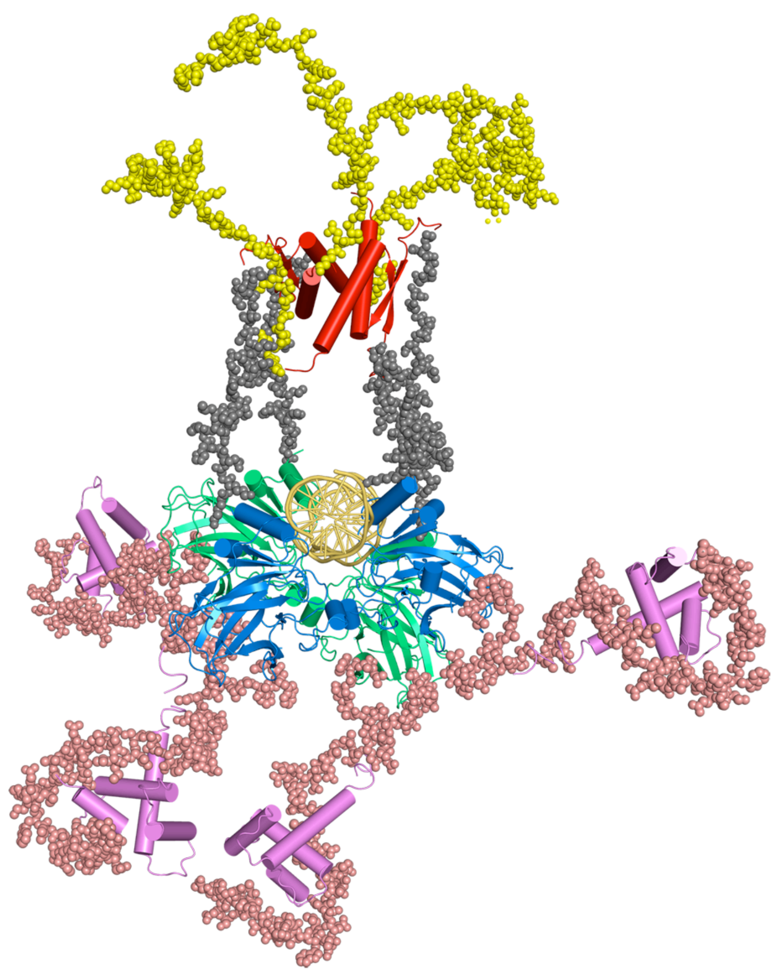
15th International p53 Workshop

Philadelphia PA
United States

October 8-12, 2010



Cover art generously provided by Alan Fersht and Andreas Joerger.

p53 globe designed by Karen Trush, FCCC, with p53 tetramer provided by Thanos Halazonetis.

T-shirt designed by Maureen Murphy and Joe Gillespie, FCCC.

Thanks to Kim Williams, FCCC, for help with the abstract booklet.

Thanks to Bob Wilkens, FCCC, for donating the tote bags.

October 8, 2010

Dear p53 Workshop Registrant:

On behalf of the Organizing Committee,
Welcome to the University of Pennsylvania School of Medicine, Philadelphia, for the 15th International p53 Workshop!

We have a very exciting research program for you, and hope that you leave here energized, enthused about science and entertained.

The organization of this meeting would not have been possible without the outstanding administrative support provided by the Abramson Cancer Center; we are so grateful to **Ms. Jennifer McGuire** and **Dr. Barbara Vance** for all of their efforts!

We gratefully acknowledge the generous sponsorship for this Workshop provided by:

Hoffmann-La Roche Inc
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Again, Welcome to Philadelphia!

Wafik El-Deiry
Penn State Hershey Cancer Institute

Maureen E. Murphy
Fox Chase Cancer Center

Donna L. George
University of Pennsylvania School of Medicine

Organizing Committee

Arnold Levine, Institute for Advanced Study, Princeton NJ

Sir David Lane, University of Dundee, Dundee, Scotland

Sam Benchimol, York University, Toronto, Ontario

Wafik El-Deiry, Penn State Hershey Cancer Center

Sir Alan Fersht, Cambridge University, UK

Donna L. George, University of Pennsylvania School of Medicine

Wei Gu, Columbia University Medical Center

Andrei Gudkov, Roswell Park Cancer Institute

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THIRD ROCK VENTURES

15th International p53 Workshop
University of Pennsylvania School of Medicine
Philadelphia PA USA

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15th International p53 Workshop
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Philadelphia, Pennsylvania USA

FRIDAY, OCTOBER 8TH 2010

2:00 pm – 7:00 pm	**WORKSHOP REGISTRATION** Houston Hall, Reading Room, 3417 Spruce Street	
7:00 pm – 11:00 pm	**KEYNOTE ADDRESSES AND OPENING RECEPTION** Irvine Auditorium, 3401 Spruce Street	
7:00 pm – 9:15 pm	**Welcome and Opening Keynote Addresses**	
7:00 pm – 7:15 pm	Wafik El-Deiry	*Welcome*
7:15 pm – 7:55 pm	Sir David Lane	*Drug discovery in the p53 pathway*
7:55 pm – 8:35 pm	Arnold Levine	*The evolution of the p53 family of genes: the roles of p53/p63 and p73 in the female germline, fertility and fidelity*
8:35 pm – 9:15 pm	Bert Vogelstein	*p53 gene mutations in the context of complete cancer gene sequencing*
9:15 pm – 11:00 pm	**Opening Reception**	

SATURDAY OCTOBER 9TH, 2010

7:30 am – 4:45 pm	**WORKSHOP PROGRAMMING** Stemmler Hall, Dunlop Auditorium, 3450 Hamilton Walk	
7:30 am – 8:30 am	**Continental Breakfast**	
8:30 am – 9:10 am	**Keynote Address**	
	Carol Prives	*Mechanisms by which the p53 tumor suppressor protein selects its target genes*
9:10 am – 10:15 am	**p53 and transcription (Chair: Carol Prives)**	
9:10 am – 9:35 am	Beverly Emerson	*Structurally diverse core promoters and p53*
9:35 am – 9:50 am	Joaquín Espinosa	*Transcriptional regulation of PUMA*
9:50 am – 10:15 am	Michael Resnick	*Expanding the DNA damage and p53 response network*
10:15 am – 10:45 am	**Coffee Break**	

10:45 am - 12:00 pm	**MicroRNAs (Chair: Hua Lu)**	
10:45 am – 11:10 am	Elsa Flores	*TAp63 suppresses metastasis through coordinate transcriptional regulation of Dicer and miRNAs*
11:10 am – 11:35 am	Leif Ellisen	*A microRNA network controlling p63/p73 homeostasis*
11:35 am – 12:00 pm	Hua Lu	*The p53-MDM2/MDMX feedback loop*
12:00 pm – 1:00 pm	**Box Lunch** Outside Seating Available	
1:00 pm – 1:50 pm	**p53 Structure (Chair: Sir Alan Fersht)**	
1:00 pm – 1:25 pm	Sir Alan Fersht	*Structure and biophysics of p53 – DNA binding*
1:25 pm – 1:50 pm	Thanos Halazonetis	*Structure of a multidomain p53 oligomer with DNA*
1:50 pm – 3:00 pm	**Life and Death Decisions (Chair: Doug Green)**	
1:50 pm – 2:15 pm	Doug Green	*Transcription-dependent and independent apoptosis*
2:15 pm – 2:40 pm	Xin Lu	*ASPP2 controls polarity and proliferation*
2:40 pm – 3:05 pm	Sam Lee	*Targeting ROS for selective killing of cancer cells*
3:05 pm – 3:30 pm	**Coffee Break**	
3:30 pm – 4:45 pm	**p53 and Stem Cells (Chair: Geoff Wahl)**	
3:30 pm - 3:55pm	Geoff Wahl	*p53, stem cells, and cancer-associated reprogramming*
3:55 pm – 4:20 pm	Varda Rotter	*Mutant p53 facilitates somatic cell reprogramming*
4:20 pm – 4:45 pm	Yang Xu	*Function and regulation of p53 in stem cells*
4:45 pm – 5:00 pm	**BOARDING FOR TROLLEY TOUR** Behind Stemmler Hall (Ticket-Holders Only)	
5:00 pm – 6:30 pm	**TROLLEY TOUR OF HISTORIC PHILADELPHIA** **(Ticket-Holders Only)** Tour will end in front of Houston Hall	
6:30 pm – 7:00 pm	**POSTER SESSION 1 SET-UP** Houston Hall, Bodek Lounge, 3417 Spruce Street	
7:00 pm – 9:00 pm	**POSTER SESSION 1 AND RECEPTION** Houston Hall, Bodek Lounge, 3417 Spruce Street *Shuttle buses available to downtown Philadelphia hotels after the event*	

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SUNDAY OCTOBER 10TH, 2010

7:30 am – 5:30 pm	**WORKSHOP PROGRAMMING** Stemmler Hall, Dunlop Auditorium, 3450 Hamilton Walk	
7:30 am – 8:30 am	**Continental Breakfast**	
8:30 am – 10:10 am	**Metabolism, Survival and Autophagy (Chair: Karen Vousden)**	
8:30 am – 8:55 am	Karen Vousden	*Regulation of migration and survival by the p53 pathway*
8:55 am – 9:20 am	Craig Thompson	*Did p53 evolve to regulate metabolism?*
9:20 am – 9:45 am	Donna George	*Inhibiting the HSP70 chaperone and p53 degradation*
9:45 am – 10:10 am	Moshe Oren	*p53-dependent regulation of autophagy protein LC3*
10:10 am – 10:30 am	**Coffee break**	
10:30 am –12:10 pm	**Animal Models (Chair: Gigi Lozano)**	
10:30 am– 10:55 am	Laura Attardi	*Mouse models for transactivation domain mutants*
10:55 am – 11:20 am	Maureen Murphy	*A mouse model for the codon 72 polymorphism of p53*
11:20 am – 11:45 am	Gigi Lozano	*Modulating p53 activity for better or worse*
11:45 am – 12:10 pm	Larry Donehower	*Comparison of non-metastatic and metastatic osteosarcomas in p53 mutant mouse models*
12:10 pm - 1:30 pm	**Box Lunch** Outside Seating Available	
1:30 pm - 3:30 pm	**Drug Discovery and Therapy (Chair: Wafik El-Deiry)**	
1:30 pm – 1:55 pm	Wafik El-Deiry	*Therapeutic targeting of p53-deficient cancers*
1:55 pm – 2:20 pm	Klas Wiman	*Pharmacological targeting of mutant p53*
2:20 pm – 2:45 pm	Galina Selivanova	*Dissecting the p53 network using small molecules*
2:45 pm – 3:05 pm	Andrei Gudkov	*p53, inflammation and cancer chemoprevention*
3:05 pm – 3:30 pm	Lyubomir Vassilev	*Activation of p53 by MDM2 antagonists*
3:30 pm – 4:00 pm	**Coffee Break**	

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4:00 pm – 5:30 pm	**p53: Targets and Control (Chair: Thea Tlsty)**	
4:00 pm – 4:25 pm	Thea Tlsty	*p53 modulates paracrine signaling and malignant phenotypes in the tissue microenvironment*
4:25 pm – 4:50 pm	Sam Benchimol	*Inhibition of p53 expression by peptide-conjugated phosphorodiamidate morpholino oligomers: effects on isoform expression and chemosensitivity*
4:50 pm – 5:15 pm	Wei Gu	*p53 modification and its regulators*
5:15 pm – 5:30 pm	J. C. Bourdon	*Biological activities of human and mouse p53 isoforms*

5:30 pm – 6:00 pm	**POSTER SESSION 2 SET-UP** Penn Museum, Upper Egyptian Gallery, 3260 South Street, 3rd Floor
6:00 pm – 8:00 pm	**POSTER SESSION 2** Penn Museum, Upper Egyptian Gallery, 3260 South Street, 3rd Floor
8:00 pm -10:00 pm	**DINNER AND MUSEUM VISIT** Penn Museum, Chinese Rotunda, 3260 South Street, 3rd Floor *Shuttle buses available to downtown Philadelphia hotels after the event*

MONDAY OCTOBER 11TH, 2010

7:30 am – 5:15 pm	**WORKSHOP PROGRAMMING** Stemmler Hall, Dunlop Auditorium, 3450 Hamilton Walk	
7:30 am – 8:30 am	**Continental Breakfast**	
8:30 am – 10:10 am	**p53 family members (Chair: Gerry Melino)**	
8:30 am – 8:55 am	Gerry Melino	*p73 is involved in neuronal development via miR-34a*
8:55 am – 9:20 am	Tak Mak	*The role of TAp73 and ΔNp73 in tumor development*
9:20 am – 9:45 am	Jennifer Pietenpol	*Regulation of the p73 cistrome by mTOR*
9:45 am – 10:10 am	Ute Moll	*Novel in vivo roles of p73 in lymphoma and neurogenesis*
10:10 am – 10:30 am	**Coffee Break**	

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10:30 am – 12:10 pm	**p53: Genetics and Disease (Chair: David Malkin)**	
10:30 am – 10:55 am	Frank McKeon	*Origins of Barrett's metaplasia*
10:55 am – 11:20 am	Lin Wu	*Clinical applications of the AmpliChip p53 test*
11:20 am – 11:45 am	David Malkin	*p53 and Li-Fraumeni syndrome*
11:45 am – 12:10 pm	Thierry Soussi	*Reassessment of the significance of p53 mutations in cancer*
12:10 pm – 1:15 pm	**Box Lunch** Outside Seating Available	
1:15 pm – 3:15 pm	**Senescence, Checkpoints & the Downstream Pathway (Chair: Scott Lowe)**	
1:15 pm – 1:40 pm	Scott Lowe	*Interrogating the p53 tumor suppressor network using mouse models and RNA interference*
1:40 pm – 2:05 pm	Curtis Harris	*Inflammation, senescence and cancer*
2:05 pm – 2:30 pm	James Manfredi	*The role of the basic C terminus in the p53 transcriptional response*
2:30 pm – 2:55 pm	Chris Kemp	*Synthetic lethal networks for the p53 pathway*
2:55 pm – 3:20 pm	Steven McMahon	*Functional consequences of p53 DNA binding domain acetylation*
3:20 pm - 3:45 pm	**Coffee Break**	
3:45 pm – 5:15 pm	**Late-Breaking Research (Chair: Maureen Murphy)**	
	Zhaohui Feng	*Negative regulation of p53 by miR-504*
	Michelle Barton	*p53 regulates cell cycle and miRNAs to drive differentiation of human embryonic stem cells*
	A. T. Tikhonenko	*Myc overexpression brings out unexpected p53-dependent and anti-apoptotic effects of miR-34a*
	Steven Grossman	*p300/CBP: Fine tuning the p53 apoptotic response*
	Kevin Ryan	*Extracellular adenosine signaling and p53-mediated death*
	Xiaolu Yang	*p53 regulates biosynthesis*
	Yi Sun	*Ribosomal protein S27-like and S27 and the p53-MDM2 axis*
5:15 pm – 6:00 pm	**BUS TO CLOSING BANQUET** Boarding Behind Stemmler Hall	
6:00 pm – 11:00 pm	**CLOSING BANQUET** National Constitution Center, 525 Arch Street *Shuttle Buses will depart for all hotels at 10:00 pm and at 11:00 pm*	

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TUESDAY OCTOBER 12TH, 2010

7:30 am – 2:00 pm	**WORKSHOP PROGRAMMING** Stemmler Hall, Dunlop Auditorium, 3450 Hamilton Walk	
7:30 am – 8:30 am	**Continental Breakfast**	
8:30 am – 10:30 am	**p53-interacting proteins (Chair: David Meek)**	
8:30 am – 8:55 am	Yanping Zhang	*An essential role for the ribosomal protein-MDM2-p53 complex in growth control and tumor suppression*
8:55 am – 9:20 am	Jiandong Chen	*MDM2 phosphorylation and DNA damage signaling to p53*
9:20 am – 9:45am	Aart Jochemson	*Functions and regulation of MdmX*
9:45 am – 10:10 am	Giannino Del Sal	*BRD7 is a candidate tumor suppressor required for p53 function*
10:10 am – 10:35 am	David Meek	*Regulation of p53 by Mage-A proteins and p68*
10:35 am -11:00 am	**Coffee Break**	
11:00 am – 12:30 pm	**Novel target genes and modifications (Chair: Gerard Zambetti)**	
11:00 am – 11:25 am	Gerard Zambetti	*Translational studies of p53 in childhood cancers*
11:25 am – 11:50 am	Xinbin Chen	*A feedback loop between p53 and RNPC1*
11:50 am – 12:15 pm	Wolfgang Deppert	*p53 regulation of LINE1 retrotransposons*
12:15 pm – 12:30 pm	Yoichi Taya	*Novel functions of p53 in the nucleus and near the plasma membrane*
12:30 pm – 1:00 pm	**Coffee Break and Snacks**	
1:00 pm – 2:00 pm	**Closing Session**	
	Arnold Levine	*The future of p53 research*
2:00 pm	**DEPARTURE**	

SPEAKER ABSTRACTS

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University of Pennsylvania School of Medicine

SPEAKER ABSTRACT

Professor Sir David Lane, Ph.D.
Chief Scientist A*STAR & Director p53 Lab
A*STAR Singapore
d.p.lane@dundee.ac.uk

Opening Keynote Address
Friday, October 8
7:15 PM - 9:15 PM
Irvine Auditorium
Main Auditorium

Co-Authors: Guo Lin, Amanda Goh and Chit Fang Cheok,
p53 Lab A*Star

Drug discovery in the p53 pathway

Small molecules that activate wild type p53 have proved active in preclinical models and are now entering clinical trial. The complexity of the upstream p53 signaling pathway offers many targets for the development of such activators and the action of Mdm2 inhibitors, kinase inhibitors, sirtuin inhibitors and low doses of actinomycin D will be described as examples. Concerns that activation of p53 might be toxic to normal tissues or that treatment with p53 activators will select for tumor cells that have mutant p53 will be discussed . Selective cyclotherapy treatment approaches for tumors that express mutant p53, in which activation of p53 in normal tissues is used to protect them from the side effects of anti-mitotic and anti-S phase drugs look promising and may be widely applicable. These developments will be helped by recently developed p53 reporter mice and zebrafish strains . The development of RFP and GFP labeled cells of different p53 status has allowed us to re-examine the selectivity of many chemotherapeutic drugs under a variety of conditions with some provocative results. Recently attempts to re-activate certain point mutant p53 proteins with small molecules that stabilize their structure have also shown promise and are the subject of intense investigation.

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SPEAKER ABSTRACT

Arnold Levine, Ph.D.
Professor
The Simons Center for Systems Biology, Institute for Advanced Study
alevine@ias.edu

Opening Keynote Address
Friday, October 8
7:15 PM - 9:15 PM
Irvine Auditorium
Main Auditorium

Co-Authors: W. Hu, A. Naqvi, Y. Sung and D. Notterman

The Evolution of the p53 Family of Genes: The Roles of p53/63 and 73 in the Female Germ Line, Fertility and Fidelity

The p53 protein responds to a wide variety of stresses by eliminating cells with DNA damage or mutations by employing apoptosis, senescence or cell cycle arrest. In this way the p53 protein acts as a tumor suppressor preventing the mutations that can lead to cancers. The p63 and p73 proteins act in a similar fashion to protect germ line cells in females (eggs). In addition the p63 protein plays a central role in the formation of epithelial cell layers and p73 plays a critical role in the formation of several structures in the central nervous system and in the immune system.

Based upon their amino acid sequences and structural considerations the present day invertebrates have two patterns of p53 family genes; either a single p63/p73 ancestor gene as observed as in sea anemone, Drosophila and C. elegans or p53/MDM-2 like genes are observed as in some mosquitoes, clams and some Placozoans. In all cases examined the role of these gene products is to function in germ cells of these animals to enforce the fidelity of DNA sequences after exposure to damage. Thus the structure and functions of this gene family have been preserved for over one billion years of evolution.

In the vertebrates the first time a clearly distinct new p53 gene arises from a p63/73 gene observed in early vertebrates is in the cartilaginous fish and in the bony fish there is a separation of the p63 and p73 gene   After that there are very limited evolutionary changes that are found in the p63 gene and only a few changes in the p73 gene DNA binding core. By contrast the p53 gene evolves rapidly and extensively obtaining the new functions of surveillance of DNA damage and cell division after stress in the somatic tissues of the body. This appears to coincide with the enhanced use of the strategy of stem cells to regenerate tissues throughout the life of the organism. P53 has an important regulatory impact upon stem cell formation in both normal tissues and cancers.

As Caucasians and Asians evolve from their African ancestors polymorphic changes in the p53 gene occur and are rapidly selected for in these populations. This results in the further selection of alleles in genes whose products interact with and regulate the p53 protein. These polymorphisms can have an impact upon the age of onset of cancers, the frequency of cancers in the elderly, the longevity of a population and even the reproductive fecundity of a population. The p53 protein is required for efficient implantation of a fertilized egg into the uterus of a female and this helps to explain the strong selection pressures upon these genes. In the mouse p63 and P73 genes play a role in the fidelity of egg development and meiosis. In humans single nucleotide polymorphisms have been identified in the p63

and p73 genes that are enriched, compared to the general population, in women over the ages of thirty-five who utilize the services of an in vitro fertilization clinic. The major reasons why these women produce poor quality eggs are aneuploidy and copy number variations. Interestingly these polymorphisms are also enriched in either the mothers or the fathers of children with autism which commonly arises from copy number variations (CNVs) in selected genes. The recombination frequencies in children whose mothers or fathers have these SNPs are much larger than in offspring without these allelic forms. In addition SNPs in genes that participate in homologous DNA repair during meiosis lead to higher recombination frequencies, higher CNVs and autism. These data support the idea that p63 and p73 enforce the fidelity of transferring information from one generation to the next.

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SPEAKER ABSTRACT

Bert Vogelstein, M.D.
Professor of Oncology and Pathology
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University
bertvog@gmail.com

Opening Keynote Address
Friday, October 8
7:15 PM - 9:15 PM
Irvine Auditorium
Main Auditorium

p53 gene mutations in the context of complete cancer gene sequencing

It was stated long ago that p53 is the most commonly altered gene in human tumors. With the advent of the ability to sequence all genes within the cancer genome, it is now possible to confirm or refute this statement. I will review these sequencing studies with that question in mind. I will also discuss what the recent cancer genome work has revealed about the p53 pathway and its clinical implications.

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SPEAKER ABSTRACT

Carol Prives, Ph.D.
Professor, Biological Sciences
Columbia University
clp3@columbia.edu

Workshop Sessions Keynote
Saturday, October 9
8:30 AM - 9:10 AM
Stemmler Hall
Dunlop Auditorium

Co-Authors: C. Prives, T. Barsotti, L. Biderman, W. Freed-Pastor, O. Laptenko, M. V. Poyurovsky, C. Priest, S. Rokudai, S. Singer and Y. Zhu.

Department of Biological Sciences, Columbia University, New York, N.Y. 10027

Mechanisms by which the p53 tumor suppressor protein selects its target genes

Depending on the initiating signal, activation of the p53 protein leads to large and varied changes in gene expression. Some genes are directly regulated as a result of p53 interaction with its response elements while others are more indirect. An increasing number of cellular processes have been shown to result from such activation. Yet some p53 outcomes are contradictory (eg survival vs apoptosis) suggesting that not all target genes are equivalently induced by p53 under conditions that produce a specific and exclusive outcome. We have spent several years striving to elucidate the basis for target gene selectivity by p53. The experimental approaches we take include biochemical and cell-based assays. We have discovered that gene expression regulated by p53 occurs at a surprising number of levels. These include: 1) binding site recognition in the context of naked vs nucleosomal DNA, 2) the extent and sites of modification of the p53 protein by acetylation, 3) selective repression by Mdm2 and MdmX, 4) initiation vs. elongation of target gene RNA and 5) post-transcriptional mechanisms. We have also identified novel p53 target genes that play roles in cell cycle progression, energy metabolism, and even possibly pro-oncogenic functions. Adding to the complexity are the different pathways and outcomes that are regulated by tumor-related mutant forms of p53. The intricacy of the p53 network poses challenges for future studies to deconstruct the key processes that are required for p53 tumor suppression.

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SPEAKER ABSTRACT

Beverly Emerson, Ph.D.
Professor
The Salk Institute
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p53 and Transcription
Saturday, October 9
9:10 AM - 10:15 AM
Stemmler Hall
Dunlop Auditorium

Co-Authors: José M. Morachis[2], Christopher M. Murawsky[1], and Beverly M. Emerson[1]

[1]Regulatory Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037

[2]Department of Pharmacology, University of California, San Diego, CA 92037

Regulation of the p53 transcriptional response by structurally diverse core promoters

p53 target promoters are structurally diverse and display pronounced differences in RNA polymerase II (RNAP II) occupancy even in unstressed cells, with higher levels observed on cell cycle arrest genes (p21) compared with apoptotic genes (Fas/APO1). This occupancy correlates well with their ability to undergo rapid or delayed stress induction. To understand the basis for such distinct temporal assembly of transcription complexes, we examined the role of core promoter structures in this process. We find that the p21 core promoter directs rapid, TATA box-dependent assembly of RNAP II preinitiation complexes (PICs), but permits few rounds of RNAP II reinitiation. In contrast, PIC formation at the Fas/APO1 core promoter is very inefficient but supports multiple rounds of transcription. We define a downstream element within the Fas/APO1 core promoter that is essential for its activation, and identify nuclear transcription factor Y (NF-Y) as its binding partner. NF-Y acts as a bifunctional transcription factor that regulates basal expression of Fas/APO1 in vivo. Thus, two critical parameters of the stress-induced p53 transcriptional response are the kinetics of gene induction and duration of expression through frequent reinitiation. These features are intrinsic, DNA-encoded properties of diverse core promoters that may be fundamental to anticipatory programming of p53 response genes upon stress.

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SPEAKER ABSTRACT

Joaquín Espinosa, Ph.D.
Assistant Professor
HHMI / The University of Colorado at Boulder
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p53 and Transcription
Saturday, October 9
9:10 AM - 10:15 AM
Stemmler Hall
Dunlop Auditorium

Co-Authors: Joaquín M. Espinosa and Nathan P. Gomes

HHMI / MCD Biology, University of Colorado at Boulder

Transcriptional regulation of PUMA involves long non-coding transcription and intragenic chromatin boundaries

The p53 transcriptional program orchestrates alternative responses to stress including cell cycle arrest and apoptosis, but the mechanism of cell fate choice upon p53 activation is not fully understood. Here we report that PUMA, a key mediator of p53-dependent cell death, is regulated by a non-canonical, gene-specific mechanism. Using Chromatin immunoprecipitation assays we found that the first half of the PUMA locus (~6 kb) is constitutively occupied by RNA polymerase II and general transcription factors regardless of p53 activity. Using various RNA analyses we found that this region is constitutively transcribed to generate a long unprocessed RNA with no known coding capacity. This permissive intragenic domain is constrained by sharp chromatin boundaries, as illustrated by histone marks of active transcription (H3K4me3 and H3K9Ac) that precipitously transition into repressive marks (H3K9me3). Interestingly, the insulator protein CTCF and the Cohesin complex occupy these intragenic chromatin boundaries. CTCF knockdown leads to increased basal expression of PUMA concomitant with a reduction in chromatin boundary signatures. Importantly, de-repression of PUMA upon CTCF depletion occurs without p53 activation or activation of other p53 target genes. Therefore, CTCF plays a pivotal role in dampening the p53 apoptotic response by acting as a gene-specific repressor.

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SPEAKER ABSTRACT

Michael Resnick, Ph.D.
Group Leader
National Institute of Environmental Health Sciences, NIH
resnick@niehs.nih.gov

p53 and Transcription
Saturday, October 9
9:10 AM - 10:15 AM
Stemmler Hall
Dunlop Auditorium

Co-Authors: Michael A. Resnick[1], Daniel Menendez[1], Maria Shatz[1], Kathleen Smoak[2], Stavros Garantziotis[3] and Michael B. Fessler[2]

[1] Chromosome Stability Group, Lab. Molecular Genetics; [2]Host Defense Group, Lab. Respiratory Biology; [3]Clinical Research Unit; National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, 27709, USA

Expanding the DNA damage and p53 response network to the human Toll-like receptor family

Over the past decade the role that the p53 tumor suppressor plays in human biology has been greatly extended beyond “guardian of the genome.” In our recent studies of response element (RE) sequences targeted by the p53 master regulatory transcription factor we have found that noncanonical REs, as well as the estrogen receptor transcriptional factor cooperating in cis, greatly expands the web of the p53 network. Based on our functionality rules for REs we anticipated a general role for p53 in the control of human Toll-like receptor (TLR) gene expression. The TLR gene family mediates innate immunity to a wide variety of pathogenic threats through recognition of conserved pathogen-associated molecular motifs. Using primary human immune cells we have examined expression of the entire TLR gene family following exposure to anti-cancer agents that induce the p53 network. All TLR genes, TLR1 to TLR10, in blood lymphocytes and alveolar macrophages from healthy volunteers were inducible by DNA metabolic stressors, even though considerable inter-individual variability was observed. We have also demonstrated similar responses in human cancer cell lines. Most of the TLR genes respond to p53 via canonical as well as noncanonical promoter binding sites. Importantly, the integration of the TLR gene family into the p53 network is unique to primates, a recurrent theme raised for other gene families in our previous studies. Furthermore, a polymorphism in a TLR8 response element identifies a p53 target sequence specifically responsible for endogenous gene induction. These findings demonstrating that the human innate immune system can be modulated by DNA metabolic stress have many implications for health and disease as well as for understanding the evolution of damage and p53 responsive networks.

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SPEAKER ABSTRACT

Elsa Flores, Ph.D.
Associate Professor
U.T. M.D. Anderson Cancer Center
elsaflores@mdanderson.org

MicroRNAs
Saturday, October 9
10:45 AM - 12:00 PM
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Dunlop Auditorium

Co-Authors: Xiaohua Su[1], Deepavali Chakravarti[1,2], Min Soon Cho[2], Lingzhi Liu[1], Young Jin Gi[1], Yu-Li Lin[1], Marco Leung[1], Adel El-Naggar[3], Chad J. Crieghton[4], and Elsa R. Flores[1,2,5]

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TAp63 suppresses metastasis through coordinate transcriptional regulation of Dicer and miRNAs

Aberrant expression of microRNAs (miRNAs) and the enzymes that control their processing have been reported in multiple biological processes including primary and metastatic tumors. The mechanisms governing this are not clearly understood. We have data indicating that TAp63 is a critical regulator of miRNA biogenesis. Using tumors from TAp63-/- mice, human head and neck squamous cell carcinomas, and lung and mammary adenocarcinomas, we have shown that TAp63 suppresses tumorigenesis and metastasis and coordinately transcriptionally regulates Dicer and miR-130b to suppress metastasis. Metastatic mouse and human tumors deficient for TAp63 expressed Dicer at very low levels, and modulation of expression of Dicer and miR-130b dramatically affected the metastatic potential of cells lacking TAp63. Importantly, TAp63 binds to and transactivates the Dicer promoter, demonstrating direct transcriptional regulation of Dicer by TAp63. These data provide a novel understanding of the roles of TAp63 in tumor and metastasis suppression through the coordinate transcriptional regulation of Dicer and miR-130b and may have important implications for the many processes regulated by miRNAs.

