isolated from 24 plasma samples from Gambian HCC patients, including 17 matched with biopsies. These specimens were collected at the time of clinical diagnosis. Next, we have investigated Ser-249 in 257 plasma samples from Qidong, collected prospectively during the follow-up of a cohort of HBV chronic carriers. DNA extracted from plasma or tissues was amplified and analysed by restriction digestion with HaeIII, that cuts within codon 249. Mutation was confirmed by sequencing. Ser-249 mutation was found in 35 % (10/29) of Gambian HCCs. The prevalence in plasma DNA was 38% (9/24). Concordance between matched tumour and plasma was 71%. In Qidong HCCs, Ser-249 was found in 15/20 cases (65%), and also in 3 of 8 non-tumoral, adjacent tissue available. In plasma samples collected prospectively, only 0.9% (2/257) contained Ser-249. In addition, the prevalence of aflatoxin exposure was low based on serum aflatoxin-albumin adduct levels. In conclusion, the prevalence of Ser-249 shows large variations between areas of high incidence of HCC. The prevalence in The Gambia is lower than reported in neighbouring Senegal. Plasma of cancer patients is a good source of material to evaluate the presence of Ser-249. However, a prospective follow-up of chronic carriers from Qidong does not show that plasma Ser-249 is a marker of early tumorigenesis. Further studies are needed to investigate correlations between plasma levels of Ser-249 mutants and levels of aflatoxin exposure.

Abstract #: 37

Lack of Inducible Nitric Oxide Synthase Gene Bilaterally Affects Lymphomagenesis of Trp53-deficient mice: Suppression of Thymic Lymphomas and Augmentation of Non-Thymic lymphomagenesis.

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Trp53-deficient mice spontaneously develop lymphomas mainly of thymic origin, although molecular mechanism of this remains largely unknown. As several interaction effects between p53 and inducible nitric oxide synthase (iNOS) activity have been reported, we hypothesized that the iNOS activity in the thymus is causally linked to lymphomagenesis in Trp53-deficient mice. In order to investigate our hypothesis, we created mouse strains having a different gene combination of Trp53 and iNOS genes. Western blot and histological analyses showed that the iNOS protein was constitutively expressed in the thymus independently of Trp53 status, and its expression was enhanced in Trp53+/- and Trp53-/- mice, when compared to Trp53-wild type mice. The iNOS homozygous disruption decreased the incidence of thymic lymphomas by almost 40% ($p=0.087$) and 90% ($p<0.05$) in Trp53-/- and Trp53+/- mice, respectively, compared to respective iNOS-wild type mice, whereas the iNOS homozygous disruption significantly ($p<0.05$) increased the development of non-thymic lymphomas in Trp53-/- and Trp53+/- mice. Although the iNOS gene disruption did not affect the phenotype of thymic lymphomas, the defect of the iNOS gene shifted the spectrum of non-thymic lymphoma from B-cell to T-cell lineage. RT-PCR analysis demonstrated the enhanced cytokines expression of interleukin (IL)-10, which could possess promoting effects on lymphomagenesis, even without any stimulation in the spleen of aging mice with gene combination of Trp53-/- iNOS-/- and Trp53+/- iNOS-/-, but not Trp53-/-iNOS+/+ or Trp53+/-iNOS+/+. These results suggest that the iNOS activity could increase the development of thymic lymphomas in Trp53-deficient mice. While iNOS activity may have protective effects against lymphomagenesis in peripheral lymphoid organs, the regulation of cytokine production by iNOS might be involved in the underlying mechanism of anti-lymphomagenesis effects in the peripheral lymphoid organ.

Abstract #: 39

The functional role of p53 in invasive bladder cancer: a correlation between gene status by GeneChip analysis and protein expression by immunohistochemistry

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Alterations in the p53 gene (mutations) or protein have been shown to be important in development and progression of bladder cancer. They have also been shown to modulate clinical outcome (survival and recurrence) of the disease. Patients with nuclear accumulation of p53 have been shown to have a significantly worse clinical outcome compared to patients who are negative for p53 expression. Previous reports have indicated that there is a significant correlation between nuclear accumulation of p53 and mutations in the p53 gene. However, these studies were carried out in relatively small patient populations, and SSCP was the method used to detect p53 mutations. In the present study, we have examined p53 protein expression (p53 phenotype) in archival paraffin embedded tissue specimens using immunohistochemistry and the p53 gene mutation status (p53 genotype) in DNA extracted from the same tissues using Roche multi-exon amplification and Affymetrix p53 gene chip in a cohort of over 100 patients who underwent radical cystectomy at the USC Norris Comprehensive Cancer Center. We have investigated the correlation, as well as the discordance, between the p53 phenotype and the p53 genotype and the functional significance of these findings. Further, we have examined the functional status of p53 by analyzing the expression of p21, a downstream target of p53, in relation to the p53 status.