15th International p53 Workshop
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University of Pennsylvania School of Medicine

SPEAKER ABSTRACT

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MicroRNAs
Saturday, October 9
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A microRNA network controlling p63/p73 homeostasis, cell survival and chemosensitivity

A central challenge in human cancer therapy is the identification of pathways that control tumor cell survival and chemosensitivity in the absence of functional p53. The p53-related transcription factors p63 and p73 exhibit distinct, p53-independent roles in development and cancer: p73 promotes genome stability and mediates chemosensitivity, while p63 largely lacks these p53-like functions and instead promotes proliferation and cell survival. We have uncovered a new and physiologically important mechanism of p63/p73 cross-talk which governs the balance between pro-survival and pro-apoptotic programs in human cancers including squamous cell carcinoma. Through comprehensive profiling of p63-regulated microRNAs (miRs), we identified a subset which target p73 for inhibition, including miR-193a-5p, a direct endogenous transcriptional target repressed by p63 and activated by pro-apoptotic p73 isoforms in both normal cells and tumor cells in vivo. Consequently, chemotherapy treatment causes p63/p73-dependent induction of this miR, thereby limiting chemosensitivity due to miR-mediated feedback control of p73. Furthermore, interrupting this feedback by inhibiting miR-193a suppresses tumor cell viability and induces dramatic chemosensitivity both in vitro and in vivo. Thus, these findings demonstrate a direct, miR-dependent regulatory circuit mediating inducible chemoresistance, whose inhibition provides a new therapeutic opportunity in p53-deficient tumors. This work suggests a general mechanism of miR-mediated cross-talk within gene families, with implications for the evolution of the p53 family.

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SPEAKER ABSTRACT

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MicroRNAs
Saturday, October 9
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New Insight Into The p53-MDM2/MDMX Feedback Loop

The p53-MDM2-MDMX feedback loop plays a crucial role in cancer development. Hence, blocking this loop is not only critical for cells to activate p53 in preventing cancerous phenotype development, but also serves as an attractive approach to eliminate cancer cells that still possess wild type, though inactive, p53 for anti-cancer drug development. Over the past years, my lab has been working on both of the aspects. On one hand, we have continued to elucidate biochemical mechanisms underlying the regulation of MDM2 by ribosomal protein L11 and regulation of MDMX by 14-3-3. Through a heavy water exchange coupled with mass spectrometry as well as a computer 3D structural modeling, we precisely mapped the L11-binding domain within MDM2 and predicted amino acid residues within both L11 and MDM2 for their physical interaction. Mutagenesis of these residues not only confirmed their direct contacts, but also identified key residues crucially important for their functional binding. We are now trying to precisely map the L5-MDM2 and L23-MDM2 domains using the same approach. In addition, we have identified a protein kinase that can phosphorylates MDMX in response to glucose depletion, leading inactivation of MDMX and consequent activation of p53. Also, we have identified several miRNA targets for the p53 family members and are now investigating their functional outcomes in cells. On the other hand, we have also identified a novel small molecule that can remarkably suppress tumor growth by activating p53 in human lung and colon cancer cells. Therefore, our recent studies provide more molecular insight into the regulation of the p53-MDM2-MDMX feedback loop and also a potential and novel small molecule as an anti-cancer drug candidate.

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SPEAKER ABSTRACT

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p53 Structure
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Structural Biology and Biophysics of p53 – DNA Binding

The availability of stabilised full-length mutant p53 is allowing novel structural and biophysical studies on its complexes with DNA that are giving new insights into its binding and recognition of DNA. I will discuss the latest biophysical measurements using a variety of single-molecule methods combined with direct structural determination.

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SPEAKER ABSTRACT

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p53 Structure
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Structure of a multidomain p53 oligomer bound to DNA

The p53 tumor suppressor is the most frequently inactivated gene in human cancer. It encodes a transcription factor that contains two independently-folding domains, a core sequence-specific DNA binding domain and a homo-tetramerization domain. Here we describe the structure of a p53 polypeptide containing both these domains on its own and in complex with DNA. The structure of the complex shows four DNA binding domains interacting with one another and with DNA. Both the DNA binding domains of p53 and the DNA undergo conformational changes upon DNA binding. These conformational changes are asymmetric, even though the p53 DNA binding site is made of four identical consensus repeats. The DNA corresponding to the center two repeats is distorted such that its helical axis shifts by more than four angstroms. This results in widening of the major groove at the center of the p53 binding site, which can thus accomodate sequence-specific contacts by both loop L1 and helix H2. The geometry of the DNA corresponding to the outer two repeats resembles better B-form DNA; this results in loop L1 adopting a recessed conformation, in which Lys120 no longer makes contacts with DNA. The structure resolves the controversies of whether p53 and its target DNA change conformation upon binding, the unusual feature of p53 that it uses both a loop and a helix to contact the major groove of DNA, and why loop L1 is not targeted by cancer-associated mutations.

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SPEAKER ABSTRACT

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Life and Death Decisions
Saturday, October 9
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Transcription-Dependent and -Independent Apoptosis by the p53 Family Proteins

The p53-family proteins act as transcription factors to regulate (activate or repress) transcription of a variety of genes involved in a range of cellular responses. These responses include apoptosis, and the role of apoptosis in the tumor suppressive effects of p53 (and probably p73) is of paramount importance. It follows that genes induced by p53 and p73 must cause apoptosis. However, this turns out to be not as simple as implied.

While stabilization of nuclear p53 triggers expression of genes that regulate apoptosis, especially PUMA, we have identified a role for cytosolic p53 in the apoptotic process as well. When stabilized in the cytosol, p53 is sequestered on Bcl-xL and thus held inactive. PUMA, but not other BH3-only proteins, causes a major conformational change in Bcl-xL that disrupts its interaction with cytosolic p53. The now free p53 interacts with the pro-apoptotic protein, Bax, to permeabilize mitochondria and cause apoptosis.

In another scenario, activation of T cells with defective NF-kappaB signaling leads to apoptosis that is dependent on p73. We have found that the NF-kappaB target DM2 is necessary and sufficient to prevent this activation-induced apoptosis. While DM2 does not induce the degradation of p73, it binds and inhibits it. Without DM2, p73 causes the death of T cells via transcriptional upregulation of the pro-apoptotic BH3-only protein, Bim, which is a p73 (but not p53) target.

These studies will be put into the context of fundamental principles regulating the mitochondrial pathway of apoptosis, and how they interface with the p53-family proteins in tumor suppression.

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SPEAKER ABSTRACT

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Life and Death Decisions
Saturday, October 9
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ASPP2: Tumour suppression through the control of polarity and proliferation

Cell polarity and proliferation are processes that play key roles in development. Loss of cell polarity and increased cellular proliferation are hallmarks of human cancers. The identification of molecules that are involved in the control of these processes is, therefore, important for our better understanding of tumour development and progression. p53 was recently found to be important in controlling the mammary epithelial stem cell pool. In addition, we recently showed that ASPP2, a previously identified regulator of p53, is an important player in the regulation of cell polarity due to its ability to bind and regulate Par-3. Interestingly, ASPP2 is also able to directly regulate cell proliferation by affecting the activity of cyclin D1, by inhibiting the nuclear localisation of SUMO-modified cyclin D1. As ASPP2 is a haploinsufficient tumour suppressor, these findings identify ASPP2 as a unique regulator of cell polarity: able to influence cell polarity one the one hand and the cell cycle machinery on the other. ASPP2's interactions with p53 and the other p53 family members and its potential role in controlling cell migration will also be discussed.

SPEAKER ABSTRACT

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Life and Death Decisions
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Targeting stress response to ROS for selective killing of cancer cells

Inducing cancer cell death with DNA-damaging chemotherapeutic agents is the most widely used strategy in cancer therapy. However, DNA-damaging drugs kill both normal and cancerous cells, which severely limits current treatment regimens. A high-throughput, cell-based screen for small molecules that activate transcription of a p53-regulated target gene in cancer cells uncovered a remarkable naturally occurring small molecule, piperlongumine (PL). PL selectively induces apoptosis in cancer cells and tumor explants regardless of p53 status while leaving normal cells and tissues unaffected. A quantitative proteomic method for target identification and mechanistic studies revealed that PL induces reactive oxygen species (ROS) in cancer cells but not normal cells by inhibiting proteins involved in ROS metabolism. In addition, PL protected normal cells from chemo-drug-mediated DNA damage response. In vivo experiments demonstrate potent anti-tumor activities of PL at low concentrations with no apparent adverse effects on normal organ and tissue function. PL-induced selective killing of cancer cells supports the theory that cancers, including their cancer stem cells in the case of ROS, have increased dependence on stress-response pathways. By targeting ROS, PL constitutes a novel strategy for cancer therapy that preferentially eradicates cancer cells.

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SPEAKER ABSTRACT

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p53 and Stem Cells
Saturday, October 9
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p53, stem cells, and cancer-associated reprogramming

The way that the p53 pathway responds to an activating stress is an important determinant of the fate of the cell in which it is activated. In a larger sense, the p53-dependent responses of cells within organs can determine whether an animal will survive or perish, develop cancer, etc. p53 is regulated at diverse levels, and we are only now beginning to get an idea of the contributions of these mechanisms to cell and organ fate in intact animals. Data from two ongoing projects will be discussed. The first involves the impact of C-terminal modifications on p53 function in vivo. The p53 C-terminus is among the most highly conserved regions of the protein, and provides an intersection for modification of the same lysine residues by ubiquitylation, neddylation, acetylation etc. We generated a mouse mutant in which arginine residues were substituted for the seven C-terminal lysines to prevent such modifications (referred to as 7KR). While the mice encoding this mutant were overtly normal, they proved to be exquisitely sensitive to low-dose radiation. Data will be presented showing that the radiosensitivity derives from the animals being born with half the number of hematopoietic stem cells, and the increased sensitivity of such cells to ionizing radiation. Mice encoding p537KR exhibit higher basal levels of p21 expression in their bone marrow, which results in a higher induced level after

ionizing radiation. While one could imagine that elevated expression of numerous p53 target genes could underlie the extraordinary radiosensitivity of these animals, deleting just one copy of p21 rescued the radiosensitivity and hematopoietic defects. This provides another compelling example of how the control mechanisms that establish p53 basal activity play critical roles in determining the biological outcome of p53 pathway activation.

The second topic concerns the relationship between p53 function and somatic cell reprogramming to a stem-like phenotype. Recently published data show that loss of p53 function enables reprogramming of somatic cells to induced pluripotent stem cells. Given the high frequency of p53 mutation, or secondary alterations that reduce p53 function in cancer, is it possible that developing cancers that lose p53 could actually become reprogrammed to a more stem-like state due to the presence of oncogenic mutations that facilitate reprogramming? We used archival microarray datasets to determine whether p53 mutant or deficient cells exhibit expression signatures enriched for genes present in human embryonic or pluripotential stem cells. We found that breast and lung cancers with p53 mutations not only exhibit stem cell-like transcriptional patterns, and they are depleted for differentiation genes induced by the polycomb repressor 2 complex. These data are consistent with a model in which loss of p53 function enables acquisition of stem cell properties, which are positively selected during tumor progression.

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SPEAKER ABSTRACT

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p53 and Stem Cells
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Mutant p53 facilitates somatic cell reprogramming and augments the malignant potential of reprogrammed cells.

p53 deficiency enhances the efficiency of somatic cell reprogramming to a pluripotent state. As p53 is usually mutated in human tumors and many mutated forms of p53 gain novel activities, here we studied the influence of mutant p53 on somatic cell reprogramming. Our data indicate a novel gain-of-function property for mutant-p53, which markedy enhanced the efficiency of the reprogramming process compared to p53 deficeincy. Importantly, this novel activity of mutant p53 induced alterations in the characteristics of the reprogrammed cells; while p53-knockout cells reprogrammed with only Oct4 and Sox2 maintained their pluripotent capacity in vivo, reprogrammed cells expressing mutant p53 lost this capability, and gave rise to malignant tumors. This novel gain-of-function of mutant p53 is not attributed to its effect on proliferation, as both p53-knockout and mutant p53 cells displayed similar proliferation rates. In addition, we demonstrate an oncogenic activity of Klf4, as its overexpression in either p53-knockout or mutant p53 cells induced aggressive tumors. Overall, our data show that reprogrammed cells with the capacity to differentiate into the three germ layers in vitro can form malignant tumors, suggesting that in genetically unstable cells such as those in which p53 is mutated, reprogramming may result in the generation of cells with malignant tumor-forming potential.

SPEAKER ABSTRACT

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p53 and Stem Cells
Saturday, October 9
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Function and regulation of p53 in stem cells

As the guardian of the genome, the tumor suppressor p53 prevents the accumulation of genetic mutations by inducing cell cycle arrest, apoptosis or senescence of somatic cells after genotoxic and oncogenic stresses. Stem cells, including embryonic stem (ES) cells and adult stem cells, can undergo extensive self-renewal but fail to exhibit some or all of p53-dependent functions observed in somatic cells. By establishing p53-/- mouse and human ES cells, we show that p53 is required for maintaining genomic stability in ESCs. In this context, p53 suppresses the self-renewal of ESCs after DNA damage, leading to the elimination of these DNA damaged ESCs from the self-renewing pool. By knocking in various acetylation site mutations into the p53 gene in human ESCs, we found that two acetylation events are required for p53 stabilization and activation after DNA damage in human ES cells. Activation of p53 in various adult stem cells leads to the depletion of these stem cells primarily by apoptosis. Therefore, p53 plays diverse and critical roles in maintaining genomic stability in stem cells.

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SPEAKER ABSTRACT

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Metabolism, Survival, and Autophagy
Sunday, October 10
8:30 AM - 10:10 AM
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Co-Authors: Patricia Muller, Arnaud Vigneron, Paul Neilson, Jim Norman and Karen Vousden

Regulation of migration and survival by the p53 pathway

Tumor-associated mutant p53s have been shown to acquire the ability to promote cell motility and migration, and so contribute to the development of metastases. The ability of these mutant p53s to promote invasion and loss of directionality when cells migrate in vitro reflects enhanced integrin and EGFR trafficking that results in constitutive activation of EGFR/integrin signalling. We have extended these observations, showing that mutant p53 also drives invasion towards HGF, in a Met-dependent but EGFR-independent manner. Cells expressing mutant p53 also show scattering that is characteristic of Met activation and loss of normal cell-cell junctions. As seen for EGFR-dependent invasion, the Met-dependent scattering exhibited by mutant p53 reflects the inhibition of p63 and is dependent on Rab coupling protein (RCP). We are currently examining the role of p63 target genes in mediating these effects of mutant p53.

Studies of the p53 binding protein ASPP1 have revealed a cytoplasmic activity that leads to the activation of YAP. ASPP1 can inhibit the interaction of YAP with the Lats1, a kinase that phosphorylates YAP and promotes cytoplasmic sequestration and protein degradation. This function of ASPP1 therefore enhances nuclear accumulation of YAP and YAP-dependent transcriptional regulation. The consequence of YAP activation by ASPP1 is to inhibit apoptosis, in part through the down-regulation of Bim expression, leading to resistance to aniokis and enhanced cell migration. These results reveal a potential oncogenic role for cytoplasmic ASPP1, in contrast to the tumor suppressive activity previously described for nuclear ASPP1.

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SPEAKER ABSTRACT

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Metabolism, Survival, and Autophagy
Sunday, October 10
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Did p53 evolve to regulate metabolism?

In comparison to unicellular organisms, metazoans face a novel challenge in how they distribute nutrients to their component cells. They must control the number of cells they feed and assure that each cell has sufficient nutrients to fuel its ability to contribute to the organism. Thus, the evolution of multicellularity created the need to regulate both cell survival and cellular nutrient uptake. In this presentation, an argument that p53 has evolved as the transcriptional regulator of the cellular decision between apoptosis and adaptation to nutrient limitation will be discussed.

SPEAKER ABSTRACT

Donna George, Ph.D.

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University of Pennsylvania School of Medicine

Metabolism, Survival, and Autophagy
Sunday, October 10
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Inhibiting the HSP70 molecular chaperone system and p53 degradation

Protein misfolding is a usual occurrence in all cells, but the frequency of such events increases in response to many protein-denaturing stresses. Among such stresses are those associated with the tumor microenvironment, including hypoxia, oxidative stress as well as an accumulation of genetic changes that alter multiple signaling pathways. Proteotoxic stress can generate potentially lethal misfolded or aggregated proteins, and cancer cells seem particularly dependent on the availability of stress-response pathways for protein quality control and survival. Among these, the HSP70/HSP90 multichaperone machinery is needed to regulate a myriad of signaling pathways by maintaining the stability, conformational maturation, and/or degradation of a large and diverse group of client proteins. We recently determined that the small molecule called pifithrin-mu, or 2-phenylethynesulfonamide (PES), interacts with HSP70 and inhibits its activities. Importantly, tumor cells are more sensitive than non-transformed cells to the cytotoxic effects of this small molecule. PES induces cell death in the absence of caspase activation and regardless of p53 status, although cytotoxicity tends to be higher in cells with mutant p53. Interestingly, PES-mediated cellular toxicity is associated with protein aggregation and an impairment of both the autophagy-lysosomal and proteasomal systems. It is well known that the maturation, stability and activity of p53, as well as other oncogenic and signaling proteins depend on an association with HSP70/HSP90. Our studies reveal that PES reduces the cellular abundance of HSP70/HSP90 complexes containing p53 and those with other signaling molecules. Because PES also impairs the autophagy-lysosome and proteasome systems, this small molecule inhibitor of HSP70 promotes the accumulation of these clients in the detergent-insoluble cell fraction, suggestive of aggregation and inactivation. Thus, targeting the functions of the stress-inducible HSP70 protein should lead to a better understanding the regulation of p53 stability and maturation, as well as the development of therapies aimed at simultaneously disabling multiple cancer-critical biological processes.

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SPEAKER ABSTRACT

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Metabolism, Survival, and Autophagy
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p53-dependent regulation of autophagy protein LC3 supports cancer cell survival under prolonged starvation

Although p53 is mutated in a high percentage of human tumors, many tumors nevertheless retain wild type (wt) p53 expression. This raises the intriguing possibility that, at least in some cases, such tumors may actually benefit from the continued presence of wt p53. Recent studies imply a role for p53 in regulation of autophagy, a catabolic pathway by which eukaryotic cells degrade and recycle macromolecules and organelles, particularly under conditions of nutrient deprivation. Here we show that, in many cell types, p53 confers increased survival in the face of chronic starvation, and implicate regulation of autophagy in this effect. In HCT116 colorectal cancer cells exposed to prolonged nutrient deprivation, the endogenous wt p53 post-transcriptionally downregulates LC3, a pivotal component of the autophagic machinery. This enables reduced yet sustainable autophagic flux. Loss of p53 impairs autophagic flux and causes excessive LC3 accumulation upon starvation, culminating in apoptosis. p53 thus increases cell fitness by maintaining better autophagic homeostasis, adjusting the rate of autophagy to changing circumstances. We propose that some cancer cells retain wt p53 to benefit from the resultant increased fitness under limited nutrient supply.

SPEAKER ABSTRACT

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Animal Models
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Distinct Transactivation Requirements Underlie p53 Action in Acute DNA Damage Responses and Tumor Suppression

Although p53 is clearly a critical transcriptional activator, the relative roles of its two discrete transcriptional activation domains are not well understood. To decipher the functions of these two activation domains in different settings in vivo, we generated knock-in mouse strains expressing different transactivation-defective mutants. These include mice expressing $p53^{25,26}$, $p53^{53,54}$, and $p53^{25,26,53,54}$ mutants, with alterations in the first, second, or both transactivation domains. Interestingly, these mutants display different extents of transactivation impairment, allowing an assessment of the importance of different transcriptional programs for p53 function in diverse contexts. In particular, the $p53^{25,26}$ mutant is dramatically compromised for transactivation of most p53 target genes but retains the ability to activate a small set of novel p53 target genes. Although this mutant cannot trigger cell cycle arrest or apoptosis in response to acute DNA damage signals, it surprisingly can still serve as a tumor suppressor. In contrast, the $p53^{25,26,53,54}$ mutant is completely deficient for transactivation and lacks p53 biological activity in both DNA damage responses and tumor suppression. These findings suggest that the first transactivation domain is critical for DNA damage responses, while two redundant transactivation domains control tumor suppression. Moreover, our findings implicate transcriptional activation of the small set of novel genes activated by $p53^{25,26}$ in tumor suppression, an area currently under investigation.

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SPEAKER ABSTRACT

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Animal Models
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The codon 72 polymorphism of p53 influences the transcription of genes involved in immunity and inflammation

A common polymorphism at codon 72 in the p53 tumor suppressor protein encodes either proline (P72) or arginine (R72). Several groups have reported that in engineered cell lines this polymorphism influences p53's transcriptional, growth arrest, and apoptotic functions. However, the impact of this polymorphism within the context of a living organism has never been examined. In efforts to understand the biological consequences of the p53 codon 72 polymorphism in a living organism, we generated P72 and R72 mice in the background of the Humanized p53 knock-in (Hupki) mouse. In Hupki mice that are extensively back-crossed into a C57Bl/6 background, we find that there are consistent tissue-specific differences in apoptotic function between the P72 and R72 variants. Within the thymus, we find that the P72 variant is associated with increased apoptosis following gamma radiation. Micro-array analysis indicates that a small subset of genes show increased transactivation in thymocytes from P72 mice compared to R72. These genes include caspase 4/11, which mediates the processing of caspase 1. Interestingly, several of these genes are also regulated by NF-kB, and play roles in immunity and inflammation. Mechanistically we show that p53 interacts with the p65 Rel A subunit of NF-kB, and further that the P72 variant of p53 interacts significantly better than R72. These data indicate that the codon 72 polymorphism affects the ability of p53 to interact with NF-kB and to cooperate in the transactivation of genes involved in immunity and inflammation.

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SPEAKER ABSTRACT

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Animal Models
Sunday, October 10
10:30 AM - 12:10 PM
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Dunlop Auditorium

Modulating p53 activity for better or for worse

The activity of the p53 tumor suppressor is normally tightly restrained by two p53 inhibitors Mdm2 and Mdm4. Deletion of either of these inhibitors in mice results in embryo lethal phenotypes that are completely rescued by concomitant deletion of p53. Additionally, subtle alterations in the pathway, haploinsufficiency for Mdm2 or Mdm4 for example, affect survival and tumor phenotypes in compromised backgrounds. A mouse model inheriting a human MDM2 single nucleotide polymorphism also shows increased Mdm2 levels, decreased p53 activity, and an increased incidence of cancer, thus providing the first direct evidence of a high frequency regulatory polymorphism contributing to cancer risk. These regulatory pathways also impinge on the gain-of-function phenotypes of mutant p53.

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SPEAKER ABSTRACT

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Animal Models
Sunday, October 10
10:30 AM - 12:10 PM
Stemmler Hall
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Comparison of Non-Metastatic and Metastatic Osteosarcomas in p53 Mutant Mouse Models

Missense p53 mutations in human tumors, besides inactivating p53 tumor suppressor function, may confer both dominant negative activities (on wt p53 family members) and dominant gain of function activities that promote tumorigenesis. To better understand the dominant effects of missense p53, we have compared biological and molecular aspects of tumorigenesis in several types of mutant p53 mice. Our focus was osteosarcomas (OS), a frequent tumor type in both p53 knockout (p53+/-) and p53 mutant knockin (p53+/R172H) mice. Also of interest was the finding that while p53+/- mice exhibited only non-metastatic OS, most p53+/R172H mutant mice developed metastatic OS. We isolated genomic DNA and total mRNA from non-metastatic (p53+/-) and metastatic (p53+/R172H) OS. BAC-array CGH to analyze global DNA copy number changes didn't indicate an increase in genomic instability in the p53+/R172H OS. Comparison of total mRNA in p53+/- and p53+/R172H OS by microarray analyses showed a dramatic decrease in extracellular matrix forming components in the p53+/R172H tumors.

Followup studies utilized mouse models with either osteoblast specific deletion of p53 or osteoblast specific expression of p53 mutant R172H. Both osteoblast p53 models resulted in mice that developed predominantly OS. As with the germline p53+/- and p53+/R172H mice, mice with osteoblast activation of a single R172H allele developed significantly more metastatic OS than those mice with osteoblast deletion of a single wildtype p53 allele. Interestingly, mice with both wildtype p53 alleles deleted in osteoblasts showed intermediate levels of metastatic OS, suggesting that complete absence of p53 affects metastatic potential nearly as much as gain of function effects produced by mutant p53. Microarray comparisons of these metastatic and non-metastatic OS also showed Wnt signaling pathway differences, with key Wnt signaling inhibitors dramatically decreased in the metastatic tumors. The results begin to point to specific pathways through which mutant p53 may influence tumor progression.

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SPEAKER ABSTRACT

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p53: Drug Discovery and Therapy
Sunday, October 10
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Therapeutic targeting of tumor suppression in p53-deficient cancers

In recent years we have employed several strategies to achieve an anti-tumor effect in tumors that are deficient in p53 signaling. The presentation will include an update on the ongoing screening to identify small molecules that restore p53 signaling in tumors with mutant p53. Candidate p53 pathway-restoring small molecules for clinical development should have attractive medicinal properties and induce potent cell death of tumor cells but not normal cells. Small molecules have been identified and initial studies document a pro-apoptotic effect towards tumor cells but not normal cells, in a manner associated with restoration of a p53 signaling pathway. A second approach involves targeting cell death in p53-deficient hypoxic tumors using small molecules. The results suggest that dual targeting of the kinasesGSK3-beta and CDK1 in combination with chemotherapy or TRAIL is an effective strategy to induce cell death in resistant hypoxic tumors. The loss of p53 function promotes therapeutic resistance under hypoxia while the restoration of sensitivity can occur independently of p53 restoration. A third approach involves targeting upregulation of TRAIL with small molecules in a p53-independent manner. The results demonstrate anti-tumor efficacy in tumor xenografts and initial proof-of-principle that upregulation of TRAIL production by both the host and the tumor is an effective anti-tumor strategy.

SPEAKER ABSTRACT

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p53: Drug Discovery and Therapy
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Pharmacological targeting of mutant p53: from molecular biology to the cancer patient

p53 is frequently mutated in human tumors, allowing evasion of apoptosis or senescence in response to DNA damage and oncogenic stress. Most p53 mutations disrupt p53 DNA binding and transcriptional transactivation of target genes such as p21, Puma and Noxa. Mutant p53 is often expressed at high levels. Therefore mutant p53 reactivation is an attractive strategy for novel cancer therapy. Restoration of wild type p53 expression in vivo causes cell death and efficient elimination of tumors. Several mutant p53-targeting small molecules have been identified, raising hopes for the development of novel anticancer drugs. PRIMA-1 was identified in a cellular screen based on induction of mutant p53-dependent apoptosis. PRIMA-1 and its structural analog PRIMA-1Met (APR-246) restore wild type conformation to mutant p53, inhibit tumor growth in mice, and synergize with chemotherapeutic drugs in cultured tumor cells and in vivo (Bykov et al., Nature Med. 8, 282-8, 2002). PRIMA-1 and APR-246 induce proapoptotic p53 target genes Bax, Puma and Noxa, along with activation of caspase-2, leading to loss of mitochondrial membrane potential, cytochrome c release, and activation of downstream effector caspases. Microarray analysis revealed that APR-246 induces changes in expression of a limited number of genes in mutant p53-expressing cells, including genes that regulate cell cycle progression and apoptosis. PRIMA-1 and APR-246 are converted to MQ, a compound with Michael acceptor activity that can modify cysteines in mutant p53 (Lambert et al., Cancer Cell 15, 376-88, 2009). This chemical activity is shared with other mutant p53-targeting compounds such as MIRA-1 and STIMA-1. APR-246 targes mutant isoforms of the p53 family members p63 and p73, presumably through a common mechanism involving homologous structural elements in the p53 family proteins. The safety and efficacy of APR-246 is currently tested in a phase I clinical trial in patients with hematological malignancies or hormone-refractory prostate cancer.

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SPEAKER ABSTRACT

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p53: Drug Discovery and Therapy
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Dissecting p53 network using small molecules as research tools

Reactivation of p53 in cancers appears to be a promising strategy to combat cancers. Several interesting compounds activating p53 have been identified. Besides being candidates for drug development, these small molecules can serve as valuable research tools, opening a possibility to get a deeper understanding of the exact mechanism of p53-mediated biological responses ranging from the induction of growth arrest and DNA repair to senescence and apoptosis. In order to address these thrilling questions of p53 biology and to identify key p53 modulators and effectors we integrated genome-wide expression profiling data and ChIP-seq data. As a model we used MCF7 breast carcinoma cells treated with RITA and nutin3a, small molecules activating p53. Although both compounds induce p53 by targeting p53/MDM2 interaction, they induce different response in MCF7 cells: nutlin3a induces growth arrest and RITA triggers apoptosis. Thus, using these compounds we can address the mechanism of alternative biological responses induced by p53 in the same background. Functional analysis of factors/genes involved in alternative biological responses by p53 will help to answer important questions of p53 biology, thus opening the way to novel therapeutic approaches.

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SPEAKER ABSTRACT

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p53: Drug Discovery and Therapy
Sunday, October 10
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p53, inflammation and cancer chemoprevention

Historically, p53 effects are mainly interpreted in the context of genotoxic stress response. However, there is growing evidence indicating that many functions of p53 are mediated via cross-talk with other intracellular regulatory pathways without involvement of DNA damage. These include the roles played by p53 in control of normal development, epigenetic stability, metabolism and inflammation. Our work is focused on pharmacological modulation of p53 activity, including the possibility of preventing cancer by using non-genotoxic pharmacological activation of p53 to suppress inflammation. Chronic inflammation is well recognized as a cancer promoting factor and the anti-inflammatory activity of p53 likely contributes to its tumor suppressor function. We and others have demonstrated mutual negative regulation between p53 and NF-κB, a major regulator of immune responses including inflammation. We found that p53 deficiency was associated with enhanced pro-inflammatory NF-κB activity[1] and that constitutively active NF-κB can contribute to p53 suppression in tumors[2]. Mycoplasma infection, known to induce constitutive TLR2-mediated activation of NF-κB, also causes partial suppression of p53, which in cultured rodent fibroblasts was sufficient to permit malignant transformation by activated Ras[3]. Moreover, we identified a strong correlation between presence of Mycoplasma hominis in prostate core biopsies and prostate cancer in 246 men examined due to elevated PSA levels[4]. This observation may set another example when chronic infection with a p53-suppressing microorganism leads to cancer predisposition and suggests that Mycoplasma hominis could serve as a new marker and/or potential target for treatment or prophylaxis of the most frequent cancer in men of the western world. We also tested whether random genetic events resulting in NF-κB activation would prevent p53 from inhibiting Ras-mediated transformation. NF-κB activating peptides were isolated through functional screening of a lentiviral library and were shown to be capable of cooperating with activated Ras in transformation of rodent fibroblasts, including acquisition of tumorigenicity[5]. This can be viewed as an in vitro model to study the role of inflammation in cancer development. Finally, we demonstrated that two classes of small molecules capable of non-genotoxic p53 activation, nutlin[6] and curaxins[7] (the latter one is also an NF-κB inhibitor), have cancer preventive activity in several models of cancer-prone mice. Taken together, these studies illustrate the impact of p53-NF-κB interplay in the regulation of inflammation and its consequences on tumorigenesis. Our data provide strong support for the potential of non-genotoxic pharmacological activation of p53 as a strategy for cancer prophylaxis.

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SPEAKER ABSTRACT

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p53: Drug Discovery and Therapy
Sunday, October 10
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Activation of p53 by MDM2 antagonists: from bench to bedside

Activation of p53 by inhibiting the p53-MDM2 interaction has long been considered an attractive approach to cancer therapy. MDM2 antagonists, the nutlins, interact specifically with the p53-binding pocket of MDM2 and release p53 from negative control, leading to its stabilization and activation of p53 signaling. Although half of human tumors retain wild-type p53, the function of the tumor suppressor could be compromised by defective signaling. Using nutlin-3a to probe downstream p53 signaling we find that many cancer cell lines expressing wild-type p53 have a defective p53-dependent apoptotic response. Cancer cell lines overproducing MDM2 due to amplification of its gene have shown most robust nutlin-induced apoptosis presumably due to intact p53 apoptotic signaling. By disrupting the p53-MDM2 autoregulatory circuit, nutlin causes substantial accumulation not only of p53 but also MDM2 protein. Nutlin binding does not affect MDM2 E3 ligase activity that is even elevated in the presence of the drug. MDMX, a known MDM2 ubiqutination target decreased with the increase of MDM2, suggesting that MDM2 contributes to antitumor activity of nutlins at least in part by facilitating MDMX degradation. However, MDMX is at very low levels in mdm2-amplified cells and does not appear to play an important role in p53 regulation. Reduction of MDM2 by RNAi decreases the apoptotic response to nutlin, suggesting that MDM2 effect is independent of MDMX. We find that highly elevated MDM2 protein levels in mdm2-amplified osteosarcoma cells contribute to the apoptotic outcome of nutlin treatment by upregulation of subsets of pro-apoptotic microRNAs. Nutlin-3a doses that shrink xenograft tumors caused relatively mild changes in p53-sensitive tissues, suggesting that MDM2 antagonists may provide an acceptable safety window for cancer therapy. A member of the nutlin family of MDM2 antagonists, RG7112, is currently undergoing Ph I clinical testing.

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SPEAKER ABSTRACT

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p53: Targets and Control
Sunday, October 10
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Inhibition of p53 expression by peptide-conjugated phosphorodiamidate morpholino oligomers: effects on isoform expression and chemosensitivity

The p53 tumour suppressor gene encodes a transcription factor that is commonly mutated in cancer. Tumours arise when premalignant cells are unable to undergo p53-dependent apoptosis, cell cycle arrest or DNA repair. The p53 signalling pathway affects not only tumour development but also the response of tumours to chemotherapeutic drugs. The role of p53 in determining drug sensitivity is complex and dependent on cell type and the drug used. In some experimental models, p53-deficiency is associated with resistance to DNA damaging agents; in other models p53-deficiency leads to enhanced drug sensitivity. In this study, we use cell penetrating peptide (CPP) conjugates of phosphorodiamidate morpholino oligomers (PPMOs) to inhibit p53 expression and report that p53 inhibition sensitizes human cancer cells with wild-type p53 to chemotherapeutic drugs. In addition, we characterize a number of endogenous p53 isoforms that are produced upon PPMO-mediated inhibition of p53 translation and splicing.

SPEAKER ABSTRACT

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p53: Targets and Control
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The Tumor Suppressor Gene p53 Modulates Paracrine Signaling to Either Promote or Suppress Malignant Phenotypes in the Tissue Microenvironment

Many studies have investigated the role of p53 and p53 mutants in the initiation and progression of malignancies. This multifunctional protein, and its family members are involved in numerous physiologic and pathologic processes. We have identified a novel function for p53 in tumor biology. We have recently reported that double-strand DNA damage or telomere malfunction results in a p53-dependent induction of a TGF-β superfamily member. This secreted protein binds to its receptor, resulting in a p38-dependent expression of proteins that induce a number of tumor-promoting phenotypes including resistance to apoptosis and enhanced cell motility in adjacent cells. We will present data that demonstrate that double-strand DNA damage has cell non-autonomous, p53-dependent effects that promote or suppress tumorigenesis in human tissue.

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SPEAKER ABSTRACT

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p53 modification and its regulators

p53 functions as a central node for organizing whether the cell responds to various types and levels of stress with apoptosis, cell cycle arrest, senescence, DNA repair, metabolic change, autophagy and aging. While the exact molecular events for p53-mediated choice of cell fate are still insufficiently explained, p53 controlled transactivation of target genes comprises an essential event in each stress response pathway although some of p53-mediated effects have been proved to be transcription independent. As a transcription factor, p53 demands an exquisitely complicated network of control and fine-tuning mechanisms to ensure correct, differentiated responses to the various stress signals encountered by cells. p53 was the first non-histone protein known to be regulated by acetylation and deacetylation. There is accumulating evidence indicating that acetylation of p53 plays a major part in activating p53 function during stress responses. Following our early findings of C-terminus p53 acetylation, we and others recently showed that p53 is also acetylated at K120 and K164 within the DNA binding domain. Subsequently, we found that loss of acetylation at all these major sites completely abolishes p53's ability to activate its mediated cell growth arrest and apoptosis in human cells. The biggest challenge remains regarding whether acetylation is indispensible for p53 function in vivo. We have recently developed an acetylation-defective (K120R for human p53/K117R for mouse p53)) point mutant knockin mouse and our preliminary studies reveal that loss of acetylation at K120 (K117 for mouse p53) dramatically abrogates p53-mediated apoptosis in vivo. Moreover, we have also developed another acetylation mutant mouse (with additional mutated acetylation sites) which is completely defective for both cell growth arrest and apoptosis of p53 in vivo. These studies demonstrate that acetylation of p53 is absolutely required for p53-mediated tumor suppression. In addition, we will update our current progress on other p53 regulators such Sirt1, HAUSP, ARF-BP1 and newly identified ARF-ubiquitin ligase ULF.

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SPEAKER ABSTRACT

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p53: Targets and Control
Sunday, October 10
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Biological activities of human and mouse p53 isoforms

The p53 pathway is altered in all cancers. However it is difficult to link p53 status with clinical outcome and cancer treatment. We previously reported that the human p53 gene expresses at least nine different p53 proteins (isoforms) due to alternative splicing, alternative translation initiation site and internal promoter usage (p53, p53β p53γ, Δ40p53α, Δ40p53β, Δ40p53γ, Δ133p53α, Δ133p53β and Δ133p53γ). This dual gene structure is a general feature of the p53 gene family members (p63 and p73) and is conserved through evolution from drosophila to man. p53 isoforms are expressed in normal human tissue in a tissue dependent manner but are abnormally expressed in many types of human cancers. We established that p53 isoforms can differentially modulate p53 transcriptional activity. Moreover, p53 isoforms play an important role in zebrafish embryo development and in senescence in human normal fibroblasts. Here we report that the Δ133p53α isoform is induced by p53 in response to stress and prevents p53-mediated apoptosis and G1 arrest without inhibiting p53-mediated G2 cell cycle arrest. It indicates that Δ133p53α does not exclusively act by inactivating p53 but rather by regulating gene expression in a p53-dependent and independent manner. Moreover, Δ133p53α expression confers cell motility and promotes angiogenesis. By analyzing p53 isoform expression in 127 primary breast tumours, we established that mutant p53 breast cancer patients expressing p53γ have as good survival rate as breast cancer patients expressing WTp53, while mutant p53 breast cancer patients not expressing p53γ have a very poor survival rate. We will discuss the mechanisms involved. The alternative splice variant of p53, p53AS, was first discovered in mice by the group of Varda Rotter in 1985. We revisited the mouse p53 gene and determined that it expresses at least 6 p53 protein isoforms, including p53AS. We will present some of their biological activities.

SPEAKER ABSTRACT

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p53 Family Members
Monday, October 11
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p73 is involved in neuronal development via miR-34a

In the last ten years, p63 and p73 have been identified as the ancestral members of the p53 family. Despite the high sequence and structural similarity, the mouse knockouts revealed a crucial role in neural development for p73 and in epidermal formation for p63. We identified several transcriptional targets, the mechanisms of regulation of cell death, and the p63 isoform involved in epithelial development. Both genes are involved in female infertility as well as in cancer formation, although with distinct mechanisms.

p73 steady state protein levels are kept low under normal physiological conditions through degradation by the 26S proteasome, mediated by the HECT-containing E3 ubiquitin ligase ITCH. We developed an ELISA high throughput screening for ITCH auto-ubiquitylation, resulting in several positive compounds that are able to modulate chemosensitivity at 10 μM concentration. These compounds could be effective in cancer treatment. In addition to this major degradation pathway, we have also described additional novel mechanisms of degradation. [1] the orphan F-box protein FBXO45 targets p73 for degradation. [2] a novel transcriptional target of TAp73, the ring finger domain ubiquitin ligase PIR2 (p73-induced Ring Finger 2) regulates the proteasomal degradation of the ΔNp73 isoforms. [3] the antizyme ubiquitin-independent, proteasome-dependent pathway targets ΔNp73 for degradation.

Here, we describe the involvement of p73 in neuronal development. TAp73 knockout mice (TW Mak G&D 2008) show hippocampal dysegensis. Conversely, ΔNp73 knockout mice (TW Mak G&D 2010) show sign of moderate neurodegeneration with a significant loss of cellularity in the cortex. TAp73 is able to drive the expression of miR-34a, acting on specific binding sites present on the miR-34a promoter. In agreement with these in vitro data, miR-34a transcript expression is significantly reduced in vivo both in the cortex and hippocampus of p73-/- mice. In keeping, we show a role for miR-34a, in parallel to TAp73 expression, during in vitro differentiation of ES cells. Expression of miR-34a increases during in vitro neuronal terminal differentiation, of ex vivo primary cortical neuronal cultures, in parallel with the expression of TAp73. Moreover, we also detect an increase ex vivo of

miR-34a steady state expression during postnatal development of the brain and cerebellum, when synaptogenesis occurs. We further confirm a role for miR-34a in synaptogenesis, as overexpression or silencing of miR-34a results in an inverse expression of a number of synaptic genes, via their 3'-UTR. In particular, miR-34a overexpression decreases synaptotagmin I and syntaxin-1A expression, and the endogenous levels of miR-34a are able to regulate only synaptotagmin I expression. Our findings show that p73 drives the expression of miR-34a during terminal, synaptic differentiation

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SPEAKER ABSTRACT

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The Role of TAp73 and ΔNp73 in tumor-development

The Trp53 gene family member Trp73 encodes two major groups of protein isoforms, TAp73 and ΔNp73, with opposing pro-and anti-apoptotic functions; consequently, their relative ratio regulates cell fate. These groups have opposing functions, and the ratio of these proteins within a cell regulates its fate. However, the precise roles of p73 isoforms in cellular events such as tumor initiation, embryonic development and cell death remain unclear. To determine which aspects of p73 function are attributable to the TAp73 isoforms, we generated and characterized mice in which exons encoding the TAp73 isoforms were specifically deleted to create a TAp73-deficient (TAp73-/-) mouse. As well we also generated the ΔNp73 specific knockout mice by deleting the ΔNp73 specific exons to complement the studies on TAp73 deficient mice. The phenotypes of these animals will be discussed.

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SPEAKER ABSTRACT

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Differential Regulation of the p73 Cistrome by mTOR Reveals Transcriptional Programs of Mesenchymal Differentiation and Tumorigenesis

p73 is a transcription factor that plays critical roles during development and tumorigenesis. It exhibits sequence identity and structural homology with p53, and can engage p53-like tumor suppressive programs. However, different pathways regulate p53 and p73, and p73 is not mutated in human tumors. p73 therefore represents a therapeutic target, and there is a critical need to understand genes and ncRNAs regulated by p73 and how they change during treatment regimens. We defined the p73 genomic binding profile and demonstrated its modulation by rapamycin, an inhibitor of mTOR and inducer of p73. Rapamycin selectively increased p73 occupancy at a subset of its binding sites. In addition, multiple determinants of p73 binding, activity, and function were evident, and were modulated by mTOR. We generated an mTOR-p73 signature that is enriched for p73 target genes and miRNAs that are involved in mesenchymal differentiation and tumorigenesis, can classify rhabdomyosarcomas by clinical subtype, and can predict patient outcome. The findings that will be presented have broad implications as mTOR inhibitors are being investigated in clinical trails, alone or in combination with other p73-activating drugs, for many tumor types.

SPEAKER ABSTRACT

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Novel in vivo roles of p73 in lymphoma and neurogenesis

Using the global p73-/- mice, we identified novel in vivo roles of Trp73 in tumor progression and development. First, we show that loss of p73 promotes dissemination of Myc-induced B cell lymphomas. Mice expressing the c-Myc transgene in B-lymphocytes (Eμ-myc) develop lethal lymphomas that frequently mutate p53. Whether p73 also functions as a tumor suppressor in vivo remains controversial. We find that non-transgenic p73-/- mice have impaired B-cell development. in Eμ-myc mice p73 loss does not substantially affect disease onset and mortality, but alters the clinical phenotype of the disease. Specifically, p73 loss decreased nodal disease, but increased widespread extranodal dissemination. We show that p53 acts as the dominant tumor suppressor during onset of B-lymphomagenesis, while p73 is a tumor modulator whose contribution lies in preventing tumor dissemination and extranodal growth. Immunophenotyping and expression profiling shows that p73 loss allows increased maturation of malignant B-cells and deregulates genes involved in lymphocyte homing and dissemination of human lymphomas. Consistent with this, p73 expression was frequently downregulated in a large cohort of human mature aggressive B cell lymphomas, and both incidence and degree of p73 downregulation correlated with their extranodal dissemination status. These data indicate that p73 is a modifier of Myc-driven lymphomas,favoring tumor dissemination, and suggest that p73 could be a biomarker for human B lymphoma dissemination.

Second, we show that p73 is an essential regulator of neural stem cell maintenance in embryonal and adult CNS neurogenesis. p73 is essential for brain development, but its precise role and scope remain unclear. Global p73 deficiency determines an overt and highly penetrant brain phenotype of cortical hypoplasia with ensuing hydrocephalus and hippocampal dysgenesis. The ΔNp73 isoform is known to act as a pro-survival factor of mature post-mitotic neurons. Here, we define another essential role of p73 in the neural stem cell compartment. In both embryonic and adult neurogenesis, p73 has a critical role in maintaining an adequate neurogenic pool by promoting self-renewal and proliferation and inhibiting premature senescence of neural stem and early progenitor cells. Thus, products of the p73 gene locus are essential maintenance factors in the central nervous system whose broad action stretches across the entire differentiation arch from stem cells to mature postmitotic neurons.

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SPEAKER ABSTRACT

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p53: Genetics and Disease
Monday, October 11
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Origins of Barrett’s Metaplasia

Esophageal and gastric adenocarcinoma together kill more than one million people each year worldwide. Both cancers arise in association chronic inflammation and are preceded by robust metaplasia with intestinal characteristics. Gastric intestinal metaplasia can be triggered by gastritis involving H. pylori infections, while Barrett’s metaplasia of the esophagus is linked to gastroesophageal reflux disease (GERD). H. pylori suppression therapies have contributed to the recent decline of gastric adenocarcinoma, although the incidence of esophageal adenocarcinoma, especially in the West, has increased dramatically in the past several decades. Treatments for late stages of these diseases are challenging and largely palliative, and therefore considerable efforts have focused on understanding the earlier, premalignant stages of these diseases for therapeutic opportunities. Cancers arise from this metaplasia via stereotypic genetic and cytologic changes that present as dysplasia, high-grade dysplasia, and finally invasive adenocarcinoma. The ontogeny of these metaplasias remains an intriguing mystery with cogent support for hypotheses involving transcommitment of resident stem cells, migration from lower gastrointestinal sites, or the reparative emergence of submucosal glands. We have used several mouse models to clarify the origins of Barrett’s metaplasia and have implicated a discrete population of cells in its initiation.

SPEAKER ABSTRACT

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Potential Clinical Applications of AmpliChip p53 Test

The tumor suppressor p53 plays a central role in preventing malignant transformation by regulating numerous cellular pathways, including cell proliferation, apoptosis and genomic integrity. Disruption of p53 function promotes checkpoints defects, genomic instability and inappropriate survival, leading to the uncontrolled growth of damaged cells. Over the past decades, many studies have investigated the prognostic and predictive value of p53 mutation status for tumor response to cancer treatment and patient outcome. Despite the large efforts, no consensus has been reached, partially, because the majority of older reports relied upon immunohistochemistry (IHC) to detect nuclear accumulation of p53 protein as a surrogate marker for mutations in the p53 gene.

The AmpliChip p53 Test, which is currently under development at Roche Molecular Systems, Inc (Pleasanton, California, USA), is designed to detect mutations, including single-base substitutions and single-base deletions in exons 2-11 and their flanking splice sites, in the p53 gene via a microarray-based re-sequencing analysis of human DNA derived from fresh frozen or formalin-fixed, paraffin-embedded human cancer tissue specimens. The test is shown to be more sensitive than the conventional Sanger sequencing in detecting mutation in the mixture with the wild type sequence, and the simple workflow of the AmpliChip p53 test offers the likelihood of standardizing the p53 mutation analysis for use in the routine clinical setting.

Recently, we conducted the p53 mutation analysis with the AmpliChip p53 test in three clinical studies and demonstrated the role of p53 mutations in managing breast cancer, CLL and Diffuse Large B-cell Lymphoma (DLBCL). We will present the results showing 1) p53 mutation is correlated to a better clinical and pathologic response of the primary tumor in women receiving neoadjuvant Docetaxel-Capecitabine (± Trastuzumab) chemotherapy for early-stage breast cancer. 2) p53 mutation is an independent prognostic marker an associated with poor response and survival as after 1st line FC and R-FC treatment in CLL. 3) p53 mutation is associated with poor survival in DLBCL patients treated with the CHOP or R-CHOP regimens. These findings provide potential clinical applications for the AmpliChip p53 test.

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p53 and the Li-Fraumeni Syndrome: From Bench to Bedside in 20 Years

LFS is the most common syndrome known to predispose both children and adults to a spectrum of histopathologically diverse malignancies. Germline p53 mutations were first reported in Li-Fraumeni Syndrome (LFS) 20 years ago. Since then, the clinical relevance of p53 genetics and biology has become increasingly important. The clinical definition of the syndrome has been redefined to account for the association of germline p53 alterations with tumor phenotypes that do not reflect the classical LFS definition. In addition, we and others have identified genetic modifiers of clinical phenotype including MDM2 SNP309, PIN3, codon 72 R>P SNP, telomere attrition and DNA copy number variation. The role that these modifiers play in determining cancer phenotype will be discussed. Recent work from our group suggests that constitutional deletions across 17p13.1 confer distinct cancer or developmental delay/congenital anomaly phenotypes depending on the precise molecular breakpoint at or near the p53 locus. Furthermore, the molecular sequence characteristics of these breakpoints suggest mechanisms of recombination that may be peculiar to this site. Utilizing high-resolution SNP/CNV arrays, we have shown that p53 mutation carriers harbor excessive inherited and de novo copy number variable regions that may identify a complementary role of genes that may be involved in malignant transformation of target tissues. The availability of germline p53 testing in at-risk individuals offers an important and unique opportunity for clinical intervention. We have developed a comprehensive surveillance protocol for p53 mutation carriers and have demonstrated it to have a beneficial effect on survival. This talk will present findings from these and emerging work that highlights the clinical relevance of germline p53 mutation analysis.

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Significance of p53 mutations in human cancer; a needed reassessment?

Partial and whole cancer genome sequencing have been launched but its led to the discovery of an unexpected landscape of in vivo somatic mutation with 10 to 20 000 bases substitutions per genome. The majority of these variations are somatic passenger mutations (or hitchhiking mutations) that have no active role in cancer progression and are co-selected by the driver mutations which are the true driving force for cell transformation. p53 alterations are the most frequent genetic event in human cancer. Recent observations have challenged several "dogma" concerning p53 mutations. Among them, are the number of p53 mutations per tumor and the biological function of p53 targeted by the various mutations. Furthermore, using novel statistical analysis, we have shown that all p53 mutation databases are polluted with either artifactual or passenger mutations that complicates their interpretation. In order to have a more accurate picture on the heterogeneity of p53 mutation in human cancer, we have undertaken the analysis of p53 status in various human tumors using massively parallel sequencing techniques as it has the potential to identify the compendia of rare subclones of genetic variants that may exist in human tumors The TP53 UMD mutation database is a paradigm, as it constitutes the largest collection of somatic mutations for a single gene (30 000 mutations from 29 000 patients with various types of cancer: http://p53.free.fr). A novel related database (LOADMUTP53 for Loss Of Activity Database for MUTant p53) has been developed. Its includes specific features related to structural (conformation, oligomerization), biological (localization, apoptosis or growth arrest) and functional (transactivation, DNA binding) activity of mutant p53. Analysis of both LOADMUTP53 and the TP53 mutation database will be presented and discussed.

SPEAKER ABSTRACT

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Senescence, Checkpoints, and the Downstream Pathway
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Interrogating the p53 tumor suppressor network using mouse models and RNA interference

Our laboratory studies tumor-suppressor networks and how their disruption influences malignant behavior. To facilitate our research, we have recently developed new mouse cancer models based on the genetic manipulation of stem and progenitor cells ex vivo followed by transplantation of the altered cells into the appropriate organ of syngeneic recipient mice. This approach allows us to rapidly study the impact of many genes and gene combinations on tumorigenesis in a “mosaic” setting where tumor-initiating cells are embedded in normal tissues. Furthermore, we have developed powerful methods for using RNAi to suppress gene function in vivo in either a stable and reversible manner. Current efforts in the laboratory strive to integrate mosaic mouse models, RNA interference, and cancer genomics to identify new components of the p53 tumor suppressor gene network and characterize their impact on tumorigenesis and treatment response. In addition, we are developing new RNAi interference methods to explore the role of tumor suppressor genes in tumor maintenance, and the cell death mechanisms involved in tumor regression.

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SPEAKER ABSTRACT

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Senescence, Checkpoints, and the Downstream Pathway
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Inflammation, Senescence and Cancer: Interweaving MicroRNA, Inflammatory Cytokines and p53 Networks

The p53 pathway is an intrinsic monitor and response pathway of telomeric attrition involved in cellular aging and senescence. Cellular senescence is tumor suppressive that can be activated by p53 in cancer cells. We are studying the molecular mechanisms of cellular senescence in normal and malignant human cells and the role of the telometric multiprotein complex, shelterin, that includes TRF2 and POT1 (1-3). Our ongoing studies have revealed that p53 and its endogenous isoforms regulate both specific microRNAs and TRF2 expression as mechanisms of replicative senescence. In addition, POT1 isoforms are functionally diverse in both maintaining telomeric integrity and preventing p53-dependent senescence induced by telomeric shortening. A switch in the expression patterns of p53 isoforms, Δ133Np53 and p53beta, is also associated with the transition of benign to malignant human cancers.

Chronic inflammation and deregulation of microRNAs have roles in human carcinogenesis (4-7). In addition to our mechanistic and genetic studies, we are investigating the expression of microRNAs and inflammatory genes as cancer biomarkers of diagnosis, prognosis, and therapeutic outcome (8-11). We are especially interested in developing prognostic classifiers of early stage cancer.

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SPEAKER ABSTRACT

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Senescence, Checkpoints, and the Downstream Pathway
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The basic C-terminus of p53 plays a key role in p53-dependent transcriptional responses

The extreme C-terminus of p53 has been a focus of study due to density of basic amino acids as well as the existence of multiple residues that are subject to a variety of post-translational modifications. In vitro studies show that this basic region has non-specific DNA binding ability and can selectively influence the interaction of p53 with one subset of its binding sites but not another. In cells, ectopic expression of various mutant p53 proteins that have substitutions or deletions in the C-terminal basic region demonstrate an obligate role for the terminal 24 amino acids in p53 transcriptional activity on all target genes tested. Further, some of these mutants have reduced activity on only a subset of p53 targets. These effects correlate with the ability of the mutant proteins to occupy the corresponding genomic sites as determined by chromatin immunoprecipitation. The C-terminal region is thus implicated in target gene selectivity by p53. This also leads to the hypothesis that combinatorial post-translational modification of the C-terminus may contribute to deciding p53-dependent cell fate outcomes. To more definitively establish a role for the p53 C-terminus under physiological conditions, a mutant mouse has been generated that conditionally expresses a truncated form of p53 lacking the last 24 amino acids. Studies will be discussed involving the characterization of the activity of this p53 protein both in vivo as well as in derived embryo fibroblasts in cell culture.

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Senescence, Checkpoints, and the Downstream Pathway
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Constructing synthetic lethal networks for p53 tumor suppressor pathways in the presence of DNA damage.

p53 deficient cells are resistant to apoptosis, which contributes to malignant progression and therapy resistance of p53 mutant tumors. For this reason, identifying and targeting p53 independent cell death (PICD) pathways is an important therapeutic goal. We previously reported that the DNA damage kinases Atm and DNA-PK exhibit p5 independent synthetic lethal interactions (Current Biology 11:191-194, 2001) and that DNA-PK regulates a DNA damage induced apoptotic response that is p53 independent (EMBO Reports, 10:87-93, 2009. To further characterize these p53 independent cell death pathways and to identify additional synthetic lethal interactions with the p53 pathway, we applied functional genomic screens using arrayed siRNAs.. We tested primary squamous cell carcinoma cells isolated from tumors from four genotypes of mice: Atm-/-, DNA-PK-/-, p19//Arf -/- and p53 -/-. We performed synthetic lethal screens with these four query cell lines against 610 siRNAs targeting the mouse kinome. Further, these screen were run in the presence of three concentrations of the chemotherapy agent doxorubicin to identify genotype specific sensitizers. We identified numerous additional synthetic partners with each query genotype, Atm, DNA-PK, p19/Arf ,and p53. These synthetic lethal interactions vary according to genotype and doxorubicin exposure, thus defining synthetic lethal pathways that sensitize tumor cells of defined genotype to genotoxic therapy. siRNAs targeting p53 sensitized both Atm and p19/Arf null tumor cells to doxorubicin and p19/Arf cells were highly sensitive to siRNAs targeting DNA repair genes in the homologous recombination and non-homologous end joining pathways. Targeting these p53 independent cell death pathways may be an effective strategy to sensitize tumors of defined genotype to existing genotoxic cancer therapies.

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SPEAKER ABSTRACT

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Senescence, Checkpoints, and the Downstream Pathway
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Functional consequences of p53 DNA binding domain acetylation

The stabilization of p53 in response to cellular stress results in part from the displacement of MDM2 that results from addition of specific phosphate groups to p53. Subsequent to stabilization, full "activation" of p53 requires the addition of a complex set of additional post-translational modifications. These modifications include acetylation, methylation, sumoylation, neddylation, ubiquitylation and ribosylation. Our group, along with the Gu laboratory, have recently demonstrated that the acetylation of specific lysine residues within the p53 DNA binding domain is of particular importance for regulating the biological effects of p53 activation. Specifically we have found that acetylation of K120 by the MYST family of enzymes is important for p53-induced apoptosis, but not p53-induced cell cycle arrest. Consistent with a role for K120 acetylation in the tumor suppressor activity of p53, this site is mutated in human cancer, in some cases resulting in the conservative substitution of an arginine. While rare, the existence of somatic K120R mutations in human tumors supports the hypothesis that acetylation at this site is important for tumor suppression. Mechanistically, the ability of p53 to induce apoptosis correlates with induction of a set of apoptosis-related target genes, and blocking K120 acetylation inhibits both the induction of this set of p53 targets and the induction of apoptosis. Recent advances in our understanding of the enzymes regulating K120 acetylation, have allowed us to manipulate levels of this p53 isoform and thereby modulate the apoptotic response. In addition, current efforts to understand the mechanism by which K120 acetylation dictates the selective induction of specific classes of p53 target genes, will be outlined.

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SPEAKER ABSTRACT

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Co-Authors: Hu W, Chan CS, Wu R, Zhang C, Sun Y, Song J, Tang L, Levine AJ

The Negative Regulation of Tumor Suppressor p53 by microRNA miR-504

Tumor suppressor p53 plays a central role in tumor prevention. p53 protein levels and activity are under a tight and complex regulation in cells to maintain the proper function of p53. microRNAs (miRNAs) are a class of endogenously expressed, small (20-25 nt) noncoding regulatory RNA molecules in cells. Recent studies have demonstrated that miRNAs play important roles in regulating various biological processes through regulation of gene expression at the post-transcriptional level. It is estimated that over 30% of all genes and the majority of genetic pathways are subject to regulation by multiple miRNAs. This raises a possibility that some specific miRNAs could regulate p53 and its pathway. To test this possibility, we screened for miRNAs which could potentially regulate p53 expression by performing an in silico search for putative binding sites of miRNAs in the 3'-UTR of human p53. Through this approach, we identified miRNA miR-504 as a novel negative regulator of p53 in cells. miR-504 represses the expression of p53 at both protein and mRNA levels through its direct binding to two sites in p53 3'-UTR. Overexpression of miR-504 decreases p53 protein levels and functions in cells, including p53 transcriptional activity, p53-mediated apoptosis and cell cycle arrest in response to stress. Furthermore, stable overexpression of miR-504 promotes tumorigenecity of cancer cells in vivo. These results demonstrate that the direct negative regulation of p53 by miR-504 is a novel mechanism for cells to regulate p53 protein levels and functions. Considering the importance of p53 in tumor suppression, our study highlights the importance of miRNAs in tumorigenesis.

SPEAKER ABSTRACT

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p53 Regulates Cell Cycle and miRNAs to Drive Differentiation of Human Embryonic Stem Cells

Disruption of p53 increases the efficiency of reprogramming adult cells to cellular pluripotency, suggesting a critical role for p53 in maintenance of differentiation. Here, we show that p53 actively promotes differentiation of human embryonic stem cells (hESCs) by a dual process of lengthening the G1-phase of cell cycle and activating micro-RNAs (mi-RNAs). During retinoic acid mediated differentiation, p53 is transiently activated by acetylation of Lys373 and dissociation from negative regulators, HDM2 and TRIM24. hESCs spontaneously differentiate when these negative regulators of p53 are depleted or when p53 is ectopically expressed. In contrast, depletion of p53 sustains pluripotency of hESCs even in the presence of retinoic acid. Global profiling of p53-interactions with chromatin shows p53-enrichment at ~11,000 sites, including mi-RNA clusters. Comparison of p53 target genes in hESCs, in response to DNA damage versus differentiation, reveals shared gene targets as well as striking differences between the two. In response to retinoic acid, activated p53 induces CDKN1A to establish a p53-dependent G1-S block in cell cycle and, in parallel, induces specific mi-RNA networks to repress Sox2 and Oct4. These studies define a dual regulatory model of "promotion and prevention" by p53, where p53 promotes differentiation by transient cell cycle arrest and prevents regression to a stem cell state, once induction of p53 ends, by activating a mi-RNA network that inhibits self-renewal of hESCs.