40% of cases showed nuclear accumulation of p53 protein by immunohistochemistry while 31% showed p53 gene mutations by GeneChip analysis. Among the cases which showed p53 mutations by the GeneChip analysis, 93.5% showed mutations in the hot spot region of the p53 gene (exons 5-8). Nuclear accumulation of the p53 protein by IHC and mutations in the p53 gene show a significant association ($p<0.01$). However, 27% cases showed a discordance between the p53 phenotype and genotype. No nuclear accumulation of p53 protein was detected in 29% cases, which showed a mutated p53 gene by the GeneChip analysis. Conversely, 26% cases, with no evidence of mutation by the GeneChip analysis demonstrated a nuclear accumulation of p53 protein. Alterations in the p53 phenotype as well as mutations in the p53 genotype were predictors of a significantly worse clinical outcome ($p<0.01$). Of the cases which showed a concordantly wild type p53 status by both IHC and GeneChip, 79.5% showed an up regulated p21 expression suggesting a functional p53 protein status. Of the cases which showed a concordantly altered p53 status by both IHC and GeneChip, 55% showed a down regulated p21 expression suggesting a dysfunctional p53 protein status. 45% of cases showed an altered p53 phenotype as well as a mutated p53 genotype, yet showed an up regulation of p21, suggesting an existence of p53-independent mechanism in the regulation of the p21 protein.

This study confirms that there is a high concordance rate between the p53 phenotype (by IHC) and the p53 genotype (by GeneChip analysis). We are expanding the study to a cohort of 220 patients to better elucidate the functional significance and clinical relevance of the disparity between the p53 phenotype and genotype.

Abstract #: 40

The IARC TP53 Database : Integration of molecular, structural, functional and clinical data.

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The IARC TP53 Database (http://www.iarc.fr/p53) compiles data on human somatic and germline TP53 variations that are reported in the peer-reviewed literature. With over 18,500 mutations and 1,000 citations in the world literature, this database is now recognized as a major source of information on TP53 mutation patterns in human cancer. It can be searched and analyzed online and is useful to draw hypotheses on the nature of the molecular events involved in TP53 mutagenesis and on the natural history of cancer.
More than 1,300 different missense TP53 mutations are compiled in the database. Some studies have shown that, in addition to loss of wild-type function, some mutants show dominant-negative effects on the wild-type p53 protein or on its two family members, p63 and p73. However, the functional impact of the various mutations described in cancer is not known. We are setting-up a battery of in vitro assays derived from FASAY to analyze in a standardized manner the functional properties of p53 mutants. The results of these assays will be used to produce standardized functional annotations of p53 mutants in the database.
Studies that have investigated the possible value of TP53 mutation as a prognostic factor in cancer are also compiled in the database. This compilation shows that the presence of a TP53 mutation has been repeatedly associated with a poor prognosis in several cancers. In breast cancer, a frequent cancer with no reliable molecular markers of prognosis, TP53 mutation is correlated with a shorter overall survival of patient. We have initiated a pilot study on a large series of European breast cancer patients which has been screened for TP53 mutation to investigate the correlation between structural and functional classes of p53 mutants and clinical parameters.
The functional and clinical data generated from these studies will be integrated in the IARC TP53 database. With these recent developments, we expect the database to become the main source of information on TP53 mutations in human cancers, available on-line for a broad range of scientists and clinicians who work in different areas:
- Basic research, to study the structural and functional aspects of the p53 protein;
- Molecular pathology of cancer, to understand the clinical significance of mutations identified in cancer patients;
- Molecular epidemiology of cancer, to analyze the links between specific exposures and mutation patterns and to make inferences about possible causes of cancer.

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