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Co-Authors: Sotillo E, Mellert H, Schelter JM, Cleary MA, McMahon S.

Myc overexpression brings out unexpected p53-dependent anti- apoptotic effects of miR-34a

Down-regulation of microRNA-34a by c-Myc is known to be essential for tumorigenesis and improve tumor cell survival. Conversely, up-regulation of miR-34a by p53 is thought to enhance its acetylation and activity and contribute to the pro-apoptotic effects of this tumor suppressor. We sought to determine whether restoration of miR-34a levels in B-lymphoid cells with c-Myc overexpression would aid therapeutic apoptosis. Unexpectedly, delivery of miR-34a, which doesn't target p53 directly, severely compromised steady-state p53 levels. This effect was preceded and mediated by direct targeting of c-Myc, which sustained p53 protein levels via the Arf-Hdm2 pathway. Consequently, in the presence of c-Myc, miR-34a inhibited p53-dependent bortezomib-induced apoptosis as efficiently as anti-p53 siRNA. Conversely, inhibition of miR-34a using antisense RNA sensitized lymphoma cells to therapeutic apoptosis. Thus, in tumors with deregulated c-Myc expression, miR-34a confers drug resistance and could be considered a therapeutic target.

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p300/CBP: Tuning the p53 apoptotic response through differential acetylation and activation/repression activities

The longstanding dogma regarding the role of the p300/CBP histone acetyltransferase (HAT)/coactivators in p53 regulation has stated that all p53-related transactivation and downstream functions require p300/CBP HAT activity, both toward p53 and toward histones at target promoters. Most data supporting this contention have been obtained through overexpression experiments. Recent data from our lab has suggested this model may be overly simplistic, as p300/CBP also encode an intrinsic E4 polyubiquitin ligase function that promotes p53 polyubiquitination and degradation in the cytoplasm. The physiologic functions of p300/CBP in regulating nuclear p53 functions, such as DNA damage signaling, have yet to be systematically addressed using RNAi technology. Using si/shRNA for p300/CBP we unexpectedly found that only p300 was required for p53-dependent apoptosis after DNA damage. CBP loss paradoxically enhanced apoptosis after DNA damage, in a p53-dependent manner. Surprisingly, neither p300 nor CBP were required for DNA damage-induced growth arrest or p21 induction. Moreover, a comprehensive survey of p53-regulated apoptosis genes revealed that only a subset were regulated by p300/CBP. In some cases, the expression of apoptosis genes increased, rather than decreased, after CBP knockdown, suggesting that CBP might also act as a corepressor of certain apoptosis genes, correlating with the hyper-apoptotic phenotype of CBP-deficient cells. Differential transcription regulation of p53 by p300 and CBP correlated with differential acetylation of p53 in vivo. CBP preferentially acetylated p53 at residues K319-321, which are known to favor growth arrest responses over apoptosis when acetylated, and p300 preferentially acetylated a cluster of C-terminal lysines known to promote apoptotic responses. Our results suggest that a nuanced interplay of activating and repressive activities of p300 and CBP at p53 apoptotic target gene promoters may have evolved to fine tune apoptotic gene expression to the level of applied stress and/or environmental conditions at the time of stress.

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SPEAKER ABSTRACT

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Extracellular adenosine sensing - a novel cell death priming mechanism downstream of p53

Changes in metabolism are a defining feature of tumour cells in order to meet their energetic and anabolic needs. It seems conceivable therefore that cells contain mechanisms to sense these changes and to link them to tumour suppressive pathways such that aberrant cells can be removed to prevent the development of malignant disease. We report here, that p53 can promote cell death by activating a novel metabolic cell death priming mechanism that senses the presence of an extracellular adenosine. Adenosine - the backbone of ATP - is frequently found at unusually high levels in the extracellular milieu of many solid tumours and is considered to arise through the increased energy demand of tumour cells. The significance of its accumulation, however, is currently unknown. We have found that activation of p53 results in the direct transcriptional activation of the adenosine receptor, ADORA2B. Activation of ADORA2B also occurs as a result of treatment with chemotherapeutic drugs and in response to metabolic/hypoxic stress. In all cases the effects are dependent on the presence of p53. Expression of ADORA2B or accumulation adenosine in tumour cells has little effect on cell viability. When combined together, however, a potent apoptotic response results which translates into a marked decrease in the clonogenic potential of the cells. In line with the physiological relevance of this observation, cell death following activation of p53 can be also enhanced by the presence of adenosine and this effect is blocked by RNAi-mediated knockdown of ADORA2B. The identification that p53 can sense the presence of extracellular adenosine not only provides a novel molecular link between the metabolic changes in cancer and tumour suppression, but also identifies a new selective cell death priming mechanism in human cancer cells. This work is supported by the Association for International Cancer Research and Cancer Research UK.

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p53 regulates biosynthesis

One of the major biochemical hallmarks of cancer cells is the high rate of glucose consumption and the preferential use of glycolysis over oxidative phosphorylation for energy production, even in the presence of adequate oxygen. This metabolic switch from previously normal quiescent cells (aerobic glycolysis or the Warburg effect) enables tumor cells to direct glucose to biosynthesis, which supports their rapid growth and proliferation. Tumor cells switch to aerobic glycolysis in a cell autonomous manner due to genetic or epigenetic alterations. However, the link between cancer genes and enhanced glucose consumption and biosynthesis is still not well understood. In particular, as an enhanced glycolytic flux does not necessarily enable biosynthesis, a pressing issue is how tumor cells direct glycolytic intermediates to biosynthesis. The pentose phosphate pathway (PPP) is a major pathway for glucose catabolism. The PPP generates NADPH, the principal intracellular reductant required for biosyntheses of lipids and cholesterol, and ribose-5-phosphate, which is essential for biosynthesis of nucleotides and several essential metabolites. Despite the vital role of the PPP in biosynthesis and its close link to glycolytic flux, the regulation of the PPP in tumor cells remains unclear. Here we report that the tumor suppressor p53, one of the most frequently mutated genes in cancer, inhibits the pentose phosphate pathway (PPP). Via the PPP, p53 suppresses glucose consumption, NADPH production, and the biosynthesis of lipid and nucleotides. Tumor-associated p53 mutations cause a loss of PPP-inhibitory activity. Thus, enhanced PPP glucose flux due to p53 inactivation may increase glucose consumption and direct glucose toward biosynthesis in tumor cells.

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SPEAKER ABSTRACT

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Ribosomal protein S27-like and S27 and the p53-MDM2 axis

Several ribosomal proteins regulate p53 function via modulating MDM2. We recently found that RPS27L, a RPS27 like protein, is a direct p53 inducible target. Here we showed that RPS27 itself is a p53 repressible target. Furthermore, either RPS27L or RPS27 binds to MDM2 via their N-terminal region on the central acidic domain of MDM2. RPS27L or RPS27 forms an in vivo triplex with MDM2-p53 and competes with p53 for MDM2 binding. Like p53, RPS27L, but not RPS27, is a short-lived protein and a novel MDM2 substrate. Degradation of RPS27L requires the RING or acidic domain of MDM2. Ectopic expression of RPS27L or RPS27 inhibits Mdm-2-mediated p53 ubiquitination and increases p53 levels by extending p53 protein half-life, whereas siRNA silencing of RPS27L decreases the p53 levels by shortening p53 half-life with corresponding reduction of p53 transcription activity. RPS27L is mainly localized in the cytoplasm, but upon p53-activating signals, a portion of RPS27L shuttled to the nucleoplasm where it co-localizes with MDM2. Both cytoplasmic and nuclear p53, induced by ribosomal stress, were reduced upon RPS27L silencing. Our study reveals a multi-level interplay among RPS27L/S27 and p53-MDM2 axis with RPS27L acting as a p53 target, an MDM2 substrate, and a p53 regulator.

SPEAKER ABSTRACT

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p53-Interacting Proteins
Tuesday, October 12
8:30 AM - 10:30 AM
Stemmler Hall
Dunlop Auditorium

An essential role for ribosomal protein-Mdm2-p53 pathway in growth control and tumor suppression

Several ribosomal proteins (RPs) have been shown to interact with and inhibit the E3 ligase function of Mdm2, thereby activating p53. The pathophysiological significance of these interactions, however, has not been established in vivo. We generated mice carrying a cysteine-to-phenylalanine (C>F) substitution in the zinc finger of Mdm2 that disrupts its binding to RPL5 and RPL11. Mice harboring this mutation (Mdm2C305F) are born with typical Mendelian inheritance and are phenotypically normal and fertile. However, while retaining normal p53 response to DNA damage the mutant mice lacked p53 response specifically to perturbations in ribosome biogenesis. To examine the role of RP-Mdm2-p53 signaling in tumorigenesis, we crossed the mutant mice with Eμ-myc transgenics, an established mouse model of Burkitt's lymphoma. The Mdm2C305F mutation accelerated Eμ-myc induced lymphomagenesis. The mean survival of Eμ-myc transgenics, 20 weeks for those with wild type Mdm2, was shortened to 9 weeks with Mdm2C305F mutation—a time frame comparable to that of Eμ-myc;p19Arf-/- transgenic mice. In addition, our recent studies show that the RP-Mdm2-p53 signaling pathway is important in coordinating nutrient stress with metabolism. Metabolomic analysis demonstrates that calorie restriction alters a plethora of metabolites in wild type but not the mutant mice. Our findings establish the RP-Mdm2-p53 axis as a bona fide in vivo signaling pathway important in growth control and tumor suppression. Updated results on the study will be presented.

15th International p53 Workshop
October 8 – 12, 2010
University of Pennsylvania School of Medicine

SPEAKER ABSTRACT

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p53-Interacting Proteins
Tuesday, October 12
8:30 AM - 10:30 AM
Stemmler Hall
Dunlop Auditorium

Co-Authors: Qian Cheng, Lihong Chen, Zhenyu Li, Baozong Li, Brittany Cross, and Jiandong Chen

Molecular Oncology Department, Moffitt Cancer Center

MDM2 phosphorylation and DNA damage signaling to p53

The p53 pathway is highly responsive to stress signals such as DNA damage and oncogene activation. Recent work showed that ATM phosphorylation of the MDM2 E3 ligase is a key step in p53 stabilization after DNA damage. ATM phosphorylates MDM2 on multiple sites near the RING domain, specifically inhibiting p53 poly-ubiquitination, thus leading to its stabilization. MDM2 phosphorylation does not inactivate its E3 ligase activity and still allows MDM2 auto-ubiquitination and MDMX ubiquitination. DNA damage does not promote MDM2 auto-degradation or reduce overall MDM2 level as widely reported, but causes phosphorylation-dependent masking of the SMP14 epitope. The selective inhibition of p53 ubiquitination is likely due to disruption of MDM2 oligomerization. Artificially induced oligomerization of MDM2 stimulates p53 ubiquitination and overcomes the inhibitory effect of phosphorylation. Therefore, MDM2 oligomerization provides a scaffold for processive ubiquitin chain synthesis on p53 and is the target of regulation by ATM. In addition to mediating p53 ubiquitination, MDM2 has recently been shown to regulate p53 transcriptional activity by recruiting histone H3 K9 methyltransferases to p53 target promoters. Ongoing study of stress signals that regulate MDM2-methyltransferase interaction will be presented.

SPEAKER ABSTRACT

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p53-Interacting Proteins
Tuesday, October 12
8:30 AM - 10:30 AM
Stemmler Hall
Dunlop Auditorium

Co-Authors: Job de Lange[1], Kristiaan Lenos[1], Anna Phillips[2], Amina Teunisse[1], Anja Wolf[3], Matt Darley[2], Kirsten Lodder[1], Matty Verlaan - de Vries[1], Suzanne Lam[1], Julia Richter, Anja Böhnke[3], Frank Bartel[3], Jeremy P. Blaydes[2]

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Functions and Regulation of Mdmx

It is assumed that all human tumors have an attenuated p53 response pathway. In approximately half of the tumors the p53 gene itself is mutated. In the remaining tumors retaining wild-type p53, upstream regulators or down-stream targets of p53 are usually aberrantly expressed. Two important upstream regulators of p53 are the Mdm2 and Mdmx proteins, expression of which is modulated in a significant percentage of human tumors.

We have been investigating the oncogenic properties of Mdmx. On the one hand we examined the activity of Mdmx in the transformation of human cells (skin fibroblasts, embryonal retinoblasts) in cell culture. We show that constitutive expression of Mdmx can to some extent circumvent the need of p53 mutation during oncogenic transformation.

In addition, we have studied the expression and function of Mdmx in uveal melanoma. Like another eye tumor, retinoblastoma, uveal melanoma never show p53 mutations. We find that a subset of uveal melanoma cell lines have high expression of Mdmx, which is essential for growth and survival of these cells. Interestingly, this growth promoting function of Mdmx is only partly dependent on wild-type p53. Results on investigations on other putative Mdmx targets will be presented.

In collaboration with Blaydes' and Bartels group we have investigated in more detail the p53-dependent transcription of the Mdmx gene. We found that every tested method of p53 activation (DNA damage, RITA, Act.D, Nutlin-3, p14ARF expression) leads to increased binding of p53 to the p53RE in the human Mdmx gene and increased transcription from the P2 promoter. Translation of the Mdmx P2 transcript leads to the synthesis of an N-terminal extended protein (Mdmx-L) with slightly altered properties compared to Mdmx. Preventing the activation of the Mdmx P2 promoter by P2-transcript specific siRNA enhances the p53-response, indicating that in addition to the p53-mediated increase in Mdm2 expression also the p53-responsive Mdmx promoter is important in a feedback loop attenuating the p53 response.

SPEAKER ABSTRACT

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p53-Interacting Proteins
Tuesday, October 12
8:30 AM - 10:30 AM
Stemmler Hall
Dunlop Auditorium

Co-Authors: Fiamma Mantovani [1], Jarno Drost [2], Mathijs Voorhoeve [3], Reuven Agami [2]

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The Bromodomain-containging protein BRD7 is a candidate tumor suppressor required for p53 function.

p53 pathway exerts a central role in tumor suppression as reflected by the presence of p53 mutations in as many as 50% of human tumors. In tumors with wild type p53, genetic lesions in other components of its pathway are often found to interfere with its activities. However in many cases the mechanisms behind the inactivation of this major tumor suppressor pathway are unknown. Recently protein remodeling complexes and non coding RNA (miRNA) have been shown to play a relevant role in regulating the p53 pathway. In search for regulators of p53, we identified BRD7, a bromodomain protein subunit of SWI/SNF chromatin-remodeling complexes, as an essential p53 cofactor in oncogene induced senescence (OIS), one of the major cellular defense mechanism in which p53 tumor suppressor pathway is crucial. BRD7 affects p53 acetylation and its transcriptional regulatory activity on the promoters of a subset of p53 target genes. Furthermore we found that low levels of BRD7 expression is specifically associated with a subgroup of human breast tumors harboring wild-type but not mutant p53, showing that in these tumors deletions of the BRD7 gene locus are frequently responsible for alterations in p53 function.

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SPEAKER ABSTRACT

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p53-Interacting Proteins
Tuesday, October 12
8:30 AM - 10:30 AM
Stemmler Hall
Dunlop Auditorium

Co-Authors: David Meek[1], Lynnette Marcar[1], Nicola MacLaine[2], Ted Hupp[2], Sam Nicol[3], Eric Wright[3], Phil Coates[3] and Frances Fuller-Pace[3]

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Regulation of p53-mediated growth arrest and apoptosis by Mage-A proteins and by the dead box protein, p68

We report on two aspects of p53 biology. In the first, we examine the interaction between p53 and members of the tumour/metastasis-associated Mage-A family first reported by Schneider’s group1. Mage-A proteins form a closely-related family that are encoded as a cluster on the X-chromosome and can act as potent p53 inhibitors. We have mapped the site of interaction of Mage-A2 to the DNA binding surface of the core domain in p53. We show that elimination of Mage-A expression leads to increased recruitment of p53 to responsive promoters and is accompanied by stimulation of p53-mediated gene expression and apoptosis in the absence of stress stimuli.

In the second part we report on conditional knock-out mice that eliminate expression of the p68 DEAD-box RNA helicase, an important transcriptional co-activator of several key transcription factors including p53 2. We show that p68 knock-out compromises the p53 response to DNA damage. In several tissues the induction of cell cycle arrest gene, p21, is abrogated; in contrast there is no inhibition in the induction of pro-apoptotic genes. These data support the idea that p68 plays a critical role in governing the choice between arrest/survival and cell death in vivo.

1. Monte M, Simonatto M, Peche LY, Bublik DR, Gobessi S, Pierotti MA, Rodolfo M, Schneider C. MAGE-A tumor antigens target p53 transactivation function through histone deacetylase recruitment and confer resistance to chemotherapeutic agents. PNAS USA 2006; 103:11160-5.

2. Bates GJ, Nicol SM, Wilson BJ, Jacobs AM, Bourdon JC, Wardrop J, Gregory DJ, Lane DP, Perkins ND, Fuller-Pace FV. The DEAD box protein p68: a novel transcriptional coactivator of the p53 tumour suppressor. EMBO J 2005; 24:543-53.

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SPEAKER ABSTRACT

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Co-Authors: Bonald Figueiredo, Raul Ribeiro & Gerard P. Zambetti

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Novel Target Genes and Modifications
Tuesday, October 12
11:00 AM - 12:30 PM
Stemmler Hall
Dunlop Auditorium

Translational Studies of p53 in Childhood Cancers

Pediatric adrenocortical carcinoma (ACC) is a rare, aggressive and often fatal malignancy with no proven effective therapy. ACC occurs at a remarkably high frequency in southern Brazil and more than 95% of these cases are associated with the germline TP53-R337H mutation. In some families, R337H selectively predisposes carriers to ACC, whereas other families are at an increased risk of diverse tumors that could be classified as LFS-L. Given the high incidence of ACC, its association with the germline R337H mutation and the poor prognosis for those who develop cancer, we conducted a general population screen of newborns (>170,000 individuals over 4.5 years) in the southern state of Parana for the presence of this mutation. Genetic counseling and clinical surveillance for early diagnosis of ACC was provided to the families of newborns who tested positive. The findings from this screen and their implications will be presented. Additional efforts to understand p53 genotype-phenotype relationships and the etiology of childhood ACC will also be discussed.

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SPEAKER ABSTRACT

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Novel Target Genes and Modifications
Tuesday, October 12
11:00 AM - 12:30 PM
Stemmler Hall
Dunlop Auditorium

A feedback loop between p53 and RNPC1, a p53 family target gene

P53 transcription factor is known to regulate a diverse array of target genes, which mediate p53 tumor suppression. To further examine p53 activity, we have identified and characterized several p53 target genes that are involved in cell death and survival, senescence and differentiation. One of the p53 target genes is RNPC1, which encodes a RRM-containing RNA-binding protein and belongs to the HuR/ELAV family. The RNPC1 gene is found to be overexpressed in breast and prostate tumor tissues and overexpression of RNPC1 is correlated with the aggressive phenotype of colon cancer. Here, we found that RNPC1 functions as a mediator of p53 in cell cycle arrest in G1 via p21 cyclin-dependent kinase inhibitor. We also showed that p21 expression is regulated through p21 mRNA stability by RNPC1. Furthermore, we found that RNPC1 cooperates with HuR to regulate p21 expression. Interestingly, our recent studies showed that RNPC1 regulates several other RNA targets and that the p53 family is the prime target of RNPC1. P63 and p73, two other members of the p53 family, are involved in many cellular processes, including growth suppression and differentiation. We found that RNPC1 modulates p53 expression via translation and p63 and p73 expression via mRNA stability. Together, our studies showed that RNPC1 modulates the p53 family expression through posttranscriptional and translation mechanisms and coordinates with HuR to regulate the mRNA stability of p21, a critical mediator of the p53 family. Thus, we conclude that as a target and regulator of the p53 family pathway, the relationship between the p53 family and RNPC1 represents a novel regulatory feedback loop.

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SPEAKER ABSTRACT

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Co-Authors: Mark-Andreas Kluth, Janosch Hildebrand, Gerald G. Schumann, Wolfgang Deppert and Genrich V. Tolstonog

Novel Target Genes and Modifications
Tuesday, October 12
11:00 AM - 12:30 PM
Stemmler Hall
Dunlop Auditorium

LINE1 retrotransposons and the genome: a symbiotic relationship secured by p53

During cellular differentiation transcriptional programs have to be rapidly altered, e.g. by transcriptional activation of differentiation-specific genes. We show that p53-dependent stimulation of bi-directional promoter activity of LINE-1 (L1) retrotransposons contributes to epigenetic regulation of cell differentiation. Non-coding transcription initiated from the L1 anti-sense promoter serves to keep the chromatin region encoding differentiation-specific genes in an open, transcription-competent state. To counteract the deleterious effects caused by L1 sense transcription on genomic integrity, p53 simultaneously stimulates transcription of “antidotes”, e.g. the Apobec3 gene, whose product blocks L1 retrotransposition. Our data reveal a novel biological activity of p53 in balancing a symbiotic relationship between L1 retrotransposons and the host genome.

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SPEAKER ABSTRACT

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Novel Target Genes and Modifications
Tuesday, October 12
11:00 AM - 12:30 PM
Stemmler Hall
Dunlop Auditorium

Novel functions of p53 in nucleus and near the plasma membrane

We have found several novel functions of p53. We previously reported that the clathrin heavy chain (CHC) exists not only in cytosol but also in nuclei and is required for the transactivation of p53-target genes forming a complex with p53. We further found that CHC and p53 bind to NuMA (Nuclear Mitotic Apparatus Protein). It is known that NuMA plays an important role in the regulation of spindle formation at mitosis. However, we found an entirely novel function of NuMA at interphase. It was found that NuMA works for induction of G1-arrest but not apoptosis genes. We have also found unexpectedly that p53 is localized near the plasma membrane and regulates endocytosis as well as cellular motility. In normal cells, if EGF is added, mesh-like structure of actin fiber is formed at the leading edge of cells. However, if p53 is knocked down, this structure is not formed. We observed by electron microscope using gold-labeled antibody against p53 that p53 is likely to be attached to F-actin fiber at the leading edge after addition of EGF. Several proteins to bind to p53 near the plasma membrane have also been identified. These observations suggest that p53 is involved in regulation of invasion and metastasis of cancer cells near the plasma membrane. ALK (Anaplastic Lymphoma Kinase) is a transmembrane tyrosine kinase. It is fused to other proteins in several human cancers including non-small cell lung cancer and lymphoma by chromosome translocations. We found that ALK-fused proteins are localized in nucleus and inactivate p53 by phosphorylation of its Tyr residues. This is the first time to show phosphorylation of p53 at Tyr residues. On the other hand, we showed that PH domain-only protein PHLDA3 is a p53-regulated repressor of Akt.

Poster Session 1
Saturday, October 10, 2010
7:00 – 9:00 p.m.
Houston Hall, Bodek Lounge, 3417 Spruce St.

Poster	Presenter
1	Alexieva-Botcheva, Krassimira, Ph.D.
2	Appella, Ettore, M.D.
3	Bakhanashvili, Mary, Ph.D.
4	Balaburski, Gregor, Ph.D.
5	Balikova, Anna
6	Barsotti, Anthony
7	Bell, Douglas, Ph.D.
8	Beyer, Ulrike
9	Biderman, Lynn
10	Boeckler, Frank, Ph.D.
11	Braun, Frederique, Ph.D.
12	Brazda, Vaclav, Ph.D.
13	Brazdova, Marie, Ph.D.
14	Burmakin, Mikhail, Ph.D.
15	Carvajal, Luis
16	Caulin, Carlos, Ph.D.
17	Cauthen, Lindsey
18	Chee, Jacqueline Lai Yuen, Ph.D.
19	Chicas, Agustin, Ph.D.
20	Dai, Mu-Shui, Ph.D.
21	Goh, Walter
22	Gurova, Katerina, M.D., Ph.D.
23	Hamard, Pierre-Jacques, Ph.D.
24	Happo, Lina
25	Harris, Bryan
26	Haupt, Ygal, Ph.D.
27	Hu, Wenwei, Ph.D.
28	Huang, Jing, Ph.D.
29	Iwakuma, Tomoo, M.D., Ph.D.
30	Jackson, James, Ph.D.
31	Joerger, Andreas, Ph.D.
32	Johannessen, Tor-Christian, M.D.
33	Jung, Yong Sam, Ph.D.
34	Karia, Bijal
35	Karni-Schmidt, Orit, Ph.D
36	King, Sharon, Ph.D.
37	Ko, Aram
38	Kumar, Pooja
39	Leu, Julia I-Ju, Ph.D.
40	Liao, Jun-Ming, Ph.D.
41	Löbel, Claudia
42	Ma, Buyong, Ph.D.
43	Madar, Shalom
44	Marcar, Lynnette, Ph.D.
45	Marcel, Virginie, Ph.D.
46	Missero, Caterina, Ph.D.
47	Nikulenkov, Fedor
48	Noll, Jacqueline
49	Ow, Thomas, M.D.
50	Post, Sean, Ph.D.
51	Poyurovsky, Masha, Ph.D.
52	Qian, Yingjuan, Ph.D.
53	Rao, Kinyali Bhavya
54	Roemer, Klaus, Ph.D.
55	Roxburgh, Patricia, MBChB
56	Sabapathy, Kanaga, Ph.D.
57	Sano, Daisuke, M.D., Ph.D.
58	Saridakis, Vivian, Ph.D.
59	Schoppy, David
60	Shatz, Maria, Ph.D.
61	Shen, Hong, Ph.D.
62	Shi, Yao
63	Singer, Stephan, M.D.
64	Stiewe, Thorsten, Ph.D.
65	Sulg, Marilin
66	Tan, Ee Hong, Ph.D.
67	Toledo, Franck, Ph.D.
68	Tollini, Laura
69	Torabi, Bez, Ph.D.
70	Tovar, Christian
71	Un, Manolya
72	Vigneron, Arnaud, Ph.D.
73	Walsh, Erica
74	Wei, Tay Seok
74	Xian, Wa
75	Yokota, Jun, M.D.
76	Yuan, Zhi-Min, Ph.D.
77	Zhang, Qiang
78	Zhou, Ge, Ph.D
79	Zhou, Muxiang, M.D.

Poster Session 2
Sunday, October 11, 2010, 6:00 – 8:00 p.m.
Penn Museum, Upper Egyptian Gallery, 3260 South St., 3rd Floor

Poster	Presenter
80	Anderson, Carl, Ph.D.
81	Arrowsmith, Cheryl, Ph.D.
82	Aylon, Yael, Ph.D.
83	Bartel, Frank, Ph.D.
84	Barton, Christopher
85	Bergholz, Johann
86	Bersani, Cinzia
87	Bertin, Joséphine
88	Birts, Charles, Ph.D.
89	Blomhoff, Heidi, Ph.D.
90	Brady, Colleen
91	Brosh, Ran, Ph.D.
92	Cheok, Chit Fang, Ph.D.
93	Cox, Miranda
94	Creamer, Bradley, Ph.D.
95	Cross, Brittany
96	El-Masry, Omar
97	Enari, Masato
98	Fei, Peiwen, M.D., Ph.D.
99	Finnberg, Niklas, Ph.D.
100	Freed-Pastor, William,
101	Gaidarenko, Olga
102	Horikawa, Izumi, Ph.D.
103	Inga, Albert, Ph.D.
104	ISSA, Samar, Ph.D.
105	Khoury, Marie
106	Kulikov, Roman, Ph.D.
107	Kulkarni, Rohan, Ph.D.
108	Kung, Che-Pei, Ph.D.
109	Laptenko, Oleg, Ph.D.
110	Leonova, Yekaterina,
111	Li, Dun
112	Li, Qin
113	Li, Xiaolong
114	Li, Yong, Ph.D.
115	Lo, Dorothy
116	Love, Ian
117	Lu, Wuyuan, Ph.D.
118	Melis, Joost
119	Mellert, Hestia
120	Menendez, Daniel, Ph.D.
121	Muller, Patricia, Ph.D.
122	Neilsen, Paul, Ph.D.
123	Pan, Yongping, Ph.D.
124	Pant, Vinod, Ph.D.
125	Patten, Nancy
126	Pishas, Kathleen
127	Priest, Christina
128	Resnick-Silverman, Lois, Ph.D.
129	Rokudai, Susumu, Ph.D.
130	Saleem, Ayesha
131	Sankala, Heidi, Ph.D.
132	Sarig, Rachel, Ph.D.
133	Shangary, Sanjeev, Ph.D.
134	Shen, Jinfeng
135	Sheng, Yi, Ph.D.
136	Shieh, Sheau-Yann, Ph.D.
137	Song, Jaewhan, Ph.D.
138	Spinnler, Clemens
139	Stambolsky, Perry, Ph.D.
140	Su, Xiaohua, Ph.D.
141	Sznarkowska, Alicja
142	Valente, Liz
143	Vaseva, Angelina
144	Vaughan, Catherine
145	Wang, Yunyuan, Ph.D.
146	Wilcken, Rainer
147	Wyendaele, Jessika
148	Xia, Mingxuan
149	Xiao, Jim Zhi-Xiong, Ph.D.
150	Xiao, Ziwei
151	Yu, Xin, Ph.D.
152	Zawacka-Pankau, Joanna, Ph.D.
153	Zhang, Jin, Ph.D
154	Zhang, Qi, Ph.D.
155	Zielonka, Elisabeth

POSTER SESSION I

POSTER 1

Krassimira Alexieva-Botcheva, **Ph.D.**
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Co-Author(s): McCorkle SR, McCombie WR, Dunn JJ, Anderson CW

Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
Houston Hall
Bodek Lounge

Whole Genome Mapping of p53 Binding Sites in Normal Human Fibroblasts Using ChIP-Seq

Comprehensive maps of p53 binding in both normal and cancer cells, before and after induction, integrated with genome-wide DNA methylation and histone modification data, are needed to determine the impacts of p53 binding, core promoter architecture and the epigenetic landscape on the p53 transcriptome. We report here the analysis of a high-resolution genome-wide p53 binding map in normal human fibroblasts after treatment with 5-FU for 6 hr using Solexa / Illumina high-throughput sequencing (ChIP-seq). Accurate interpretation of the ChIP-seq data requires a reference chromatin sample because of the systematic biases present in the data and because the chromatin background is not uniform throughout the genome. We prepared and sequenced a matching Input sample (chromatin isolated prior to immunoprecipitation) and used it for the analysis of our ChIP-seq data. We obtained 7-11 millions sequence reads per library, 85-87% of which mapped uniquely to the human genome. More than 750 ChIP-seq peaks representing genomic loci occupied by p53 with high-confidence were identified. Randomly selected peaks were validated using quantitative PCR (qPCR), and a very good correlation was observed between the ChIP-Seq peak height and qPCR-measured enrichment. We also compared the p53 binding profile observed in normal human fibroblasts (IMR-90) with that reported previously for human colorectal cancer cells (HCT-116) under the similar experimental conditions. In addition to identifying significantly more sites than previous studies, clear evidence for cell type-specific p53 binding in response to treatment with 5-FU was observed. Bioinformatics analysis of the bound loci revealed that p53 occupies distinct types of core promoters in the two cell types. We correlated our experimental data with epigenetic modifications reported by others as an initial step in developing a comprehensive and precise analysis of p53 binding to the human genome in the natural context of chromatin with respect to promoter composition and the epigenetic landscape at the identified loci.

POSTER 2

Ettore Appella, **M.D.**
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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
Houston Hall
Bodek Lounge

Quantitative Proteomics Analyses of the Effects of p53 Post-Translational Modifications on the Response of Mouse Thymocytes to Ionizing Radiation

The critical roles of the tumor suppressor p53 in the induction of cell cycle arrest, apoptosis, DNA repair, and cellular senescence are highly regulated by post-translational modification. Exposure to a variety of stresses causes p53 to become extensively post-translationally modified, resulting in its activation and stabilization, including phosphorylation of its N-terminus and acetylation of its C-terminus. Among these modifications are phosphorylation of mouse Ser18 (human Ser15) and acetylation of Lys317 (human Lys320). To better understand the role of these modifications in the response of p53 to DNA damage, quantitative mass spectrometry was used to determine the global effects of ionizing radiation (IR) on the levels of proteins in thymocytes of wild type mice and p53(S18A) or p53(K317R) knock-in mice. We found that both mutations had widespread effects on specific protein levels following IR, including affecting the levels of proteins involved in apoptosis. Pathway analysis of differently regulated proteins suggested that TGFβ signaling was also decreased in p53(S18A) thymocytes, whereas TNFα signaling was decreased in the p53(K317R) thymocytes. In the canonical pathway of TGFβ signaling, Smad proteins are primarily responsible for the transcriptional effects of signaling, and we propose that phosphorylation of human p53 at Ser15 may affect Smad activity in one of two ways: modulating the phosphorylation status of Ser9, known to be important for p53-Smad binding or by mediating recruitment of transcriptional cofactors known to be important for Smad function. We hypothesize that p53 modulates the activity of TNFα by affecting the nuclear localization of the glucocorticoid receptor and by sequestering needed transcriptional co-factors. The results further suggest that the cross-talk with the TGFβ and TNFα pathways are specific to the N- and C-termini of p53, respectively. These results indicate that post-translational modification of p53 modulates both transcriptional and non-transcriptional functions of p53, influencing signaling along different pathways in the cell.

POSTER 3

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
Houston Hall
Bodek Lounge

Differential expression of exonuclease activity with ARE-RNA substrate in cytoplasm by activated p53 protein

The tumor suppressor p53 protein is expressed at low levels under normal conditions. In response to diverse stress-inducing signals, p53 level increases and this is accompanied with increased activities of p53. The abundance of p53, sub-cellular localization, and the interaction with cofactors play a central role in the regulation of its different biochemical functions. p53 in cytoplasm exerts intrinsic 3' -> 5' exonuclease activity with various RNA and DNA substrates. p53 may act as an external proofreader for errors introduced by exonuclease-deficient DNA polymerases. p53 can remove 3'-terminal nucleotides from RNA substrates containing an adenosine and uridine-rich (ARE) element (localized to the 3' un-translated region of many mRNAs). The sub-cellular localization of p53 and its functions are influenced by various external stimuli. In an effort to understand how different signals induce p53 exonuclease activity, cytoplasmic fractions of human cell lines treated by different drugs or following γ-irradiation were analyzed for expression of exonuclease activity. Here, we show that various signals we examined (induction by doxorubicin, etoposide, γ-irradiation or DL-α-difluoromethyl-ornithine-DFMO) resulted in increased level of p53 in cytoplasm. Interestingly, the exonuclease activity with various ARE-RNA substrates in cytoplasmic extracts of etoposide, doxorubicin or DFMO-treated cells was lower than in controls. Conversely, there was no decrease in exonuclease activity with DNA substrates, indicating that, the observed reduction in exonuclease activity with RNA substrates after drug treatment is not a general phenomenon. Remarkably, the increase in the level of p53 is concomitant with an increase in constitutive excision capacity in IR-exposed cytoplasmic extracts with ARE-RNA and DNA substrates. The results demonstrate the difference in expression of exonuclease activity in cytoplasmic fractions when p53 is stabilized under various stress scenarios. The data suggest that the binding of ARE-RNA by RNA binding protein HuR, and/or post-translational modifications of p53 decrease RNA binding and ssRNA exonuclease activity of p53.

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POSTER 4

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
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Identification of novel small molecule inhibitors of the inducible heat shock protein Hsp70

p53 is a well established tumor suppressor protein that controls genomic homeostasis by inducing cell growth arrest or apoptosis in response to periods of genotoxic stress or oncogene activation. Because the majority of human tumors inactivate the p53 pathway, there is an urgent need for anti-cancer compounds that are selectively cytotoxic to tumor cells, and which function independent of p53 status. Pifithrin mu (phenylethynesulfonamide, or PES) was first identified as a compound that inhibits the trafficking of p53 to mitochondria. We recently identified this compound as one that selectively binds and inhibits the stress-induced chaperone protein Hsp70. Our published data indicate that PES interacts with Hsp70, disrupts the interaction between Hsp70 and its co-chaperones, and inhibits Hsp70 function at the lysosome. We find that PES shows selective cytotoxicity to tumor cells, kills p53-null or mutant tumor cells effectively, and inhibits autophagy, a key survival pathway utilized by tumor cells. In this work, we sought to identify the domain of Hsp70 that interacts with PES, to model the interaction between PES and Hsp70, and to use this information to design and test analogues of PES. Using deletion analysis, we mapped the Hsp70-PES interaction to the C-terminal, substrate-binding domain of this protein. We used homology modeling and drug-docking programs in order to model the Hsp70-PES interaction. We used this information to design compounds predicted to possess superior binding affinity and clinical efficacy. We tested several of these compounds, and report that one possesses superior toxicity in cancer cell lines. Notably, through analysis of these PES analogues, we find that there is a consistent correlation between the ability of PES analogues to inhibit autophagy and their cytotoxicity in tumor cells. The combined data support the premise that inhibition of Hsp70 and autophagy may be a viable and attractive modality for cancer therapy.

POSTER 5

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
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The impact of tumor suppressor p53 on the expression of mucin-like leukocyte marker CD43 in human cancer cells

An increased proliferation is the common feature of cancer cells, which can be achieved due to the simultaneous activation of oncogenes and the inactivation of tumor suppressors. Both leucosialin CD43 and tumorsuppressor p53 are shown to be present at the early stages of different cancer types. We have previously shown that in cancer cells the overexpression of CD43 causes the accumulation of active p53, which is an ARF-dependent process, and induction of apoptosis. When cells are not expressing functional p53 or p14ARF, CD43 induces cells to undergo faster proliferation. Thus, CD43 has similar features of an oncogene and thereby can potentially promote tumorigenesis. We demonstrate now that in human cancer cell lines p53 specifically downregulates the expression of endogenous and exogenous CD43 whereas p14ARF alone is not able to downregulate CD43 expression. Transactivating properties of p53 are necessary to affect the expression of exogenous CD43. Supposedly, p53 activates some other genes that in turn modulate CD43 expression. p53 also represses endogenous CD43 mRNA expression levels. The transrepression possibly involves histone deacetylation of the CD43 gene surrounding areas. The levels of endogenous CD43 are probably regulated both at the level of transcription and post-translational modifications. Hence, we demonstrate for the first time a new feedback-loop between CD43 and p53 pathways, which we believe to have an important role in preventing tumorigenesis.

POSTER 6

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
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p53-mediated regulation of PVT1 and miR-1204

The tumor suppressor p53 inhibits the expression of a substantial number of genes whose protein products serve to promote cell survival or cell cycle progression, thereby ensuring efficient execution of p53-dependent apoptosis and cell-cycle arrest. p53 uses several distinct strategies to directly or indirectly inhibit specific gene expression. Indirect regulation of gene repression by p53 often involves induction of intermediary factors that fall into several categories: proteins (e.g. p21), microRNAs (e.g. miR-34a), and lincRNAs (lincRNA-p21). While p21's ability to mediate gene repression has been well-documented, the functional connection between p53 and microRNAs as well as other regulatory RNAs (e.g. lincRNAs) has brought the potential importance of indirect gene repression into sharp focus. We have identified PVT1 as a p53-inducible target gene. PVT1 is a very large (>300 kb) locus located downstream of c-myc on chromosome 8q24. Intriguingly, within the PVT1 locus is a cluster of microRNAs, miR-1204, miR-1205, miR-1206, miR-1207 and miR-1208. Preliminary results indicate that p53 induces both PVT1 mRNA and several transcripts corresponding to the primary sequence of the microRNAs listed above. The primary transcript of miR-1204, located closest to the 5' end of PVT1 of all the microRNAs, is induced to the greatest extent by p53. Further, chromatin immunoprecipitation (ChIP), electrophoretic mobility shift assay (EMSA) and luciferase-assays have revealed that p53 binds and activates a canonical response element within the vicinity of miR-1204. As such, we hypothesize that miR-1204 is the functional target of p53 within this locus. PVT1 has been linked to oncogenesis; it is translocated in variant Burkitt's lymphoma and frequently displays copy number gains in multiple cancer types. In contrast, a recent report demonstrated an induction of miR-1204 levels during senescence of normal human fibroblasts. We aim to determine the function of miR-1204 and other PVT1-associated transcripts within the context of the p53-pathway.

POSTER 7

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Poster Session 1
Saturday, October 9
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Human SNPs Alter p53 Sequence-Specific Binding at Gene Regulatory Elements: Use of a Microsphere Assay for P53 Binding (MAPD), HapMap Gene Expression and ChIP-Seq to Examine Allelic Effects

p53 coordinates the expression of an intricate network of genes in response to stress signals. Sequence-specific DNA binding is essential for p53-mediated tumor suppression. Objective: We evaluated the impact of single nucleotide polymorphisms (SNPs) in p53 response elements (p53RE) on DNA binding and gene expression in response to DNA damage. Approach: Using a bioinformatics approach based on incorporating p53 binding strength into a position weight matrix, we selected 32 SNPs in putative and validated p53REs. The microsphere assay for protein-DNA binding (MAPD) and allele-specific expression analysis was employed to assess the impact of SNPs on p53-DNA binding and gene expression, respectively. We monitored p53 binding in nuclear extracts from doxorubicin- or IR-treated human lymphoblastoid and U2OS osteosarcoma cells. Results: Significant increases in p53 binding were observed for most REs tested. p53 bound several candidate elements at levels comparable to the positive control. Alleles that were predicted to lower p53 binding indeed reduced binding in 25 of the 32 sequences. We used p53-Chromatin Immunoprecipitation-Sequencing (ChIP-Seq) of DOXO treated HapMap lymphoblastoid cell lines to confirm p53 binding to eight of these polymorphic p53 elements and demonstrated that the candidate SNPs occurred within the p53 binding sites. In addition, five polymorphisms were associated with altered gene expression following doxorubicin treatment of HapMap cell lines. Significance: Our findings demonstrate an effective strategy to identify and evaluate SNPs that may alter p53-mediated stress responses.

POSTER 8

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Poster Session 1
Saturday, October 9
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An endogenous retrovirus drives novel pro-apoptotic p63 isoforms in the male germline of humans and great apes, likely promoting their evolution by increasing genome stability across generations

TAp63, but not its homologue p53, eliminates oocytes that suffered DNA damage. An equivalent gene for guarding the male germ line is currently not known. Here we identify hitherto unknown human p63 transcripts with novel 5'-ends derived from incorporated exons upstream of the currently mapped TP63 gene. These unique p63 transcripts are highly and specifically expressed in testis. Their most upstream region corresponds to a long terminal repeat (LTR) of the human endogenous retrovirus 9 (ERV9). The insertion of this LTR upstream of the TP63 locus occurred only recently in evolution and is unique to humans and great apes (Hominidae). A corresponding p63 protein is the sole p63 species in healthy human testis, and is strongly expressed in spermatogenic precursors but not in mature spermatozoa. Mouse testicular germ cells lack integrated retroviral sequences near Trp63. Consequently, the relative abundance of p63 in murine sperm precursors is lower than in human testis, and this difference is most pronounced in spermatogonia. In response to DNA damage, this human male germ cell-encoded TAp63 protein (designated GTAp63) is activated by caspase cleavage near its carboxyterminal domain and induces apoptosis via transactivation of target genes and mitochondrial association. Of note, human testicular cancer tissues and cell lines largely lost p63 expression. Importantly, however, pharmacological inhibition of histone deacetylases completely restores p63 expression in testicular cancer cells (>3000-fold increase). Our data support a model whereby testis-specific GTAp63 protects the genomic integrity of the male germ line and acts as a tumor suppressor. In Hominidae, this guardian function was greatly enhanced by integration and selection of an endogenous retrovirus upstream of the TP63 locus that occurred 15 million years ago. By providing increased germ line stability, this event may have contributed to the evolution of humans and great apes and enabled their long reproductive periods.

POSTER 9

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Poster Session 1
Saturday, October 9
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MdmX is required for full activation of the Mdm2 gene after cellular stress

The tumour suppressor protein p53 is activated following DNA damage and other cellular stresses, protecting the integrity of the genome through inducing cell cycle arrest or apoptosis. p53 activity is tightly controlled by its main negative regulator the Mdm2 E3 ubiquitin ligase, which inhibits p53's transcriptional activity and targets it for degradation via the proteasome pathway. The Mdm2 homologue MdmX was also identified as an inhibitor of p53 activity. It does so through binding to the p53 trans-activation domain. Although it lacks E3 ligase activity, MdmX affects Mdm2-mediated auto-ubiquitination as well as ubiquitination of p53. We have discovered that MdmX plays a positive role in p53-mediated activation of the Mdm2 gene after DNA damage, ribosomal and replication stresses. Down-regulation of MdmX by siRNA resulted in lower levels of mature and nascent Mdm2 transcripts following cellular stress while having no or minimal effect on the expression of p21 or several other p53 target genes. Additionally, we found that this requirement for MdmX depends on the existence of p53:Mdm2 complexes in the cell. To further explore the mechanism behind the requirement of MdmX for Mdm2 transcription we showed that p53 binding to the Mdm2 promoter is inhibited in the absence of MdmX. In vitro, we discovered that Mdm2 inhibits p53:DNA interaction to a much greater extent than does MdmX. Thus, our data both show that MdmX is not a global inhibitor of p53 transcriptional activity and reveal an additional layer of regulation of Mdm2 transcription in the cell.

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POSTER 10

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Poster Session 1
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Inhibitors from inside – In silico and biophysical screening for ligands of human MDM4 reveal an endogenous substance interacting with the p53 binding site

MDM4 is a protein involved in regulating the tumor suppressor p53 and is found to be overexpressed in many cancer cell lines [1]. The N-terminal transactivation domain of p53 binds to a hydrophobic cleft on MDM4. Three p53 residues (F19, L22 and L26) are most relevant for this interaction [2]. Impeding the MDM4/p53 interaction with small molecule drugs arises as a potential anticancer strategy[3, 4]. We used a combined approach to screen for small molecule inhibitors of MDM4 comprising in silico and biophysical methods. Starting with a virtual database of 3,600,000 ready-to-order molecules we first selected compounds with desirable physical properties and/or structural elements. A total of 87,430 molecules were then docked to the N terminal domain of MDM4 using GOLD and two independent scoring functions (goldscore and chemscore) by means of four crystal structures (pdb codes: 2vyr, 3dab, 3fe7 and 3fea). Docking scores were normalized and docking poses were evaluated using an expert system. About 325 promising compounds were subsequently tested in a fluorescence polarization anisotropy assay. Hits were validated using 1H/15N HSQC NMR experiments and, if possible, isothermal calorimetry (ITC) experiments. The best inhibitors were found to have low micromolar binding constants. Interestingly, the most potent inhibitor of this series is an endogenous substance, naturally occurring in the human body. Whether this finding is coincidental or has any physiological relevance needs to be evaluated.

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POSTER 11

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Poster Session 1
Saturday, October 9
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Survival function of p21 in response to serum-nutrient starvation and to Bcl-2/Bcl-xL inhibition

The cyclin-dependent kinase inhibitor p21 (Cip1) regulates many cellular functions. It is critically involved in cell growth arrest and protects cells from genotoxic and other cellular stresses downstream of p53 (1-7). We show here, using HCT116 cells and their isogenic p53 -/- or p21-/- counterparts, that p21 is also part of a p53-independent survival response to serum-nutrient starvation. Our data demonstrate that p21 exerts a survival function against starvation-induced apoptosis mediated by the pro-apoptotic proteins Puma and Bax, independently of p53. Enhancement of starvation induced cell death by knock down of p21 was also observed in human breast cancer cells MCF-7 cells, that lack functional caspase 3, indicating that the anti-apoptotic role of p21 is unrelated to its reported ability to bind top, and inhibit, this effector caspase. Actually, our data demonstrate that p21 inhibits the induction, by starvation, of a Bax/Puma mediated mitochondrial apoptotic pathway. We previously shown that ABT 737, a potent inhibitor of the BH3-binding activity of Bcl-xL induced a Bax mediated apoptosis in p21-/- cells involving the Puma BH3 only protein (Gallenne T, et al, J Cell Biol. 2009), Here, we found that the anti-apoptotic function exerted by p21 in starved cells could be bypassed by silencing of Bcl-xL or by the inhibition of its BH3-binding activity even in cells that contained a p53 mutation. We hypothesized that the survival function of p21 might deal with the ability of Bcl-xL to interact with Puma and Bax. Taken together, our data indicate that p21 exert oncogenic properties independently from p53, by playing a role in the adaptive response of tumors to a low-nutrient environment that typically characterizes the central region of solid tumors. They may account for the fact that high cytoplasmic levels of p21 are associated with poor prognosis.

POSTER 12

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
Houston Hall
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The cruciform structure formation as determinant for efficient p53 sequence-specific binding

p53 is a multifunctional protein and pleiotropic transcription factor with implications in regulating a wide range of important cellular processes such as growth arrest or apoptosis. Its role as a tumor suppressor is evident by binding to DNA and regulating the transcription of various genes. p53 binding to DNA is strongly dependent on the topology of DNA. DNA topology has a fundamental importance for a great variety of biological processes including DNA transcription, replication, recombination, control of gene expression and genome organization. Here we investigated the presence of inverted repeats in artificial p53-responsive sequences and natural p53-responsive sequences from promoter regions of p21, RGC, mdm2 and gaad45. We also looked into the presence of cruciform structure in these sequences and effects of topology on p53-DNA binding. We demonstrated by gel retardation analyses, ELISA assay and magnetic immunoprecipitation assay that the p53 protein binds preferentially to negatively supercoiled plasmid DNAs with p53-responsive sequence presented in cruciform structure. p53-DNA binding is improved by not only the appearance of cruciform structures but also the opportunity to form non-B structure in p53-responsive sequences. Our data show strong correlation between the change in negative superhelix density and extra enhancement of p53-DNA binding to natural p53-responsive sequences. The best target for p53 protein by these assays are the sequences with inverted repeats which form cruciform structure in topologically constrained DNA.

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Specific binding of p53 proteins to non-B DNA structures and supercoiled DNA in vitro and its influence on transcriptional repression in vivo

Tumor-associated hot spot mutant p53 proteins exert tumor progression gain-of-function activities. In our work we concentrated on investigation contribution of mutp53-DNA recognition on anti-apoptotic gain of function. Mutp53 has lost wtp53 sequence specificity in DNA binding activity, but retained the property to recognize non-B DNA and interact preferentially with repetitive sequences[1]. So far direct DNA binding of mutp53 is assumed to be involved in mutp53-mediated repression of e.g. proapoptotic genes (CD95/Fas/Apo1, MSP/MST1, BAX etc. (2-4)). In our study, we analyzed the interaction of six hot spot mutp53 proteins with DNA containing mutp53 recognition sequences (mutp53BS) and /or non-B DNA structures in vitro and in vivo. The influence of DNA topology on mutp53 driven repression of the MSP/MST1 and BAX genes, as studied by luciferase reporter assays, correlated with the ability of mutp53 proteins (G245S, R248W, R273C, R273H and R282W) to preferentially bind DNA at native negative superhelix density (scDNA, forming non-B DNA structures), .The preference of mutp53 for scDNA was further substantiated by competition experiments with linear or relaxed DNA in vitro and in cells. Also, mutp53 proteins preferred more tightly packed DNA topoisomers with a higher number of negative superhelix turns, as shown here for the first time by two-dimensional electrophoresis. Our results indicate that preferential binding of hot spot mutp53 proteins to scDNA and non-B DNA structures contributes to transcriptional repression. This work was supported by QLGA-CT-2001-52001(EC), MERG-6-CT-2005-014875 (EC), 204/06/P369, P301/10/2370 and 204/08/1560 (Grant Agency of Science of CR) to M.B, Research Centre No. LC06035.

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POSTER 14

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Poster Session 1
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Development of novel therapeutic strategy for neuroblastoma: reactivation of the p53 tumor suppressor function by small molecules

Upon development of metastasis and therapy resistance neuroblastoma (NB) becomes the most deadly tumor of childhood. The tumor suppression function of p53 is crucial for the prevention of tumor development as well as for the response to anticancer therapy. Restoration of the p53 function in tumors is a good therapeutic strategy, because it should trigger massive apoptosis in tumor cells. Overexpression of the oncogene N-myc is a driving force for the development of a large fraction of NB, in part via inactivating p53 by inducing the expression of p53's own destructor HDM2. Therefore, we tested whether rescue of p53 can kill NB cells.

We have previously identified the small molecule RITA that binds to p53 and disrupts p53's interaction with several inhibitory proteins, including HDM2, Parc and iASPP. In current study we explore reactivation of p53 in NB tumor cell lines by RITA.

1μM RITA efficiently kills NB cells, as assessed in nine NB lines with or without MYCN amplification. We studied the expression of p53 and its transcriptional response in NB cell lines and found that RITA induced p53 accumulation and expression of p53 target genes, suggesting that p53 is reactivated by RITA in NB lines. After 48 hours of RITA treatment several p53 target genes were induced, including pro-apoptotic BAX and PUMA as well as cell-cycle arrest p21 gene. Further, RITA is able to inhibit crucial oncogenes in NB cells, such as N-myc, HDMX, WIP-1, MCL-1, BCL-2. The growth suppression by RITA was p53-dependent, since we did not observe any effects of RITA after knockdown of p53 in SHSY5Y cells. RITA does not kill NB cells pre-treated with chemical p53 inhibitor pifithrin-α. These data indicate p53-dependent induction of apoptosis upon RITA treatment. Moreover, RITA showed strong antitumor activity effect, resulting in a more than twofold decrease in the growth rate of SHSY5Y xenografts in mice. Taken together, our results suggest that targeting of p53 by RITA or its analogues might serve as a good strategy to treat NB.

POSTER 15

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Poster Session 1
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The cyclin-dependent kinase inhibitor p21 plays an obligatory role in p53-dependent transcriptional repression independent of effects on Rb family members

The cyclin-dependent kinase inhibitor p21 is a target for transcriptional activation by the tumor suppressor p53. It has a well-characterized role in triggering a G1 cell cycle arrest. However, its definitive role in the G2 checkpoint has been difficult to study since cancer cells lacking a functional p53 pathway retain the ability to arrest in G2 in response to genotoxic stress. Previous studies have led to the notion that an intact p53-p21 network is necessary for maintaining the G2 checkpoint rather than initiating cell cycle arrest. DNA damage induces transcriptional downregulation of a number of genes involved in the G2 to M transition of the cell cycle. Ablation of p21 expression in tumor cells expressing wild-type p53 prevents transcriptional repression by p53 of the genes encoding Cdc25C, Survivin, Cdc20, Cdc2, Cdk2 and Cyclin B1 accompanied by a decreased number of cells in the G2/ phases and an increase in apoptosis. This suggests that p21 plays an important role in the repression of genes involved in the G2/M transition and it is an essential component of the G2 checkpoint maintenance pathway. Indeed, ectopic expression of p21 is sufficient to downregulate Cdc25C, Survivin, Cdc20, Cdc2, Cdk2 and CyclinB1 and to induce a G1 and G2 arrest. Promoter analysis has identified a previously characterized element, the CDE/CHR, as mediating these effects of p21. Ablation of all three Rb family members (pRb, p107, and p130) has no effect on p21-mediated repression. Further, these p21-dependent repression targets do not appear to be cell cycle regulated in their expression. Thus, the role of p21 in transcriptional repression is likely not an indirect consequence of cell cycle effects. Understanding the role of p21 in transcriptional repression of genes involved in the G2 to M transition is an important step in order to assign a definitive role for p21 in the G2 checkpoint.

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POSTER 16

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
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Mutant p53 promotes head and neck cancer progression in response to DNA damage

Head and neck cancer is the sixth leading cancer worldwide with over 500,000 cases diagnosed every year. Advanced head and neck cancers exhibit high frequency of relapse and recurrent tumors are extremely aggressive and refractory to therapies, largely limited to chemotherapy and radiation. The p53 gene is mutated in about half of the head and neck cancers, and potential p53 gain-of-function mutations are associated with poor prognosis and lack of response to chemotherapy. To define the role of p53 gain-of-function mutations during head and neck cancer progression, we generated a mouse model for sporadic head and neck cancer based on the co-activation of the endogenous p53 gain-of-function mutation p53R172H and oncogenic K-rasG12D. Using this model we found that p53R172H, but not loss of p53, cooperates with K-ras during oral tumor initiation, accelerates tumor growth and promotes progression to carcinoma. This oncogenic function of mutant p53 is associated with the induction of cyclin B1 and cyclin A. Analysis of cell lines derived from the mouse oral tumors revealed that upregulation of these cyclins by mutant p53 is promoted by DNA damage and oncogenic K-ras. Additionally, we found that oral tumors expressing p53R172H accumulate double strand breaks, suggesting that mutant p53 can compromise the DNA damage response induced by oncogenic K-ras. Finally, we observed that p53R172H confers resistance to DNA damage induced by the chemotherapeutic drug doxorubicin and blocks Chk1 activation in response to DNA damage. Consequently, oral tumor cells that survive exposure to doxorubicin accumulate chromosomal aberrations and acquire increased tumorigenic potential. These findings indicate that mutant p53 promotes head and neck cancer progression by attenuating the DNA damage response induced by oncogene activation and in response to chemotherapy and suggest that p53 profiling may predict the response of head and neck cancers to chemotherapy, a step toward personalized cancer treatment.

POSTER 17

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Poster Session 1
Saturday, October 9
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Bodek Lounge

A role for p53 in regulation of reprogramming factors during liver regeneration

Pluripotency factors occupy an important role in regenerative medicine and disease development. Previous studies demonstrated p53-dependent regulation of pluripotency factors in cell culture; however, the role of p53 in regulation of pluripotency factors in normal proliferating cells remains elusive. Liver regeneration (LR) following 2/3 partial hepatectomy (PH) in mice is an outstanding model to study proliferation of normal cells. We hypothesized that p53 regulates expression of pluripotency factors Pou5f1 (Oct3/4), Nanog, Klf4, and Myc in quiescent and proliferating liver cells. During LR, Myc expression is significantly upregulated in WT livers at 4h post-PH (2.5 fold) compared to control surgery (Sham). However, in p53-/- mice this upregulation is insignificant (1.5 fold), demonstrating that Myc activation during LR is p53-dependent. Expression of Myc returns to basal (Sham) levels by 48h post-PH in WT mice. Interestingly, in p53-/- livers, expression of Myc at 48h decreases >2 fold compared to Sham, suggesting that p53 is important for maintaining basal levels of Myc expression in normal proliferating hepatocytes. However, there was no change in Myc expression in WT compared to p53-/- livers at T=0, demonstrating that, unlike during proliferation, Myc expression is supported by a p53-independent mechanism in quiescent hepatocytes. Klf4 expression showed a 3-fold upregulation in both WT and p53-/- livers at 4h post-PH, suggesting that Klf4 expression is regulated in a p53-independent manner. Expression of Oct3/4 was detected in WT but not in p53-/- livers, suggesting a p53-dependent activation of basal expression of this gene in hepatocytes. Nanog expression did not change in WT liver at 48 hours PH compared to Sham; however in p53-/- liver, Nanog expression increased 5-fold, demonstrating that p53 represses Nanog during LR. Ongoing studies will determine the mechanism of p53-mediated regulation of pluripotency factors in normal quiescent and proliferating hepatocytes.

POSTER 18

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
Houston Hall
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p53 as a Novel Inhibitor of Caspase-9

p53 is a transcriptional factor known to induce apoptosis via transactivating the expression of pro-apoptotic proteins and by directly activating the mitochondria apoptotic pathway. p53 is also found to be mutated in 50% of human cancers with some of these tumours overexpressing both wild type (WT) and mutant p53. Overexpression of the p53 protein has been implicated with the more aggressive nature of these tumour cells, suggesting a possible gain-of-function of such mutants. In this study, the involvement of p53 in apoptosis via the caspase pathway was investigated. WT-p53 and three mutant p53 with mutations at the 1) N-terminus (D42Y), 2) central domain (R175H) and 3) C-terminus (R337H) were selected for this study. The data collected demonstrated that p53 was able to inhibit the cleavage of caspases early in the apoptotic pathway. In vitro assays showed that addition of recombinant WT and the mutant p53 inhibited the cleavage of caspase-9, -3 and subsequently PARP, while overexpression of these p53 in mammalian cells yielded the same inhibition profile in vivo. Conversely, removal of p53 via siRNA and immunodepletetion showed accelerated caspase-9 activity and cleavage. Immunoprecipitation experiments and recombinant assay systems suggest that the inhibition by p53 is targeted at the active cleaved caspase-9. In addition, the presence of p53 (WT or mutant) in p53-null cells were able to confer higher survival rate. These data therefore demonstrate that p53 may have an additional anti-apoptotic role via the inhibition of caspase-9, which is often masked by its pro-apoptotic functions. This anti-apoptotic role could hence manifest in a cancer background with mutant p53 which has lost its transcriptional activities i.e. the ability to induce apoptosis. As such, this newly discovered role of p53 could potentially explain the aggressive nature of cancers with overexpression of p53.

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POSTER 19

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Poster Session 1
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Dissecting the role of the RB and p53 pathways during cellular senescence

Cellular senescence is potent tumor suppressive mechanism that is associated with the activation of the RB and p53 tumor suppressor pathway. We have used RNAi to dissect the contribution of these pathways to cellular senescence. We show that suppression of RB leads to a delayed cell cycle arrest with an ultimate arrest characterized by the accumulation of cells with 4N DNA content and hyperactivation of p53 and its target p21. To test if the p53 pathway could serve as a second barrier to bypass senescence, we developed a new RNAi technology that allows us to express two or more shRNAs. Using this technology, we show that suppression of RB and the p53 target p21 bypass cellular senescence. This bypass is associated with the accumulation of polyploid cells indicating that the consequence of bypassing cellular senescence is rampant genomic instability, a hallmark of cancer cells. These results reveal how loss of RB and p53 cooperate to bypass senescence.

POSTER 20

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Poster Session 1
Saturday, October 9
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Regulation of p53 stability and activity by ribosomal protein S27a and Otub1

Ribosomal proteins (RPs) play a critical role in coordinating p53-dependent cell cycle checkpoint with ribosomal biogenesis. A number of RPs, including L5, L11, L23, L26, and S7, have been shown to inhibit MDM2 activity, leading to p53 activation, in response to ribosomal stress. Here we show that S27a, one of the two RPs synthesized as a C-terminal extension of ubiquitin (Ub), can also bind to MDM2 and inhibit MDM2-mediated p53 suppression. Knockdown of endogenous S27a significantly attenuated actinomycin D (Act D)-induced p53, suggesting that S27a is a non-redundant MDM2 regulator. We further show that MDM2 ubiquitinates S27a in cells treated with Act D, leading to its proteasomal degradation. Thus, MDM2 forms a mutual regulatory loop with S27a, which may play an important role in cells' dynamic response to nucleolar stress. Interestingly, a Diamond-Blackfan Anemia (DBA)-derived mutant of the Ub-S27a, S57P, failed to activate p53, suggesting that this mutant could impair the normal p53 signaling. Second, we have identified that the ovarian tumor (OTU) domain-containing ubiquitin aldehyde-binding protein 1 (Otubain 1, Otub1 thereafter) is a novel p53 deubiquitinase. Otub1 binds to p53 at its central DNA-binding domain and deubiquitinates p53 in cells and in vitro. Overexpression of Otub1 drastically stabilizes and activates p53, leading to apoptosis and marked inhibition of cell proliferation in a p53-dependent manner. A point mutation, Otub1D88A, in which Asp 88 is mutated to Ala, completely abolished Otub1's activity to deubiquitinate and stabilize p53. Strikingly, overexpression of Otub1D88A or ablation of endogenous Otub1 by siRNA drastically impaired p53 activation induced by diverse stress-inducing agents. Further, Otub1 expression is significantly decreased in tumor tissues vs matched adjacent normal tissues from 12 out of 18 breast cancer patients tested. Altogether, our results reveal a crucial role for Otub1 in regulating p53 stability and activity in response to stress.

POSTER 21

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Poster Session 1
Saturday, October 9
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Novel in vitro profiling of p53-DNA interactions

p53's role as a transcription factor in mediating cell fate in response to various stress stimuli hinges critically on its ability to bind different DNA response elements, and consequently, drive the transcription of downstream effector genes. The ever growing list of target genes, their accompanying DNA response elements, and apparent sequence degeneracy have long mandated detailed analyses of the p53-DNA interactome. We describe here a novel, non-radioactive assay, to measure p53-DNA binding which involves the sequential use of in vitro transcription/translation (IVT), immunoprecipitation and quantitative PCR. The use of IVT precludes the requirement for purified protein, enabling rapid characterization of the binding properties of p53 variants. Uniquely, it offers the opportunity to add compounds during translation that might modulate and activate p53, and also the possibility of examining post-translational modifications due to p53's un-modified state. The method reliably enables the detection of sequence specific DNA binding of full-length p53 at low concentrations of physiologically important Res. Furthermore, we demonstrate multiplexing of 4 different Res in a single binding reaction. When compared to prevailing protein-DNA binding assays, this method exhibits comparable or higher sensitivity, in addition to an expansive dynamic range afforded by the use of real-time PCR. A further extrapolation of its utility is demonstrated when the addition of a peptide known to activate p53 increased its binding to a consensus RE, consistent with published data.

POSTER 22

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Poster Session 1
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Targeting transcriptional regulators for simultaneous modulation of p53 and NF-kappaB in cancer treatment

We used cell phenotype-based readout to screen for chemicals capable of activation of dormant p53 in tumor cells. As a result, we isolated two classes of compounds that simultaneously inhibit NF-kappaB through previously unknown mechanism and activate p53. These compounds appeared to have strong anticancer activity in all tested human tumor xenografts grown in mice and were named Curaxins. The mechanisms of activity of Curaxins involves targeting of “facilitate chromatin transcription” (FACT) complex, which is required for transcription from NF-kappaB-inducible genes. In the presence of Curaxins, FACT is trapped in chromatin thereby blocking NF-kappaB-mediated gene expression what results in CK2-mediated activation of p53 through its phosphorylation at a single C-terminal serine residue. Besides NF-kappaB, Curaxins suppress transcription driven by Heat Shock Factor-1 (HSF1), thereby blocking activation of cell response to proteotoxic stresses (heat shock response). Constitutive activation of NF-kappaB and HSF1 is frequently observed in tumors making them “addicted” to these pro-survival pathways. Extensive testing of Curaxins did not reveal DNA damaging activity and activation of conventional DNA damage signaling pathways. However Curaxins bind DNA and cause changes in chromatin structure in cells. The signal to p53 activation from FACT in Curaxin treated cells suggest existence of p53 pathway sensitive to chromatin structural alterations what may be important for p53-dependent control of epigenetic stability. In summary, Curaxins are triple-targeted non-genotoxic anticancer drug candidates acting through the inhibition of SSRP1 function via non-genotoxic DNA binding. The Curaxin story indicates principle advantages of phenotype-driven “non-targeted” drug discovery strategy, which enables finding new anticancer targets and approaches that could not be predicted based on the preexisting knowledge.

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POSTER 23

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
Houston Hall
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The C-terminal basic region of p53 influences its target gene selectivity

Although an important body of data has been generated over the years from studying the C-terminal basic region, its functional significance remains elusive. The activity of ectopically expressed wild-type p53 was compared to that of a truncated mutant lacking the terminal 24 amino acids (Δ24) as well as mutants in which five lysines were replaced by arginine (5KR) or glutamine (5KQ). The Δ24 mutant is transcriptionally defective on all target genes tested and this correlates with diminished DNA binding, as assessed by chromatin immunoprecipitation. It has been proposed that a lysine to glutamine mutation mimics acetylated lysine and several reports have suggested that acetylation within the C-terminus of p53 activates the protein. Surprisingly, the 5KQ mutant is less active than the wild-type protein on a subset of target genes, whereas the 5KR mutant is as active as wild-type. Intriguingly, the defect in the 5KQ mutant is seen on a subset of targets, namely p21 and TIGAR, but is not observed with either Mdm2, PUMA, or Sestrin 2. Additional mutants were generated: lysine to alanine (5KA), lysine to leucine (5KL) and lysine to glutamic acid (5KE). These mutants behaved similarly to the 5KQ mutant. Thus it is likely that the observed effects are due to an alteration in charge being a key determinant for p53 activity and target gene selectivity. The basis for this affecting one set of target genes and not another remains unclear. It does, however, raise the intriguing possibility that combinatorial modification of the C-terminus may contribute to determining p53-dependent cell fate choices. To further analyze the role of the p53 C-terminus, a mutant mouse has been generated that conditionally expresses a truncated form of p53 lacking the last 24 amino acids. This genetically modified mouse and embryo fibroblasts derived from it are currently under investigation.

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POSTER 24

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Poster Session 1
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Maximal killing of lymphoma cells by DNA-damage inducing therapy requires not only the p53 targets Puma and Noxa, but also Bim

Defects in apoptosis can impair response of tumor cells to cancer therapy. The apoptotic response to a diverse range of stress requires the Bcl-2-regulated pathway, regulated by the pro- and anti-apoptotic members of the Bcl-2 family. The pro-survival members inhibit apoptosis by keeping Bax and Bak in check, thereby preventing mitochondrial outer membrane permeabilization and activation of the caspase cascade. The pro-apoptotic BH3-only Bcl-2 family members are essential for apoptosis initiation in a death stimulus- and cell type-specific manner. Commonly used chemotherapy drugs, such as cyclophosphamide (CTX) and etoposide, invoke DNA damage and hence, p53-mediated apoptosis. BH3-only proteins, Puma and Noxa are direct transcriptional targets of p53, and mediate p53-mediated apoptosis in non-transformed cells. The mechanisms for killing of malignant cells by DNA-damaging drugs are however, still poorly defined. Loss of Puma, but not loss of Noxa protected Eμ-myc lymphoma cells from etoposide-induced killing in vitro and CTX-induced killing in vivo, however, loss of Puma afforded less protection than did p53 deficiency. Interestingly, combined deficiency of both Puma and Noxa resulted in no greater resistance than lack of Puma alone. A third BH3-only protein, Bim, was also up-regulated in Eμ-myc lymphomas incurring DNA damage (although not known to be a direct target of p53) and retroviral knock-down of Bim levels markedly increased the drug resistance of Eμ-myc/noxa-/-puma-/- lymphomas. Remarkably, Myc-driven lymphoma cell lines derived from noxa-/-puma-/-bim-/- fetal liver cells were as resistant as those lacking p53, whilst combined loss of Puma and Bim resulted only in mid-resistance to etoposide-induced lymphoma cell death. Together, these results show that Puma, Noxa and, unexpectedly, Bim are all required for optimal apoptotic responses of Myc-driven lymphomas to two DNA-damaging chemotherapeutic agents commonly used in the clinic, suggesting that levels of these proteins may be used as bio-markers for therapeutic outcome.

POSTER 25

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Poster Session 1
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p53 dependent immune cell infiltration for clearance of apoptotic cells requires Thrombospondin-1

The importance of immune cells in tumor progression is marked by complexity, displaying both pro and anti-tumor roles. One critical function for these cells during tumorigenesis is phagocytosis of apoptotic cells, mediated by neutrophils and macrophages. Tumors treated with radiation undergo DNA damage, activating the tumor suppressor p53 and triggering tumor cell apoptosis. Previous studies show that neutrophils and macrophages migrate towards and phagocytose apoptotic cells in tumors. However, the signals produced by apoptotic cells that mediate chemotaxis are not well understood. We examined the role of the p53 target thrombospondin-1 (TSP-1) during immune cell infiltration, utilizing mice with targeted deletions of Tsp-1 and p53. Our studies found that TSP-1 is transactivated by p53 only after sustained DNA damage in thymocytes. While the cell cycle arrest gene p21 is upregulated immediately after p53 activation, TSP-1 expression is detected 2 days after treating thymocytes with doxorubicin. Surprisingly, ChIP analysis demonstrated p53 binding to the TSP-1 promoter 6 hours after DNA damage; however, TSP-1 mRNA and protein upregulation were not detectable until 2 days. These studies implicate a gradient of p53 target activation with differential activation during sustained DNA damage, and that TSP-1 transactivation by p53 may require additional co-factors. In the absence of TSP-1, we find defective neutrophil and macrophage infiltration into the thymus and significant increases in the number of apoptotic cells. The lack of apoptotic clearance may lead to important consequences for the host by enhancing inflammation, favoring tumor growth. Our results indicate that p53 regulated TSP-1 is critical in the migration of neutrophils to genotoxically stressed tissue. This novel role TSP-1 plays in regulating immune cell infiltration to the tumor microenvironment may play a significant role in tumor cell clearance during treatment and response to therapy. Targeting both p53 and TSP-1 may allow us to increase treatment efficacy.

POSTER 26

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Poster Session 1
Saturday, October 9
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Houston Hall
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Regulation of the p53-PML tumour suppression circuit

The p53 and the promeylocytic leukemia (PML) tumour suppressors network play important role in the cellular response to stress. PML is essential for the formation of PML nuclear bodies (NBs), which have been implicated in the regulation of growth inhibition, senescence and apoptosis. P53 and PML form a positive regulatory loop. PML protects p53 from Mdm2 and this is achieved by facilitating the phosphorylation of p53 on N-terminal sites. More recently, we found that PML also regulates mutant p53. PML interacts with mutant p53 and enhances its transcriptional activity. Unexpectedly, PML is required for some of the gain of function activities of mutant p53 in cancer cells. Down-regulation of PML expression inhibits the growth of mutant p53 expressing cancer cells. To define the interplay between PML and mutant p53 we extended out study to the mouse models and screened human cancer patients for a correlation between PML levels and the status of p53. We will present these results and discuss their implication to human cancer. While much has been revealed about the regulatory role of PML, the regulation of PML and the factors governing this regulation are incompletely understood. We have recently found that the mammalian E3 ligase E6AP (HPV E6 Associated Protein) promotes PML for degradation in the ubqiquitin proteasome system. E6AP mediates the ubiquitination of PML in vivo and in vitro. Importantly, E6AP also regulates the number and intensity of PML-NBs, and the susceptibility of cells to genotoxic stress. The role of E6AP in cancer development has been examined in the Eμ-Myc mouse models for lymphomagenesis. Further the correlation between E6AP and PML has been examined in human prostate cancer. These results will be presented and discussed.

POSTER 27

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Poster Session 1
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The regulation of human maternal reproduction by p53 and its family members

p53 and its family members play important roles in maternal reproduction in mice. p53 regulates implantation through transcriptional regulation of leukemia inhibitory factor (LIF). p63 and p73 play crucial roles in maintaining genomic stability of female germ cells and ovary function. To determine whether these observations could be extended to humans, a list of single nucleotide polymorphisms (SNPs) in p53 family members and the p53 pathway was employed to study their impact on human fertility. The p53 allele encoding proline at codon 72 (P72) was found to be significantly enriched in in vitro fertilization (IVF) patients, and serves as a risk factor for implantation failure. Uterine LIF levels are significantly lower in human p53 knock-in (Hupki) mice carrying the P72 allele than carrying the arginine (R72) allele, especially at the implantation stage, which may contribute to the decreased implantation and fertility associated with the P72 allele. Selected alleles in SNPs in LIF, Mdm2, Mdm4, and Hausp genes, each of which regulates p53 levels in cells, are also enriched in IVF patients. Selected alleles of SNPs in p63 and p73 genes are found to be enriched in IVF patients. Interestingly, the at-risk alleles of SNPs in the p53 pathway for human fertility mainly act on young patients, who are more likely to have impaired implantation; and the at-risk alleles in the p73 gene act on older patients, who are more likely to have decreased quality of oocytes and ovary function. The at-risk allele in the p63 gene is enriched in both older and young patients. Taken together, these findings strongly suggest that the p53 family members regulate the efficiency of human reproduction through different mechanisms. These results also provide a plausible explanation for the evolutionary positive selection of some alleles in p53 family members and the p53 pathway.

POSTER 28

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Poster Session 1
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Genome-wide approaches to decipher the gene network of p53

The gene network of p53 is critical for its tumor suppressive function. To decipher the gene network of p53, we utilized genome-wide approaches, such as chromatin-immunoprecipitation-based microarray (ChIP-chip) and gene expression microarray. The integration of these two platforms allowed us to successfully discover a novel anti-differentiation function of p53 through regulating the Wnt signaling pathway in mouse embryonic stem (ES) cells (ref1). However, the regulation of the Wnt signaling pathway by p53 is ES-specific. Therefore, the tumor suppressive function of p53 could not be explained by this p53-Wnt axis. Based on these previous studies, we further identified p53 target genes in somatic cells. Combining the datasets of p53 target genes in both ES and somatic cells, we generated a human-mouse conserved p53 gene signature. By interrogating about 100 tumor datasets of gene expression microarray, we found that some of the p53 target genes are also direct targets of oncogene, such as cMyc. These results suggest that the gene-network of p53 could be invaded by oncogenes in cancers. These "corrupted" p53 target genes could serve as therapeutic targets in cancer treatment.

1. Lee KH, et al. A genomewide study identifies the Wnt signaling pathway as a major target of p53 in murine embryonic stem cells. PNAS, 2010, Vol 107

POSTER 29

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Poster Session 1
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Crucial role of Mdm2 Binding Protein in mitotic progression and chromosome segregation

MDM2 Binding Protein (MTBP) was originally isolated as a protein that interacts with MDM2. Although MTBP has been implicated in cell proliferation, the underlying mechanisms and its relationship with the p53 pathway remain unclear. We previously generated MTBP knockout mice and demonstrated the p53-independent role of MTBP in embryo development and tumor progression. Here, we have found the crucial role of MTBP in mitotic progression. Analyses of MTBP expression during cell cycle progression revealed that MTBP protein levels were rapidly degraded during mitosis, specifically after late prometaphase. MTBP overexpression delayed mitotic progression at prometaphase to metaphase regardless of p53 status and caused chromosome segregation errors such as lagging chromosomes, chromosome bridge, and multipolar chromosome segregation during anaphase. On the other hand, MTBP down-modulation resulted in abbreviated metaphase, insufficient mitotic arrest, and chromosome segregation errors. Consequently, MTBP down-modulation caused a decrease in cell proliferation with cell death, senescence, and aneuploidy, similarly to Mad2 down-modulation. Intriguingly, MTBP down-modulation inhibited accumulation of Mad1 and Mad2, but not BubR1, at the kinetochores upon nocodazole treatment, suggesting the possibility that MTBP participates in the Mad1-Mad2-mediated mitotic checkpoint. Taken together, this study, indicating that an appropriate MTBP level is crucial for proper mitotic progression and faithful chromosome segregation, provides new insights into the regulation of mitotic checkpoint. Additionally, our findings may provide mechanistic insight to previously reported phenotypes associated with altered MTBP expression such as cell proliferation, tumor progression, p53-independency, and embryonic lethality of Mtbp-null mice.

POSTER 30

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Poster Session 1
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p53 mediated senescence impairs the apoptotic response to chemotherapy and clinical outcome in breast cancer

How p53 mediates response to chemotherapy induced DNA damage in breast cancer is not well understood with few mechanistic studies addressing the conflicting reports in the literature. We have examined the role of p53 in the biochemical, cellular and clinical response of MMTV-Wnt1 breast tumors to doxorubicin. We found that treatment of p53 wild type breast tumors, or heterozygous tumors that retained the wild type allele, resulted in an initial mean tumor regression of only 35%, followed by stable disease (~1 to 2 weeks) and relapse by 11 days. Surprisingly, we found that breast tumors with mutant p53 responded better to treatment, with regression of 70% and 24 days to relapse. Investigating the mechanism of these responses, we found that p53 wild type tumors harvested 24 hours after final treatment had elevated levels of p53 targets when compared to untreated tumors, were negative for proliferation marker Ki67, and did not induce apoptosis. p53 mutant tumors did not induce p53 targets after treatment or enter proliferative arrest, but did undergo apoptosis, consistent with tumor regression data. In examining the partial response, we found p53 wild type tumors harvested during the stable arrest phase (7-14 days) after treatment had elevated markers of senescence, including p21, Dec1, Dcr2, p15, p16 and PML, compared to relapsed or resistant tumors. Many senescence associated cytokines were also elevated, and exhibited autocrine/paracrine activity in the tumor. Lastly, we found that many of the same senescence-associated genes elevated in our mouse model were also elevated in neoadjuvant treated human tumors with wild type TP53. These data show that p53 mediated senescence is the primary outcome in treated breast tumors, resulting in an incomplete response, stable disease and eventual early relapse, while treated p53 mutant tumors undergo cell death and regression.

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POSTER 31

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Poster Session 1
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Structural evolution of p53, p63, and p73: implication for heterotetramer formation

Oligomerization of members of the p53 family of transcription factors (p53, p63, and p73) is essential for their distinct functions in cell-cycle control and development. To elucidate the molecular basis for tetramer formation of the various family members, we solved the crystal structure of the human p73 tetramerization domain (residues 351-399). Similarly to the canonical p53 tetramer, p73 forms a tetramer with D^2 symmetry that can be described as a dimer of dimers. The most striking difference between the p53 and p73 tetramerization domain is the presence of an additional C-terminal helix in p73. This helix, which is conserved in p63, is essential for stabilizing the overall architecture of the tetramer, as evidenced by the different oligomeric structures observed for a shortened variant lacking this helix. The helices act as clamps, wrapping around the neighboring dimer and holding it in place. In addition, we showed by mass spectrometry that the tetramerization domains of p63 and p73, but not p53, fully exchange, with different mixed tetramers present at equilibrium, albeit at a relatively slow rate. Taken together, these data provide intriguing insights into the divergent evolution of the oligomerization domain within the p53 family, from the ancestral p63/p73-like protein toward smaller, less promiscuous monomeric building blocks in human p53, allowing functional separation of the p53 pathway from that of its family members[1].

[1]Joerger AC et al. (2009) Proc. Natl. Acad. Sci. U S A 106:17705-10.

POSTER 32

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Poster Session 1
Saturday, October 9
7:00 PM - 9:00 PM
Houston Hall
Bodek Lounge

Application of a novel p53 reporter assay in human glioblastoma cells

Even though 50% of all human tumors retain expression of wild type (wt) p53 (encoded by TP53), functional abrogation of the p53 pathway can yet occur by overexpression of the MDM2 oncoprotein. In this work, we employed a novel lentiviral-based p53 reporter assay to assess p53 activation in response to temozolomide and the MDM2 antagonist nutlin-3 in glioblastoma cells with distinct MDM2 expression levels. We have developed an assay system where green fluorescent protein (GFP) is placed under the regulation of a p53 binding sequence so that changes in p53 activity can be directly observed over time in living cells. Our results show that MDM2 amplification significantly impairs temozolomide from inducing the p53 pathway in glioblastoma cells whereas nutlin-3 was generally more efficient in activating wt-p53 compared to temozolomide. In addition, the combination of temozolomide and nutlin-3 resulted in increased levels of p53 induction compared to temozolomide or nutlin-3 alone. In agreement with our findings using the p53 reporter assay, immunoblotting demonstrated increased levels of p21, MDM2, p53 and PUMA following treatment with nutlin-3 or temozolomide plus nutlin-3 in glioblastoma cells harbouring MDM2 amplification. Cell viability assays showed that nutlin-3 displayed increased cytotoxicity in temozolomide resistant cells with MDM2 amplification, indicating that the p53 pathway remained intact and could still be exploitable for therapy even in these chemoresistant cells. Combination therapy with temozolomide and nutlin-3 resulted in increased cytotoxicity compared to either drug alone. Taken together, our data demonstrate that increased MDM2 levels prevent temozolomide from inducing wt-p53 in glioblastoma cells in vitro, and that this may contribute to chemoresistance. However, the presented results indicate that the p53 pathway is still exploitable for therapy using MDM2 antagonists such as nutlin-3 since combination therapy with temozolomide and nutlin-3 further enhances cellular cytotoxicity.

POSTER 33

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Saturday, October 9
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Houston Hall
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The RING finger protein Pirh2 promotes the proteasome-dependent degradation of p73

The transcription factor p73, the structural and functional homologue of p53, induces cell cycle arrest and apoptosis in response to genotoxic stress. The cellular activity of p73 is controlled by not only the ubiquitin/proteasome-dependent protein degradation but also through the ubiquitin-independent mechanisms. Here we found that p73 is physically as well as functionally associated with the RING finger E3 ubiquitin ligase Pirh2. Pirh2 binds specifically to TAp73α triggering its proteasome-dependent degradation. Importantly, Pirh2 promotes the poly-ubiquitination of TAp73α both in vivo and in vitro. Ectopic expression of Pirh2 decreased the half-life of TAp73α in association with a significant inhibition of the TAp73α-mediated proapoptotic activity. Moreover, siRNA-mediated depletion of Pirh2 stabilizes TAp73α and cocurrently induces cell death in a p53-independent manner. Taken together, our present findings imply that Pirh2 might promote the proteasomal turnover of p73 in an ubiquitin-dependent manner, and also suggest that Pirh2 might play an important role in the regulation of p73-mediated apoptosis.

POSTER 34

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P53 Suppression of Homologous Recombination and Tumorigenesis

DNA is the genetic material which encodes the genes of an organism. Therefore, maintaining genomic stability is critical for organismal fitness, however, damage to one's DNA is a normal occurrence. Consequently, the absence of tumor suppressor gene activity, such as p53, results in increased genomic instability and increased cancer predisposition. This may either be due to a lack of damage recognition or lack of repair. Homologous recombination (HR), as measured by the in vivo pun assay, is a DNA repair mechanism that our laboratory uses to measure genomic instability. Previously, we have shown that in the absence of p53 the normal frequency of spontaneous HR is significantly elevated. However, the mechanism by which p53 suppresses HR is unclear. To understand the functionality by which p53 modulates HR frequency in vivo, we extended our previous studies to determine the capability of two p53 mutant mouse models to suppress HR. The p53 R172P mutant mice retains limited transcriptional functionality (regulating cell cycle genes but not apoptotic genes) while the p53 R172H mutant mice lack any transcriptional activity but retain some protein: protein interaction capability. Using the pun assay, we observed significantly increased HR frequency in the p53 R172H mutant versus the p53 R172P mutant mice. This suggests that p53 regulation of cell cycle genes but not apoptotic genes may be responsible for its ability to suppress HR frequency. It has been previously reported that R172H mutant mice come down with early aggressive tumors compared to the R172P mutant mice. This correlates with the increased HR frequency we observed in the R172H mutant mice implicating p53 suppression of genomic instability as a major mechanism for p53 tumor suppression.

POSTER 35

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Distinct Expression Profile of p63 During Urothelial Development and Bladder Cancer Progression

In contrast to the TP53 tumor suppressor gene, the related TP63 gene is rarely mutated in cancer. Due to alternative promoter usage and alternative splicing, TP63 is expressed as at least six different isoforms with different N-(TA and ΔN) and C-(alpha, beta and gama) termini. The extent to which all of these isoforms are expressed at the protein level in development or in normal and cancerous tissues is not well characterized. In the present study, we used a set of well defined antibodies to examine different p63 protein isoforms in the mouse and human bladder during embryogenesis and tumor progression, respectively. Notably, we report for the first time that p63-null mice develop the bladder but the urothelium is composed of a single cell layer that exclusively comprises umbrella cells. Here we also report that wild-type mice express distinct p63 isoforms in the urothelium during development, and provide evidence that the first variant to be expressed at a detectable level is the TAp63 isoform. However, ΔNp63 is only detected in the urothelium of newborn and adult mice. In contrast, we found that normal human bladder urothelium displays undetectable ΔNp63 protein levels. Regarding bladder carcinomas, we report that a significant proportion of papillary superficial and invasive tumors express ΔNp63, while others lesions completely lose the expression of all p63 variants, a phenotype significantly associated with tumor progression. Importantly, we found striking clinical implications when comparing the expression of p63 isoforms in bladder cancer patients, which emphasizes the importance to extensively analyze p63 specific variants. Finally, we found that after treatment with various types of genotoxic stress, ΔNp63 is downregulated at the protein level in bladder cancer cell lines. Our study suggests that there may be a significant role for the expression levels of p63 isoforms during urothelial development and bladder tumor progression.

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POSTER 36

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p53 dependent repression of PLK1 in breast cancer

We investigated the repression of PLK1 in a number of cell lines. PLK1 is an important regulator of the cell cycle as reflected by its expression. PLK1 is expressed as low levels at G1, increasing into S phase and becoming most elevated in G2/M where it acts as a checkpoint to allow G2/M transition. In addition, p53 is required for maintenance of the G2/M checkpoint and without p53 cells are driven into mitosis. Previous research has shown that as p53 levels increase, PLK1 decreases in expression, however the mechanism for this remains unclear. We have examined the effects of DNA damage in cells and confirm that PLK1 expression is repressed following p53 up regulation. In addition successful knockdown of p21 by siRNA shows that PLK1 depletion is independent of p21. Furthermore we find that p53 is recruited to the PLK1 promoter under conditions of etoposide induced stress and when linked to a luciferase reporter gene, PLK1 promoter activity is repressed. These findings suggest that at the G2/M checkpoint, PLK1 is suppressed in a p53 dependent manner. Over expression of PLK1 is often observed in tumour cells and consequently may offer therapeutic interest. As a result we have optimised an antibody against PLK1 for use in immunohistochemistry studies. This will allow us to investigate further any significance between plk1 and p53 status in hundreds of breast tumours as well as gaining a better understanding of the distribution of PLK1 in normal and tumour tissue.

POSTER 37

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Hdm2 negatively regulates telomerase activity by functioning as an E3 ligase of hTERT

In this study, we identify post-translational regulation of hTERT by the E3 ligase Hdm2. The telomerase activity generated by exogenous hTERT in U2OS cells was reduced upon adriamycin treatment. The overexpressed levels of hTERT were also decreased under the same conditions. These processes were reversed by treatment with a proteasome inhibitor or depletion of Hdm2. Furthermore, intrinsic telomerase activity was increased in HCT116 cells with ablation of Hdm2. Immunoprecipitation analyses showed that hTERT and Hdm2 bound to each other in multiple domains. Ubiquitination analyses demonstrated that Hdm2 could polyubiquitinate hTERT principally at the N-terminus, which was further degraded in a proteasome-dependent way. A hTERT mutant with all five lysine residues at the N-terminus of hTERT mutated to arginine became resistant to Hdm2-mediated ubiquitination and degradation. In U2OS cells, depletion of Hdm2 or addition of the Hdm2-resistant hTERT mutant strengthened the cellular protective effects against apoptosis. Similar results were obtained with the Hdm2-stable H1299 cell line. These observations indicate that Hdm2 is an E3 ligase of hTERT.

POSTER 38

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p63-Expressing Stem Cells in Repair of H1N1 Infected Lung

The adult lung is a complex organ made up of multiple epithelial cell types. The tracheobronchial epithelium mainly consists of Clara ,ciliated and goblet cells while the alveoli in the deep lung include Type 1 and Type2 pneumocytes. The search for stem cells that give rise to the multiple cell types in the lung is critical for understanding the development and repair of lung tissue following infection and environmental damage and is an area of intense investigation. For the deep lung, bronchioaveolar stem cells (BASCs) have been implicated as a common progenitor of Clara cells and type 2 pneumocytes, and have been identified by four markers (Sca-1+, CD45-, Pecam-, CD34+). Our studies have focused on p63-expressing basal cells found in association with bronchial, tracheal, and nasal epithelium that express none of the markers reported for BASCs. Following H1N1 influenza virus-induced lung damage, we have observed a significant proliferation of these p63 cells and their clustering in interbronchial clusters in regions of massive lung damage, and are tracking these cells via lineage tracing to determine if they contribute to alveolar repair. We have also cloned p63-expressing cells from both normal and infected lungs and have observed a significant rise in clonogenic cells from the infected lungs. We are presently analyzing the transcriptome of these cloned cells as well as assessing their differentiation potential in vitro to determine if they are stem cells for airway repair in the infected lungs. The characterization of these p63 expressing stem cells will lay a foundation for understanding adult lung stem cells and facilitate their therapeutic applications.

POSTER 39

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Codon 72 Variants of p53 and Metabolic Stress

The human p53 tumor suppressor protein has a well-documented role in tumor development and progression. It also is a determinant of transformation-related metabolic stress responses. Importantly, the existence of naturally occurring p53 variants in the human population reinforces the need to determine if, and how, different p53 forms may impact altered metabolic responses associated with diet-induced pathophysiology. To address this question, we have utilized a mouse model for the codon 72 polymorphism of p53. This knock-in mouse model contains a humanized version of p53 (Humanized p53 knock-in, or Hupki), encoding either the P72 or R72 variant. Previous studies have shown that the human codon 72 polymorphic forms of p53 can exhibit functional differences under some conditions. Data from our group, for example, indicate that these naturally occurring variants can differentially affect the p53-mediated cell death response. We have now begun to explore whether the p53 codon 72 polymorphism also influences the hepatic response following exposure to dietary stress. In initial studies, we have found that the R72 and P72 Hupki mice differentially exhibit enhanced multifocal, mixed, aggregated inflammatory cell infiltration in the livers following exposure to a diet-mediated metabolic stress. We also observed differences in the expression of a p53-responsive hepatic survival factor in livers of the R72 and P72 Hupki mice following exposure to a diet challenge. Interestingly, the aberrant expression of this survival factor has been noted in patients with non-alcoholic steatohepatitis. Such findings provide the foundation to utilize this Hupki mouse model to better understand the role of p53, and the codon 72 variants, in regulating the hepatic metabolic response.

POSTER 40

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A potential role for RPL11 in facilitating the recruitment of RISC to target mRNAs

miRNAs are a class of small non-coding regulatory RNAs that have been shown to inhibit gene expression. The functional form of miRNA is the RNA-induced silencing complex (RISC), basically consisting of TRBP2, Ago, Dicer and miRNA. Since discovery of miRNAs, more and more target genes of miRNAs have been identified. However, how exactly miRNAs find their specific targets remains to be an outstanding question. Although all miRNAs rely on their seeding sequences (9-10 bases in length) to complementarily associate with their specific mRNA target sequences, it is hard to fathom how each specific mRNA effectively and efficiently distinguishes a specific mRNA sequence in the "ocean" of mRNAs in a cell. Hence, we hypothesize that there might be the existence of other protein players that could facilitate the identification of a specific target mRNA sequence by a specific miRNA. Our previous study showing that knocking down ribosomal protein L11 (RPL11) increases the c-Myc mRNA level. Hence, we speculate that RPL11 may play a role in regulating c-Myc mRNA via RISC. Here we show that RPL11 can bind to TRBP2 and increase the half-life of TRBP2 by inhibiting the ubiquitination of TRBP2. Furthermore, we show that RPL11 can pull-down Ago and Dicer as well. In flag-RPL11 stable 293 cells, anti-RPL11 antibodies could also pull-down c-Myc mRNA, but not 5S RNAs. In addition, over-expression of RPL11 reduced the level of exogenous c-Myc. These results imply that RP L11 may recruit RISC to c-Myc mRNA, and by doing so, RPL11 may help miRNAs target c-Myc mRNA, consequently leading to degradation of c-Myc mRNA. This function of RPL11 resembles that of an E3 ubiquitin ligase, which assists the E1-E2-ubiquitylation holoeznyme in identifying a specific protein substrate for ubiquitination. We will further investigate this novel function of RPL11 and possibly other RPs in our future study.

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Frequent Detection of MDMX Isoforms in Human Ovarian Carcinomas

The p53 tumor suppressor pathway is inactivated in many human tumors either by p53 mutations or through overexpression of negative regulators, such as MDM2 and MDMX. In analogy to MDM2, besides the full-length mRNA several transcript variants, alternatively, as well as, aberrantly spliced transcripts of MDMX have been identified in different tumor cell lines. Here we screened 34 snap frozen ovarian tumor samples, using a high-temperature First-Strand cDNA synthesis kit to avoid reverse transcriptase artefacts. We identified in addition to MDMX-S numerous new MDMX transcripts in 16 of the 34 tumor samples that were either spliced at genuine exon/intron boundaries or at cryptic splice sites within exons. Furthermore, we could detect the transcript variants MDMX-A and MDMX-XALT2 firstly in ovarian cancer tissue. The MDMX splice variants can be classified into two groups: some isoforms have only retained the p53 binding domain, such as MDMX-S; in contrast, other splice forms consist of an intact C-terminus including the RING-finger domain, such as MDMX-XALT2. We have found that expression of a splice variant containing the complete RING-finger domain (designated as MDMX-Var4) in p53-wt cells results in the stabilization of p53 and subsequently in increased p21-expression. After treatment of these cells with cisplatin, we observed a significant decrease of p21-levels, suggesting a switch from cell cycle regulation to apoptosis. In contrast, a splice variant that consists of the complete p53-binding domain (designated as MDMX-Var6) does not have an impact on p21 expression. However, after addition of cisplatin we observed a stronger induction of p53 compared with full-length MDMX but a significantly lower induction of p21 expression. Noteworthy, both splice variants sensitize p53-wt MCF-7 cells to cisplatin treatment. In ongoing studies, we are further investigating the role of frequently detected MDMX splice forms regarding their impact on chemoresistance in ovarian cancer.

POSTER 42

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p53 Somatic Mutations Correlate with Gain and Loss of Amino Acids in Human SNPs

Background: Cancer is an evolutionary process with cancer cells gaining growth advantage in tissues. TP53 has the highest prevalence of cancer mutations. p53 gain-of-function mutations are similar to driver mutations in cancer genes, with both promoting tumorigenesis. It was suggested that conservation of p53 correlates with positive selection of p53 mutational hotspots. Most previous studies focused on residues lost by mutations, providing information related to a dominantly-negative effect. However, to understand gain-of-function mutations, it is also important to investigate what are the distributions of residues gained by mutations.

Methods: We compile available p53/p63/p73 protein sequences and construct a non-redundant dataset. We analyze the amino acid and dipeptide composition of p53/p63/p73 proteins across evolution and compare them with the gain-loss of amino acids and dipeptides in human p53 following cancer-related somatic mutations. Results: We find that the ratios of amino acids gained via somatic mutations during evolution to those lost through p53 cancer mutations correlate with the ratios found in single nucleotide polymorphisms in the human proteome. The dipeptide mutational gain/loss ratios are inversely correlated with those observed over p53 evolution but tend to follow the increasing p63/p73-like dipeptide propensities. We successfully simulated the p53 cancer mutation spectrum using the dipeptide composition across the p53 family accounting for the likelihood of mutations in p53 codons.

Conclusions: We find that the mutation spectrum is dominated not only by p53 evolution but also by reversal of evolution. The p53 gain-of-function relates to the loss of p53 ancestral function and could interfere with p63/p73 transactivation because p53 mutants are more similar to p63/p73 than to wild type p53.

POSTER 43

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ATF3 duality in tumors and stromal fibroblasts and its interaction with p53

ATF3 is known to have a dual role in cancer progression acting either as tumor suppressor or as an oncogene. However, the underlying mechanism by which ATF3 exerts its activity is still obscure. In this study we provide a broad over-view regarding the role of ATF3 in cancer. We show that ATF3 is highly expressed in a large variety of clinical cancer samples both in the tumor cells and in their surrounding stroma. Ectopically expressing ATF3 fibroblasts, exhibited enhanced proliferation, promoted tubulogenesis of endothelial cells and enhanced tumor growth of adjacent tumor cells. Utilizing a genome wide approach we were able to unravel a distinct gene expression program facilitated by ATF3 in fibroblasts. Through Chromatin IP assay we show that ATF3 specifically binds their promoters. Co-injection of stroma and cancer cells into mice revealed that ATF3 represses both tumor suppressors and oncogenes and that its net effect on tumor growth is greatly dependent on the expression levels of its target genes. Finally, we demonstrate a mutually exclusive expression pattern between ATF3 and mutant p53 in cancer samples and cell lines and that wild-type p53 might counteract ATF3 activity in cancer. Taken together, our study suggests that ATF3 is elevated in cancer cells and in their surrounding stroma and modulate specific target genes to either promote or attenuate cancer progression.

POSTER 44

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The Mage-A family of cancer/testis antigens inhibit p53 by blocking its interaction with chromatin

The tumour suppressor p53 has been shown to interact with numerous proteins which in turn modulates the activity of p53. One novel class of proteins that has been recently shown to interact with p53 is the melanoma antigen family A (Mage-A). Mage-A genes were originally identified in melanoma but were subsequently found to be expressed in various tumour types. They are members of the broader family of cancer/testis (CT) antigens that are expressed only in germ cells and cancer, but not in normal tissues. Reactivation of their expression in tumours suggests that they may have a potential role in the progression of tumour. There are 11 Mage-A proteins in homo sapiens which show striking similarity to each other. They are highly immunogenic and, accordingly, are considered to be promising targets of drug therapy. However, little is known about how Mage-A proteins contribute to the process of tumourigenesis. We shall present data showing that Mage-A2 interacts independently with both p53 and MDM2. We have mapped the binding site for Mage-A2 to the DNA binding surface of the core domain of p53. Consistent with the idea that this may sterically block the interaction of p53 with its cognate sites in responsive promoters we have shown that suppresion of Mage-A expression leads to increased promoter occupancy, gene expression and p53-mediated apoptosis in the absence of an additional stress stimulus. We also show that Mage-A2 interacts with the N-terminus and RING finger domain of MDM2. The implications of these findings for p53 regulation and tumour development will be discussed.

POSTER 45

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The Δ133p53 transcript encodes two p53 isoforms: Δ133p53 and Δ160p53

The TP53 gene contains an internal promoter P2, which regulates the expression of Δ133p53a mRNA. This transcript encodes Δ133p53a isoform, which lacks the whole transactivation domain and part of the DNA-binding domain. It has been reported that Δ133p53a inhibits p53-mediated replicative senescence, apoptosis and G1 arrest through modulation of gene expression. Interestingly, Δ133p53a does not inhibit p53-mediated G2 arrest. By siRNA transfection and site-directed mutagenesis, we identified a fourth N-terminal p53 isoform, Δ160p53a. This novel p53 isoform is produced by internal initiation of translation at ATG160 using Δ133p53a transcript. We detected endogenous Δ160p53a protein in three different cell lines: U2OS, T47D and K562. In K562 cells, the TP53 gene presents an insertion at codon 136 leading to a premature stop. Thus, K562 cells do not express p53 or Δ133p53a but retain the ability to express Δ160p53a protein. Two C-terminal splicing variants of Δ160p53 were detected in K562, Δ160p53a and Δ160p53b. In addition, we observed that Δ160p53b protein expression is regulated by hemin treatment, which induces erythrocyte differentiation. Therefore, we described for the first time that the human Δ133p53transcript encodes two proteins by alternative initiation of translation at ATG133 and ATG160.

POSTER 46

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Characterization of a knock-in mouse model carrying a clinically relevant mutation in the p63 gene

AEC syndrome (or Hay-Wells syndrome) is a rare autosomal dominant disorder characterized by cleft palate, ectodermal dysplasia, and severe skin defects. The disorder is caused by missense mutations in the p53 family member, p63. Although p63 null mice have contributed significantly to the elucidation of p63 function of p63 in stratified epithelia, little is known about the pathogenesis of AEC syndrome. To dissect the molecular defects underlying AEC syndrome, we generated a unique knock-in mouse model (p63+/L514F) that carries a clinically relevant point mutation in the SAM (sterile-alpha-motif) domain of the p63 alpha isoform. p63+/L514F mice exhibit severe cleft palate of the secondary palate, ectodermal dysplasia and epidermal defects, but no limb abnormalities, thus faithfully recapitulating the defects observed in AEC syndrome. In mutant embryos the palatal shelves initiate and elevate properly, although there are unable to meet to form the secondary palate due to their hypoplasia. Similarly, p63+/L514F epidermis and hair follicles are hypoplastic in spite of normal cell proliferation, terminal differentiation, and cell survival rate. Interestingly, a reduced number of stem cells accounts for the hypoproliferative phenotype. At the molecular level, only a specific set of target genes is affected in the AEC mouse mutant epidermis, suggesting a selective effect of the SAM domain mutation on p63 function. Taken together these data indicate that molecular defects in AEC syndrome are likely to involve as-yet poorly explored cellular mechanisms downstream of p63, involving a specific subset of p63 target genes.

POSTER 47

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Genome-wide analysis of transcriptional activity of pharmacologically activated p53

p53-mediated induction of apoptosis, growth arrest and senescence in response to oncogene activation and DNA damage serves to eliminate tumor development, therefore it is not surprising that p53 or its pathway is inactivated in the majority of human cancers. Thus, reactivation of p53 by small molecules appears to be a promising strategy to combat cancer. Using a cell-based assay we have screened a chemical library and identified a small molecule RITA (reactivation of p53 and induction of tumor cell apoptosis), which suppresses the growth of tumor cells in a p53-dependent manner. Using microarray analysis we studied changes in gene expression profiles induced by RITA in HCT116 and MCF7 cells. Our results demonstrated that the response of tumor cells to RITA is entirely p53-dependent. At the same time, microarray data showed that around 1500 genes affected by RITA treatment in tumor cells, which exceeds the number of predicted p53 target genes. Thus, our data suggests that a number of genes are affected as a result of secondary effects, i.e., modulation of expression of certain transcriptional factors which in turn affect the expression of their target genes. In order to distinguish between the primary and secondary effects of pharmacologicaly reactivated p53 we performed ChIP-Seq analysis in MCF7 cells upon treatment with RITA, nutlin3a and 5-FU, all known to induce p53. Bioinformatic analysis of Chip-Seq data allowed us to identify more then 11,000 genomic sites ($p < 0.05$) occupied by p53 in vivo. Moreover, we identified around 3,000 p53-bound sites which are common for all three treatments. Among those 1,600 sites are located in the promoters of genes, which potentially represent the set of direct p53 target genes. Further identification and validation of these targets is currently ongoing. Charting the p53 transcriptome will facilitate deeper understanding of p53 biology.

POSTER 48

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ANKRD11 suppresses mutant p53 gain of function by disrupting its interaction with p63 and p73

Mutations in the TP53 gene not only lead to the expression of a protein unable to impart its normal tumour suppressor ability, but also provide the protein with a gain of function (GOF) which is critical for tumour progression. To facilitate the analysis of GOF of mutant p53, we have established a panel of ecdysone-inducible p53 derivatives of the p53 null H1299 cell line. All of the eight common p53 "hotspot" mutants demonstrate expression that is dose responsive to the inducing agent. Previously, we identified ANKRD11 (ankyrin repeat domain 11) as a novel coactivator of wild type p53, and established that ANKRD11 could restore normal transactivation potential to the p53 R273H mutant. We have now utilised our panel of ecdysone-inducible p53 mutant cell lines to extend these findings. Induction of p53 GOF mutant expression in H1299 cells drives a mesenchymal-like phenotype with random, disordered growth of scattered cells at low cell density with an enhanced invasive capacity into matrigel. Co-expression of ANKRD11 in this system suppresses the ability of mutant p53 to drive mesenchymal transformation and invasion, providing evidence that ANKRD11 can suppress the GOF characteristics of mutant p53. One mechanism for the invasive and metastatic GOF of mutant p53 has been shown to result from its newly-acquired interaction with p63. We have shown that an 80aa region of ANKRD11 impedes the formation of both the mutant p53-p63 and mutant p53-p73 complexes. This 80aa region of ANKRD11 was shown to interact with the TET and C-terminal domains of p53, which was the specific region of mutant p53 previously shown to be required to drive invasion. These findings provide a possible mechanism through which ANKRD11 may be able to suppress mutant p53 gain of invasive function and present a potential target for the development of new cancer therapies.

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Disruptive p53 mutations are associated with poor outcome among patients with advanced head and neck squamous cell carcinoma treated with surgery and post-operative radiation

Objective: To correlate p53 mutations with prognosis in patients with advanced head and neck squamous cell carcinoma (HNSCC) treated with surgery and post-operative radiation.

Approach: p53 mutations disruptive to the L2 and L3 DNA-binding domain (disruptive mutations) have been reported to correlate with poor outcome in HNSCC. We have recently found that these mutations were associated with aggressive tumors in an orthotopic mouse model of HNSCC. We also observed that HNSCC cell lines with splice-site and insertion/deletion mutations were more indolent than those with other mutations. The current study aimed to validate these observations in a cohort of patients. The TP53 gene was sequenced from tumors obtained from 86 patients with advanced HNSCC, each treated with surgery and post-operative radiation. Survival and recurrence data were reviewed from patient records. p53 mutation was then correlated with outcome. Patients were categorized into p53 wildtype, disruptive, non-disruptive, and null groups, and patient outcomes between these groups were compared.

Results: Patients with mutant p53 had decreased disease-free survival at five years compared to patients with wild-type p53 (30% vs. 47%, respectively $p = 0.03$), whereas overall survival was not significantly different ($p = 0.12$). Disruptive p53 mutations correlated with decreased overall survival (18% disruptive vs. 53% wildtype, $p= 0.028$) and disease-free survival at five years (18% disruptive vs. 47% wildtype, $p = 0.012$). Furthermore, the group of patients with splice-site and insertion/deletion mutations appeared to have a more favorable outcome.

Conclusion: Our results validate the predictive value of a disruptive and non-disruptive classification system of p53 mutation for patients with advanced HNSCC. We have extended the classification system to consider splice-site and insertion/deletion mutations as markers for better prognosis. As gene sequencing becomes increasingly available in the clinical setting, our work has important implications for p53 mutation as a prognostic biomarker in HNSCC.

POSTER 50

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Poster Session 1
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A high-frequency regulatory polymorphism in the p53 pathway accelerates tumor development

The p53 pathway is disrupted in most human cancers. MDM2, a key negative regulator of p53, is present at high levels in many cancers that retain wild-type p53, suggesting that elevated MDM2 levels are sufficient to impair p53 functions. A single nucleotide polymorphism (SNP) in the human MDM2 promoter increases the affinity of the Sp1 transcription factor, resulting in elevated MDM2 protein levels. Numerous studies have shown a correlation between MDM2SNP309G and an increased cancer risk. To directly address the impact of Mdm2SNP309G on tumorigenesis, we generated mice carrying either the wild-type MDM2SNP309T or the polymorphic MDM2SNP309G allele. Cells from Mdm2SNP309G/G mice exhibit elevated Mdm2 levels, reduced p53 levels, and decreased apoptotic activity. Importantly, some Mdm2SNP309G/G mice succumbed to tumors before one year of age, suggesting that the Mdm2SNP309G allele alone increases tumor risk. Additionally, the Mdm2SNP309G allele potentiates the tumor phenotype in mice inheriting a p53 hot-spot mutation and alters tumor spectrum. These data provide the first direct causal evidence for increased cancer risk in carriers of the Mdm2SNP309G allele.

POSTER 51

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The C-terminus of p53 binds the N-terminal domain of Mdm2

The p53 tumor suppressor interacts with its negative regulator Mdm2 via the former's N-terminal region and core domain. Yet the main region of p53 that contains lysines ubiquitinated by Mdm2 is at the extreme p53 C-terminus and C-terminal ubiquitination and acetylation of p53 are refractory to Mdm2-p53 association. Expanding upon these observations, we report that the C-terminus of p53 interacts directly with the N-terminus of Mdm2. Supporting this conclusion are several lines of evidence. First, the interaction between Mdm2 and p53 is decreased upon deletion, mutation, or acetylation of the p53 C-terminus. Second, Mdm2 decreases the association of full length but not C-terminally deleted p53 with DNA spanning the major p53 response element in the p21 promoter in vitro and in cells. Third, peptides derived from the C-terminus of p53 bind the N-terminus of Mdm2 in native gels, ELISA and fluorescence anisotropy experiments. Interestingly, while Nutlin-3 treatment leads to virtually complete dissociation of full length p53 and Mdm2 in vivo, it is much less efficient in doing so in vitro. This discrepancy is resolved by our observations that p300-acetylated p53 binds inefficiently to Mdm2 in vitro and that Nutlin-3 treatment of cells induces C-terminal modification(s) of p53.

POSTER 52

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ΔNp63α, a novel target of DEC1, mediates DEC1 function in keratinocyte differentiation

p63, a member of the p53 family transcription factors, plays an important role in cell cycle arrest, differentiation, apoptosis, and maintenance of the proliferative capability of epithelial cells. The p63 gene is expressed as TA isoforms, which contain the N-terminal transcriptional activation (TA) domain and conserved among the p53 family, and ΔN isoforms, which lack the TA domain. ΔNp63 is the predominantly isoform and expressed in cells of epithelial origin. Interestingly, ΔNp63 is frequently overexpressed in human tumors and downregulation of ΔNp63 is correlated with keratinocyte differentiation. However, it is still not certain what regulates p63 expression, especially isoform-specific transcriptional regulation of p63. In this study, we found that ΔNp63α is trascriptionally regulated by DEC1, a p53 family target gene which is not regulated by ΔNp63α. Specifically, we found that overexpression of DEC1 upregulates ΔNp63α, whereas knockdown of DEC1 downregulates ΔNp63α in multiple cell lines, including MCF7, MCF10A, and HaCaT cells. In addition, we found that DEC1 binds to, and activates, the P2 promoter of the p63 gene from which ΔNp63 isoforms are produced. Moreover, we found that a proximal Sp1 site in the P2 promoter is essential for DEC1 to activate the P2 promoter. Finally, overexpression of DEC1 inhibited, whereas knockdown of DEC1 promoted, keratinocyte differentiation via modulating ΔNp63α expression. Taken together, it is the first time to show that DEC1 plays a role in keratinocyte differentiation via regulating its target ΔNp63α.

POSTER 53

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Evaluating Actinomycin D and VX-680 combination for p53-based Cyclotherapy strategy.

Actinomycin D (ActD) is an old antineoplastic agent used to treat Wilm's tumour and rhabdomyosarcomas for more than 40 years. ActD inhibits RNA synthesis by binding to GC-rich regions in DNA and inhibiting DNA-dependent RNA polymerase. It is a potent p53 activator and has been used as a positive control for in vitro p53 activation screens for many years. Recently it has been shown that low doses of cytotoxics can be used to protect normal tissues. In this novel approach called Cyclotherapy, nutlin-3 (a potent inhibitor of the p53-mdm2 interaction) has been shown to protect mice from neutropenia induced by the PLK1 inhibitor, BI2536. The p53 based Cyclotherapy approach exploits the key genetic difference between a normal cell (wildtype p53) and a tumour cell (mutant p53). Since nutlin-3 is still to be approved in the clinic and ActD is an approved drug, we sought to compare ActD with nutlin-3 for this novel approach. We decided to use the aurora kinase inhibitor VX-680 as our second drug. VX-680 causes extensive endoreduplication in the absence of cytokinesis resulting in polyploidy and cell death. Normal cycling cells are susceptible to this effect. We show that pre-treating cells with ActD at doses between 2 to 4 nM before VX-680 addition can protect normal cycling cells from nuclear morphology abnormalities induced by VX-680. We also show that cell lines carrying p53 mutations are mildly sensitive to low doses of ActD and less protected by ActD from VX680 treatment. ActD and Nutlin-3 are very different with regards to their mechanisms of action. In spite of these differences, we can conclude from our observations that because it is extremely potent and an approved drug, ActD is a suitable candidate for pre-clinical models for p53 activation-based cyclotherapy.

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POSTER 54

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Poster Session 1
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Novel cellular factors impacting the p53-MDM2-MDMX-network

OBJECTIVE: Identification and characterization of novel pathways talking to p53 and MDM2.

APPROACH: Proteomic, biochemical and cell biological analyses of p53/MDM2-interacting proteins.

RESULTS: Acetylation and ubiquitinylation of lysines function in part as antagonistic posttranslational modifications on many transcription factors. We have previously identified, by Proteomics and Mass-spec, the ubiquitously expressed, nucleolus-nucleoplasm shuttle protein NIR (Novel INHAT Repressor) as an inhibitor of histone acetylation by p300/CBP that is recruited to promoters by p53, TAp63 and TAp73 [1,2,3]. We report here that NIR is also an inhibitor of p53-acetylation by p300/CBP and of p53-S15-phosphorylation, and furthermore, that NIR can directly bind to the N-terminal and central portions of MDM2. NIR-MDM2 association in vivo results in the accumulation of both proteins. Co-expression of NIR, MDM2 and p53 causes the accumulation of all three. We also identified Np9, a 9 kDa nuclear protein encoded by Type I Human Endogenous Retroviruses (HERVs) and expressed exclusively in transformed cells[4], as a further protein that can shuttle between the nucleolus and the nucleoplasm, and that can bind to and regulate MDM2.

CONCLUSION, SIGNIFICANCE: The identification of NIR and Np9 as further p53/MDM2-impacting proteins hosted by the nucleolus again points the importance of this organelle for the regulation of the p53 pathway.

[1]Hublitz, P. et al., Genes & Dev 2005, 19(23): 2912-24.

[2] Heyne, K. et al., Nucl Acids Res 2010, 38(10) : 3159-71.

[3]unpublished results

[4]Armbruester, V. et al., Clin Cancer Res 2002, 8(6) : 1800-7.

POSTER 55

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Poster Session 1
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Pharmacodynamic biomarkers of Mdm2 inhibition

The p53 pathway is dysfunctional in most cancers, so restoration of the pathway may be an effective cancer therapy. Mouse models have demonstrated that reactivation of p53 in established tumours results in tumour shrinkage. p53 responds to multiple stresses to initiate the appropriate p53 response. Traditional cytotoxic agents cause cell death via genotoxic stress resulting in p53 activation, but this strategy has a narrow therapeutic window since there is concurrent genotoxic damage to normal cells. As mechanistic understanding of the p53 pathway improves efforts to reactivate p53 for tumour treatment focus on non-genotoxic methods of p53 stabilisation (i.e. removal of the inhibitory effects of Mdm2 on p53). We have been assessing compounds that inhibit Mdm2 ubiquitin ligase activity, and have evidence that they can function to stabilize p53 by directly interacting with the RING domain of the Mdm2/MdmX heterodimer. Some traditional cytotoxic agents (5-fluorouracil and actinomycin D) can stabilise p53 by non-genotoxic means at low-dose. We are using samples from patients receiving these cytotoxic drugs to develop biomarkers of p53 stabilisation, which may be valuable in future development of Mdm2 inhibitors. We also plan to use these biomarkers to test wither low-dose 5-fluorouracil or actinomycin D may be used as pre-treatment to protect normal cells from the cytotoxic effects of subsequent cell-cycle specific chemotherapy agents. Peripheral blood mononuclear cells (PBMCs) and plucked hair follicles were taken from patients before and after cytotoxic therapy. These samples were fixed and stained with antibodies against p53, p53 targets and markers of proliferation and DNA damage. PBMCs were also quantified by FACS analysis. Both techniques allow comparison of pre and post treatment samples and demonstrate that the p53 stabilising effects of therapy can be measured by these methods. This approach has the potential to accelerate validation of the Mdm2 inhibition strategy in clinical practice.

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Understanding the functional properties of mutant p53 - the R246S knock-in mouse model

Tumor-derived p53 mutants have been thought to facilitate cellular growth and promote carcinogenesis through mechanisms involving both loss of tumor-suppressive functions and gain of oncogenic functions. In addition, in vitro studies have alluded to the dominant-negative property of these mutants in inhibiting the remaining functional allele, though physiological relevance of this property has not been well established. To decipher the mechanistic basis of mutant p53 function, we have generated a knock-in mouse strain which carries the hot-spot R249S equivalent (R246S) p53 mutation, generally found in human hepatocellular carcinomas. Homozygous mutant mice are born alive but succumb to spontaneous tumors, similar to that noted with the absence of p53, indicating loss of wild-type p53 function. Expression profiling analysis of tumors (mutant p53 vs. p53 null) indicated that R246S mutant p53 expression leads to global changes in gene expression, suggesting a gain-of function. Importantly, R246S-dependent genetic changes appear to be different from the profiles of other hot-spot mutants. Biologically, the R246S mutant appears not to accentuate tumorigenesis in a mdm2-/- background, unlike other mutants, highlighting mutant specificity. The consequence of the differential gene expression will be discussed. Analysis of dominant-negative property of this mutant indicated that this mechanism is operative only in some cell types, suggesting that dominant-negative property may be cell-type specific. Moreover, this property is manifested primarily in response to acute p53 activation, and has no impact on long-term tumor formation, and hence, requires consideration during therapeutic response. Detailed analysis of the effects of this mutation on oncogene and irradiation-induced tumorigenecity, and its biochemical functions will be discussed.

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Poster Session 1
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Disruptive and non-functional p53 mutations in head and neck squamous cell carcinoma are associated with aggressive tumor behavior in an orthotopic animal model of oral tongue cancer

Objective: To characterize tumor growth and metastatic potential in 49 head and neck squamous cell carcinoma cell lines and to investigate how different types of p53 mutations correlate with tumor growth and regional metastasis of these lines in an orthotopic murine model of oral tongue cancer.

Approach: The tumorigenicity and lymphatic metastatic potential of forty-nine HNSCC cell lines was determined by orthotopic injection of 5 x104 of HNSCC cells into the oral tongue of nude mice. Tumor growth and mouse survival over a period of sixty days was evaluated. The presence of cervical lymph node metastases was determined grossly at necropsy and on histologic review. P53 mutation status was evaluated with direct sequencing from genomic DNA and p53 protein expression was evaluated with western blot. Cell lines were determined to be either p53 mutant or p53 wild-type. P53 mutations were then classified using two systems: either disruptive and nondisruptive based on significant alteration of the L2 or L3 DNA-binding domain, or non-functional or functional based on transactivating activity. Tumor growth, lymph node metastasis, and survival were then compared in the context of these groups.

Results: While there was no significant difference in survival between the wild-type p53 group and mutant p53 group (P=0.1252), mice injected with cells harboring disruptive and non-functional p53 mutations showed significantly decreased survival (P=0.0004, vs. wild type group, P<0.0001, vs. other mutations group, respectively). Tumors with disruptive p53 mutations also showed an increased incidence of cervical lymphatic metastasis compared with the wild-type and non-disruptive p53 groups of cell lines (P=0.0008, P<0.0001, respectively).

Conclusion: Our observations suggest that there is a correlation between p53 mutation status and biological behavior of tumors grown in an orthotopic nude mouse model of HNSCC. We provide evidence that disruptive and non-functional p53 mutations play key roles in tumor progression and regional metastasis in HNSCC.

POSTER 58

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Further Insight into Substrate Recognition by USP7: Structural and Biochemical Analysis of the HdmX and Hdm2 Interactions with USP7

The deubiquitinating enzyme, herpesvirus associated Ubiquitin Specific Protease 7 (USP7), modulates the levels of p53, Hdm2 and HdmX. The crystal structures of the N-terminal domain of USP7 (USP7-NTD) complexed with p53, Hdm2 and HdmX peptides have revealed that these peptides all bind to the same surface of USP7 demonstrating the complex nature of the interactions between USP7 and its substrates. We have already shown that USP7-NTD binds two closely spaced 4-residue sites in both p53 and Hdm2 and have now extended these studies with HdmX. However, unlike p53 and Hdm2, HdmX interacts with USP7-NTD at two distinct sites rather than two closely spaced sites. We have also identified a novel USP7-NTD interaction site in Hdm2. Structural and biochemical studies including GST pull downs, size exclusion chromatography and fluorescence binding assays have indicated a preference for a P/AxxS motif in peptides that bind USP7 whereby contacts made by serine residues are critical. Analysis of USP7-interacting peptides on filter arrays confirmed the importance of these serine residues and showed that phosphorylation of serines within the binding motif prevents this interaction. The data provide a structural framework for understanding the mechanism of substrate recognition by the N-terminal domain of USP7 and its involvement in the regulation of the p53/Hdm2 pathway.

POSTER 59

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ATR suppression as a therapeutic approach to p53-deficient malignancies

Disruption of p53 signaling is one of the most frequent alterations in sporadic cancers, and loss of this critical tumor suppressor pathway often confers resistance to conventional chemotherapeutics. Much effort has thus focused on developing novel strategies to target p53-deficient cancers, and several studies have suggested that the ATR-CHK1 pathway may be a viable therapeutic target. Interference with ATR-CHK1 signaling sensitizes p53-null cells to chemotherapeutic treatments in culture and lethally disrupts tissue homeostasis when combined with p53 deficiency in adult mice. Although drugs that inhibit CHK1 along with other kinases are in early-stage clinical trials, it remains untested whether selective inhibition of the ATR-CHK1 pathway can effectively target established p53-deficient malignancies while sparing normal tissue function. We have developed a system to conditionally reduce ATR expression to 10% of normal levels in adult mice (ATRflox/seckel Cre-ERT2+) and have investigated the impact of this suppression on both non-malignant proliferative tissues and p53-deficient cancers. Notably, hypomorphic levels of ATR support normal bone marrow and intestinal homeostasis, suggesting that appreciable reduction of this pathway is not generally toxic to adult tissues. In contrast, murine models of acute myeloid leukemia and fibrosarcoma that harbor defects in p53 signaling are strongly selected against following similar conditional reduction of ATR expression in vivo. Progression of these malignancies is limited by induced ATR suppression even after the development of significant tumor burden, indicating that ATR pathway inhibition is capable of controlling tumorigenesis well after the initial stages of transformation. In aggregate, these results genetically define a level of ATR-Chk1 pathway inhibition in which p53-deficient malignancies can be suppressed with minimal impact on normal tissue homeostasis.

POSTER 60

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The p53 tumor suppressor is a regulator of innate immunity genes

The p53 tumor suppressor can function as a sequence-specific transcription factor to regulate genes associated with a wide range of cellular functions among which are apoptosis, cell cycle arrest, senescence, DNA repair, differentiation and glycolysis. In this study we have extended this list to include the Toll-like receptor (TLR) set of innate immunity genes, suggesting that p53 can modulate the human immune response by p53. The TLR family comprises membrane glycoproteins that recognize pathogen-associated molecular patterns (PAMPS) and mediate innate immune responses. Our studies with several human cell lines establish that doxorubicin, 5-Fluorouracil, UV and ionizing radiation can elicit changes in TLR expression that are cancer cell line and damage specific. Importantly, the effect of DNA stressors on TLR expression was at least partially mediated by p53. Consistent with this, we found that increased p53 resulting from DNA damage or nutlin treatment enhanced TLR pathway mediated production of cytokines. Interestingly, synthetic TLR agonists are being used as adjuvants in specific cancer treatments. Overall, our findings suggest that regulation of innate immunity in humans is responsive to DNA damage and metabolic stresses, which has implications for both cancer induction and treatment.

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Persistent p21 expression after Nutlin-3a removal is associated with senescence-like arrest in 4N cells

Nutlin-3a is a pre-clinical drug that stabilizes p53 by blocking the interaction between p53 and MDM2. In our previous study Nutlin-3a promoted a tetraploid G1 arrest in two p53 wild-type cell lines (HCT116 and U2OS) and both cell lines underwent endoreduplication after Nutlin-3a removal. Endoreduplication gave rise to stable tetraploid clones resistant to therapy-induced apoptosis. Prior knowledge of whether cells are susceptible to Nutlin induced endoreduplication and therapy resistance could help direct Nutlin-3a-based therapies. In the current study, Nutlin-3a promoted a tetraploid G1 arrest in multiple p53 wild-type cell lines. However, some cell lines underwent endoreduplication to relatively high extents after Nutlin-3a removal while other cell lines did not. The resistance to endoreduplication observed in some cell lines was associated with a prolonged 4N arrest after Nutlin-3a removal. Knockdown of either p53 or p21 immediately after Nutlin-3a removal could drive endoreduplication in otherwise resistant 4N cells. Finally, 4N arrested cells retained persistent p21 expression, expressed senescence-associated beta-galactosidase, displayed an enlarged, flattened phenotype, and underwent a proliferation-block that lasted at least 2 weeks after Nutlin-3a removal. These findings demonstrate transient Nutlin-3a treatment can promote an apparently permanent proliferative block in 4N cells of certain cell lines associated with persistent p21 expression and resistance to endoreduplication.

POSTER 62

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p53-dependent DNA Damage Response Contributes to the Apoptosis Induced by RITA

We have previously identified the small molecule RITA which binds p53 and prevents MDM2-mediated degradation of p53. RITA induces p53-dependent apoptosis in a variety of tumor cell lines. Here we demonstrated that in wild-type p53 carrying HCT116, MCF7 and U2OS cell lines, DNA damage signaling was significantly induced by 1μM RITA, not upon 0.1μM RITA treatment. We also showed that the induction of DNA damage signaling by RITA was p53-dependent, as it was not induced in p53-null cell lines, HCT116 TP53-/-, Saos-2 and H1299. Interestingly, there was no DNA damage signaling induction in normal nontransformed cell lines 184A1 and MCF10A either, which correlates with the absence of growth inhibition by RITA. Notably, we found that the induction of DNA damage by RITA was independent of apoptosis. P21 has been shown to play a pivotal role in cell cycle arrest and DNA repair. And, Wip1 is a phosphatase, which could de-phosphorylate ATM, Chk1, Chk2, p53 and γ-H2AX. Wip1 has been demonstrated to be involved in the DNA damage response. We elucidated that p21 and Wip1 were two key players in DNA damage signaling induced by RITA. The decline of protein levels of p21 and Wip1 correlated with DNA damage induction by RITA. Using shRNA to knock down p21 and Wip1, we observed that inhibition of these two genes synergized with 0.1μM RITA to induce DNA damage signaling. We also demonstrated that 0.1μM RITA induced DNA damage signaling to the same extent as 1μM RITA in HCT116 p21-/- cell line, which further strengthened our hypothesis that p21 might contribute to DNA damage signaling by RITA. Furthermore, we observed that apoptosis was induced in HCT116 p21-/- after long-term 0.1μM RITA treatment while no apoptosis was induced by this concentration of RITA in parental HCT116 cell line.

POSTER 63

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Nup98 regulates select p53 target gene expression on a post-transcriptional level

We previously reported that the nuclear transport factor CAS binds to a subset of p53 target genes and is required for their full activation (Tanaka et al., Cell 2007). Preliminary data also suggested that the nucleoporin Nup98 may be involved in directing p53 target gene preference. Following siRNA mediated depletion of Nup98 in camptothecin treated HepG2 cells (wild-type p53) we observed reduced p21mRNA and protein accumulation but little or no effect on several other p53 target genes including Puma. As Nup98 has been reported to be involved in transcription as well as mRNA export, we sought to determine at which level p21 expression is regulated by Nup98. We first used H1299 cells (p53 null) engineered to inducibly express p21 cDNA in a p53 independent manner and found that Nup98 knock-down again reduced p21 mRNA and protein induction. We then discovered that in HepG2 cells, Nup98 knockdown affects accumulation of mature but not nascent p21 mRNA. Thus, Nup98 regulation of p21 mRNA occurs by a post-transcriptional mechanism such as mRNA stability or export. Consistent with these observations, RNA-IP experiments revealed that Nup98 interacts with the 3' UTR of p21 mRNA but not Puma mRNA. Using an in silico approach to screen for motif similarities in the 3' UTRs of other mRNAs of the p53 transcriptome, we identified 14-3-3' as another p53 target mRNA regulated by and co-immunoprecipitated with Nup98. Our data showing Nup98 regulates a subset of p53 target genes by a post-transcriptional mechanism may have relevance to human cancer. Specifically, Nup98 mRNA expression levels in patients with hepatocellular carcinoma, when compared to non-tumorous liver tissue, was reduced in 26% (7/27) of the cases examined. The requirement of Nup98 for full activation of select p53 target genes and its reduced expression in tumor tissue suggests a role as a tumor suppressor.

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DNA binding cooperativity is essential for p53-induced apoptosis

p53 limits the proliferation of precancerous cells by inducing cell-cycle arrest or apoptosis. How the decision between survival and death is made at the level of p53 binding to target promoters remains unclear. Here we demonstrate by global chromatin immunoprecipitation that the cooperative nature of DNA binding serves to extend the genome-wide binding profile of p53 to degenerate response elements in proapoptotic genes. Mutations that reduce cooperativity do not compromise the cell-cycle arrest response but strongly reduce binding of p53 to multiple proapoptotic gene promoters (BAX, PUMA, P53AIP1, NOXA, CASP1) and impair the apoptotic response of tumor cells to chemotherapy. Furthermore, knock-in mice engineered to carry a cooperativity-reducing point mutation in the endogenous p53 gene locus show a selective loss of p53's apoptotic function in vivo. Because mutations, which impair cooperativity, are genetically linked to cancer susceptibility in patients with Li Fraumeni syndrome, we conclude that DNA binding cooperativity contributes to p53's tumor suppressor activity.

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POSTER 65

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DNA damage and p53 response in the inner ear sensory cells

The inner ear sensory cells, the hair cells, are critical for hearing and balance functions. It is known that the maintenance of postmitotic state is critical for their life-long survival. A general concept is that differentiated cells rapidly die following unscheduled cell cycle re-entry. Our aims were: 1) to study the response of cochlear (auditory) and utricular (balance) hair cells to forced cell cycle reactivation and 2) to study the role of p53 and its primary regulators Mdm2 and Mdm4 in hair cell survival. Explant cultures of the inner ear sensory epithelia of postnatal mice were prepared, and hair cells were forced into cell cycle through HPV-16 E7 misexpression. This oncogene induced DNA damage and p53 upregulation in cochlear outer hair cells. These cells were rapidly lost, before entry into mitosis. The death was attenuated by p53 inactivation. In contrast, despite DNA damage and p53 upregulation, utricular hair cells showed long term survival and a proportion of them progressed into mitosis. Consistently, elevation of p53 levels by nutlin-3a, a non-genotoxic compound, caused rapid death of cochlear outer hair cells, while other hair cell populations were death-resistant. Thus, the different sensory cell types of the inner ear show a remarkably different response to p53 induction, despite their common origin and function. We have further studied whether the differential p53 sensitivity depends on Mdm2 and/or Mdm4. Acute Mdm2 or Mdm4 inactivation was induced in explants prepared from Mdm2 and Mdm4 floxed mice using viral-mediated Cre expression. The differential p53 sensitivity was maintained following Mdm2 and Mdm4 disruptions, pointing to the importance of the downstream components in the p53 apoptotic cascade in the regulation of hair cell death. Together, these data have important clinical implications as they show the importance of keeping p53 function in check in sensory cells that are essential in hearing function.

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Poster Session 1
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Co-operation of APC and p53 mutations lead to invasion in a novel colorectal carcinoma mouse model.

Aim: The p53 tumor suppressor is commonly mutated in sporadic colorectal cancer (CRC), but the properties conferred by this mutation remain unclear. p53 mutation occurs relatively late in colorectal carcinogenesis, suggesting a role in tumor progression.

Methodology/Principal Findings: To test this we used cre-lox technology to delete p53 alone, and in the presence of Apc mutation in the murine intestine. Whilst p53 loss alone did not drive intestinal tumorigenesis, a rapid acceleration of tumorigenesis was seen in Apcfl/+ p53fl/fl mice. Furthermore a marked increase in the number of invasive tumors was observed and indeed metastasis was observed. Mechanistically, p53 deficient tumors recapitulated human CRC, with high levels of B-catenin, c-Myc and Wnt targets such as LGR5, Sox9, Fascin at the invasive front. Importantly, these tumors also expressed high levels of ARF at the invasive front, which was absent from p53 proficient tumors. These data suggested that in p53 deficient animals, ARF upregulation could be tolerated and may even confer a survival advantage. To test this, we overexpressed ARF in HCT116 p53 knockout cells. ARF overexpression conferred a selective advantage to the p53 deficient HCT116 cells, increasing both tumor growth and invasion.

Conclusions/Significance: Taken together, these studies suggest that p53 mutation permits deregulation of the Wnt-Myc pathway at the invasive front. This increased signaling drives pro-invasive targets, including Fascin and ERK alongside ARF. These studies suggest that there are two thresholds of Wnt-Myc signaling activation which must be reached in colorectal cancer: the first drives adenoma formation, and the second transition to adenocarcinoma.

POSTER 67

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p53 regulation: Lessons from mouse models expressing only a subset of p53 isoforms

TP53, the gene encoding p53, is mutated in more than half of human cancers, and many other tumors present alterations of proteins interacting with p53. Understanding the regulation of p53 is therefore of major clinical importance. It was recently found that p53 isoforms may participate in the regulation of the p53 full-length protein (FL-p53). In humans, TP53 encodes 9 isoforms, from the use of 2 promoters, 3 translation start sites and 3 alternate splicing events. At least 3 of these isoforms appear to have a significant biological role : FL-p53, Δ133p53 (expressed from an internal promoter in intron 4), and p53β (with a distinct C-terminal domain resulting from alternative splicing). Overexpression of Δ133p53 or p53β in cultured human cells was found to affect the p53 transcriptional response, and both isoforms are misregulated in some cancers. The mouse Trp53 gene also comprises an internal promoter in intron 4 and an alternative splicing (AS) exon encoding a distinct C-terminal domain. To analyze the role of p53 isoforms in vivo, we decided to target at the Trp53 locus the specific deletion of either the internal promoter, or of the alternative splicing exon. We thus generated two mouse models with conditional mutations: one may express all p53 isoforms but those from the internal promoter, and the other may express all p53 isoforms but those with the AS C-terminal domain. A preliminary analysis of these mutants is presented.

POSTER 68

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Function of the Mdm2 Zinc Finger in Tumorigenesis

Previously, in vitro studies have shown inhibition of ribosomal biogenesis to activate p53 via ribosomal protein (RP) - mediated suppression of Mdm2 E3 ligase activity. To study this concept in an in vivo setting, providing information on the physiological significance of the RP-Mdm2 interaction, knockin mice carrying a cysteine-to-phenylalanine substitution in the zinc finger of Mdm2 were generated. The Mdm2C305F mutation results in disruption of Mdm2 binding with RPL5 and RPL11, while retaining the ability of Mdm2 to bind to L23 and p53. Mice carrying the Mdm2C305F mutation were shown to retain normal body weight and physiology as compared to wild type mice, as well as normal p53 response to DNA damage, however, a lack of p53 response to perturbations in ribosome biogenesis was observed. To determine the role of RP-Mdm2-p53 signaling in tumorigenesis, Eμ-Myc; Mdm2C305F compound mutants were generated and observed for morbidity and lymphoma development. As compared to Eμ-Myc; Mdm2+/+ mice, Eμ-Myc; Mdm2C305F/C305F mice demonstrated phenotypically indistinguishable lymphomas and significantly decreased survival, suggesting RP-Mdm2-p53 signaling as an important stress response barrier. To further investigate the potential relationship between the RP-Mdm2-p53 pathway and the p19Arf-Mdm2-p53 pathway, immunopreciptation and western blotting were used to demonstrate that both Eμ-Myc; Mdm2+/+ and Eμ-Myc; Mdm2C305F/C305F mice exhibit elevated p19Arf expression, despite the inability of Mdm2C305F mutant protein to interact with L11, indicating that induction of p19Arf does not require L11-Mdm2 binding. Through utilizing the Mdm2C305F knockin mouse model, in vivo evidence has been provided supporting a p53 checkpoint mediated by RP-Mdm2 interaction to monitor the integrity of ribosome biogenesis and activate p53 accordingly. In addition, this data suggests that the RP-Mdm2-p53 pathway serves as a parallel barrier to the p19Arf-Mdm2-p53 signaling pathway in defense against oncogenic c-Myc-induced tumorigenesis.

POSTER 69

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Sp1 transcriptionally regulates Bid independent of p53 and modulates sensitivity to UV and TRAIL

Lowering levels of transcription factor Sp1 with shRNA significantly reduces apoptosis. We and others have reported that the over-expression of Sp1 can lead to apoptosis. Taken together, these observations suggest that Sp1 may exert these effects by modulating transcription of a downstream target(s). We have identified pro-apoptotic Bcl-2 family member Bid as a transcriptional target of Sp1. When Sp1 is depleted in cells using shRNA, there is a significant reduction of Bid mRNA and protein levels as measured by qRT-PCRand Western blot, respectively. In contrast, over-expression of Sp1 led to up-regulation of Bid RNA and protein levels. Furthermore,Sp1 up-regulated a Bid-promoter-luciferase activity suggesting that Sp1 regulation of Bid is at the transcriptional level. We used chromatin immunoprecipitation analysis to confirm that Sp1 binds to the BID promoter in a region overlapping that previously shown to be bound by p53. We believe that the regulation of Bid by Sp1 is p53 independent, based on finding that Sp1 over-expression increased Bid levels in p53-/- SAOS-2 cells; moreover, the silencing of Sp1 had no effect on p53 levels, but significantly reduced Bid. In addition, there was a correlation between Sp1 and Bid levels but not p53 and Bid levels in multiple cell lines. Knockdown of Sp1 or Bid resulted in significant reduction of apoptosis in response to UV and TRAIL. These findings suggest that Sp1 may modulate apoptosis through regulation of Bid. Many traditional chemotherapies and new therapeutic approaches target apoptosis initiation. The regulation of Bid and apoptosis by Sp1 may provide a therapeutic target for cancer therapy.

POSTER 70

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Poster Session 1
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MDM2 antagonists augment antitumor effect of androgen withdrawal: Implications for therapy of prostate cancer

Hormone therapy is the standard of care for newly diagnosed or recurrent prostate cancers. It uses anti-androgen agents, castration, or both to eliminate cancer promoting effect of testicular androgen. The p53 tumor suppressor controls a major pathway that can block cell proliferation or induce apoptosis in response to diverse forms of oncogenic stress. Activation of the p53 pathway in cancer cells expressing wild-type p53 has been proposed as a novel therapeutic strategy and recently developed MDM2 antagonists, the nutlins, have validated it in preclinical models of cancer. The crosstalk between p53 and androgen receptor (AR) signaling suggest that MDM2 antagonists could augment antitumor outcome of androgen ablation in prostate cancer. Using charcoal-stripped serum as a cellular model of androgen deprivation, we show an increased apoptotic effect of p53 activation by nutlin-3a in the androgen-dependent LNCaP cells and to a lesser extent in androgen-independent but responsive 22Rv1 cell line. This effect is due, at least in part, to an enhanced downregulation of AR expression by activated p53. In vivo, androgen deprivation followed by two weeks of nutlin administration in LNCaP-bearing nude mice led to a greater tumor regression and dramatically increased survival. Since majority of prostate tumors express wild-type p53, its activation by MDM2 antagonists in combination with androgen depletion may offer an efficacious new approach to prostate cancer therapy.

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Patz1/MAZR binds to K382-methylated p53

Known as the "Guardian of the Genome," p53 lies in the center of an intricate signaling network, where it establishes a context-appropriate cell fate in response to genotoxic stress. Recent findings show that p53 function is tightly regulated by site-specific post-translational modifications. The C-terminal lysine residues in the regulatory region of p53 are of special interest, as differential methylation is correlated to the activation or repression of p53 transcriptional activity and of protein stability. This phenomenon renders p53 unique, as one of the few non-histone regulatory targets of lysine methylation. We investigated the biological significance of p53 K382 methylation during T lymphocyte development; where double-stranded DNA breaks (DSBs) naturally occur as a result of somatic VDJ recombination. To this end, we identified co-mediator proteins that are involved in DNA damage induced p53 K382 methylation. Using computer-simulated homology modeling, we predict that isoform 001 of Patz1/MAZR can interact with mono- or di-methylated p53 K382. Patz1 is a BTB-POZ domain zinc finger transcription factor, which is expressed in CD4-CD8- double negative (DN) and CD4+CD8+ double positive (DP) thymocytes, but is progressively downregulated following the generation of a functional TCR. Our computational model predicted that the p53-Patz1interaction is mediated by an isoform 001-specific binding pocket generated by the 6th and the 7th C2H2 zinc finger motifs that is not present in other alternatively spliced Patz1 isoforms. We confirmed this prediction by co-immunoprecipitation studies, where ectopically expressed Patz1-001 interacts with both ectopically expressed and genotoxic stress-induced endogenous p53. Our findings show that Patz1, a transcriptional repressor of the CD8 gene locus, may have a novel role in the p53-regulated steps of T cell development.

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Regulation of the Hippo pathway by ASSP1

ASPP1 is a p53 binding protein that functions as a coactivator of p53, specifically promotoing the activate the expression the apoptotic p53 target genes. However, immunofluorescence experiments and cell fractionation have revealed a cytoplasmic localisation of ASPP1 indicating some other functions for this protein. Our experiments have shown an unexpected role of ASPP1 in the control of the Hippo pathway via the binding and the inhibition of the Lats1 kinases. ASPP1 expression leads to an increase of YAP translocation to the nucleus and transcription of its target genes, resulting in a reduced sensitivity to different stress like dNTP depletion, low serum or anoikis. The regulation of YAP by ASPP1 also lowers the expression of LATS2, another important regulator of YAP and p53, and prevents the induction of p21 by the LATS2/p53 pathway. This activity of YAP reduces the induction of senescence and increase the clonogenic potential of cells treated by different inducers of p53 like DNA damaging treatment or ROS induction. These functions of cytoplasmic ASPP1 confer a potential oncogenic role to this protein, balancing previous results showing a tumour suppressive role for nuclear ASPP1.

POSTER 73

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Inhibition of phosphorylation in the MDM2 central acidic domain activates p53 and Rb

MDM2 is an ubiquitin E3-ligase critical for proteasome-mediated degradation of p53 and retinoblastoma protein (Rb). It has been shown that the MDM2 central acid domain is indispensable for p53 ubiquitination and degradation. Notably, the MDM2 central acid domain is targeted for phosphorylation by several kinases, including GSK3b. We have previously shown that MDM2 binds to retinoblastoma protein (Rb) through the central acidic domain and facilitates Rb protein degradation. In this study, we show that MDM2 phosphorylation is critical for MDM2-Rb interaction and for MDM2 to bind to the C8 subunit of the 20S proteasome. Interestingly, the Rb-binding segment in the MDM2 central acidic domain contains an $S_{256}XXXS_{260}$ motif, which is known to be preferentially targeted by GSK3b. In this study, we show that a S_{256}-A substitution leads to reduced MDM2 activity toward p53 ubiquitination and degradation. In addition, this single point mutation reduces MDM2 binding to Rb and thus results in Rb stabilization. Furthermore, pharmacological inhibition of GSK3b leads to a significant decrease in MDM2-S_{256} phosphorylation, concomitant with reduced MDM2-Rb interaction and Rb protein stabilization. We also show that MDM2-S_{256} is required for MDM2 to inhibit Rb growth suppression function. Together, these data suggest that phosphorylation of MDM2 in the central acidic domain is critical for its function toward both p53 and Rb, and that GSK3b may play an important role in modulating the p53 and Rb pathway.

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POSTER 74

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"p53 Signatures," Stem Cells, and High-Grade Ovarian Cancer

High-grade ovarian cancer is almost always a lethal process for which no treatment or early detection exists. Probably the most exciting finding in the search of potentially treatable, nonmalignant precursors of this disease is that of early, mitotically dormant clusters of secretory cells in the fallopian tube. Significantly, these cell clusters uniformly express a mutant, stabilized version of p53 ("p53 signatures") of the same allele that in some instances has been documented in patient-matched peritoneal serous carcinoma. This association is the link establishing a precursor relationship of the p53 signatures to the serous carcinoma. Very recently, additional secretory cell outgrowths (SCOUTs) have been documented in the tube that lack mutations in p53 yet share with the p53 signature functional alterations in ovarian cancer associated genes. The major goals of our efforts are to uncover the origin(s) of these precursors and dissect the genetic changes that lead to their evolution with the ultimate hope of developing strategies to either eradicate them before they can progress or exploit their biologic differences to identify women at risk. To accomplish these aims, we integrated the approaches of genomics, genetics, pathology, and stem cell biology. We have recently cloned stem cells from the human fimbria and we are currently comparing its expression properties with those of SCOUTs and p53 signatures to access the potential links between stem cells and cancer precursors.

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Association of p53 Gene Polymorphisms With Response To Platinum-Based Doublet Chemotherapy In Non-Small Cell Lung Cancer Patients

In lung cancer, p53 mutations play an important role in the phenotypic conversion from non-invasive tumors to invasive ones. p53 is also a representative genes associated with lung cancer risk. The risk (72Pro) allele for the p53-Arg72Pro single nucleotide polymorphisms (SNPs) encodes a protein with a weaker apoptotic activity, thus, allows survival of cells with DNA-damages than the 72Arg allele. Here we show that p53 SNPs are also involved in chemotherapeutic responses in non-small cell lung cancer (NSCLC) patients. To identify polymorphisms in DNA repair genes that affect responses to platinum-based doublet chemotherapy in NSCLC patients, 640 NSCLC patients, who received platinum-based doublet chemotherapy and whose responses were evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST), were subjected to an association study between the response and genotypes for 30 SNPs in 27 DNA repair genes. Candidate SNPs were selected by a discovery set of 201 cases and their associations were validated in an independent set of 439 cases by pre-specified p-value criteria. Homozygotes for the p53-72Pro allele of the p53-Arg72Pro SNPs showed a better response rate (54.3%) than those for the p53-72Arg allele (29.1%)(P = 4.4x10-5), and p53-72Pro allele homozygotes had significantly longer progression-free and overall survivals than p53-72Arg allele homozygotes (hazard ratios=0.85 [95% confidence interval (CI) = 0.74-0.98], P = 0.020; and 0.86 [95% CI = 0.74-0.99], P = 0.039). These results indicate that polymorphisms in the p53 genes are involved in inter-individual differences in the response to platinum-based doublet chemotherapy in NSCLC patients.

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POSTER 76

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Poster Session 1
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The MDM2/MDMX complex is required for suppression of lethal p53 activity in vivo

Two distinct models are currently proposed to explain why both MDM2 and MDMX are required in p53 control, with a key difference centered on whether these two p53 inhibitors work together or independently. To test these two competing models, we generated knock-in mice expressing a MDMX(C462A) mutant defective in MDM2 binding. This strategy enables a targeted disassociation of MDM2/MDMX complex without affecting both the binding of MDMX to p53 and the intrinsic E3 ligase activity of MDM2, two activities that are thought primarily responsible for p53 inhibition. Significantly, mdmxC462A/C462A homozygous mice died at around day 9.5 of embryonic development and the lethality was completely rescued by concomitant deletion of p53. Our data clearly demonstrates that MDM2 and MDMX function as an integral complex in p53 control, providing new insights into the non-redundant function of MDM2 and MDMX.

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The effect of PRIMA-1MET on mutant p53 aggregation

p53 is a key tumor suppressor which is inactivated by a point mutation in around 50% of human tumors. Most of them are single missense mutations clustered in the p53 DNA binding domain (residues 100-300) PRIMA-1MET / APR-246 is a novel anticancer compound that reactivates mutant p53 through binding to its core domain. Currently, it is tested in a Phase I clinical trail. Since protein aggregation could interfere with biological activity of p53, and mutant p53 proteins are prone to aggregation, we want to investigate whether PRIMA-1MET /APR- 246 can prevent aggregation and/or disrupt aggregates of mutant p53 in cells. Our preliminary experiments using glutathione as a model have shown that treatment with PRIMA-1MET /APR-246 prevents SH-based dimerization of glutathione. Obstructing the formation of intermolecular disulfide bonds may protect proteins from aggregation. Subsequently, we have studied p53 aggregation in BL41-ts p53 cells carrying temperature-sensitive mutant p53. That mutant is expressed as unfolded non-functional mutant p53 at 37°C but it acquires fully functional wt conformation at 32°C. We identified high molecular weight p53 aggregates, by native gel electrophoresis and Western blotting in BL41-ts p53 cells grown at 37°C, and found that these p53 aggregates were disrupted upon temperature shift to 32°C. The ability to disrupt aggregates might be a new way of circumventing inactivation of p53 by a point mutation. It may also open an opportunity to treat diseases which are triggered by a protein aggregation.

POSTER 78

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Poster Session 1
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Gain-of-Function p53 Mutants Inhibit AMPK Signaling in Head and Neck Squamous Cell Carcinoma

p53 mutation is one of most frequent genetic alterations in many cancers, including head & neck squamous cell carcinomas (HNSCC). It is well established that in addition to loss of wild-type p53 function as a tumor suppressor, or function as a dominant negative factor of wild-type p53, certain p53 mutants actually gain oncogenic functions to promote tumor growth and tumor progression. However, the mechanisms involved are still largely unknown. In order to investigate the mechanistic role of gain-of-function (GOF) p53 mutants in HNSCC, we over-expressed p53 mutants in p53-null HNSCC cells, and down-regulated mutant p53 expression in several HNSCC cell lines. In support of the oncogenic role of GOF p53 mutations, our results demonstrated that expression of certain p53 mutants contributes to malignant phenotypes of HNSCC tumor cells in both in vitro 3-D culture and an orthotropic animal HNSCC model. Our study further indicates that although GOF p53 mutants do not affect AKT and MAPK signaling, they negatively modulate AMP-activated kinase (AMPK) signaling under metabolic stress in HNSCC cell lines. In addition, we systematically sequenced p53 in 49 HNSCC cell lines, and compared them with tumorigenic and metastatic potentials of each cell line in an orthotropic animal tumor model. Our results showed that the tumor growth and metastatic potential of different HNSCC cell lines are well correlated with the ability of each GOF p53 mutant to inhibit AMPK signaling. Taken together, our findings have provided an important insight into a metabolic signaling pathway (AMPK signaling) that is affected by GOF p53 mutants in HNSCC cell lines. These results are of great significance as they help us to delineate the mechanism through which certain p53 mutations contribute to tumor progression in HNSCC. We anticipate that these findings will have important implications for personalized therapy for patients with HNSCC.

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Berberine induces p53-dependent apoptosis in cancer cells by degrading MDM2

Berberine, an isoquinoline alkaloid derived from a plant used in Chinese herbal medicine, is reported to exhibit anti-proliferative and pro-apoptotic effects on human cancer; however, its mechanism of action is not clearly defined. Herein, we demonstrate that berberine induces a p53-dependent apoptosis in acute lymphoblastic leukemia (ALL) cells by downregulating the MDM2 oncoprotein. Our investigation revealed that the cytotoxic and apoptotic effects of berberine were closely associated with both the MDM2 expression levels and p53 status of a set of ALL cell lines. The most potent apoptosis was induced by berberine in ALL cells with both MDM2 overexpression and a wild-type (wt) p53, while no pro-apoptotic effect was detected in berberine-treated MDM2-negative ALL cells that had a p53-null phenotype. In contrast to the conventional chemotherapeutic drug doxorubicin, which induces p53 activation and a subsequent upregulation of MDM2, we found that berberine strongly induced persistent downregulation of MDM2 followed by a steady-state activation of p53, which resulted in potent apoptosis of the MDM2-overexpressing ALL cells tested, even if they were doxorubicin-resistant. We discovered that berberine's downregulation of MDM2 in ALL cells occurred at the post translational level through demethylation of RASSF1A, which disrupted the MDM2-DAXX-HAUSP interactions, thereby promoting MDM2 self-ubiquitination and subsequent MDM2 degradation. Given that wt-p53/MDM2-overexpressing ALL cells are commonly chemotherapy-resistant due to MDM2-regulated repression of the p53 function our finding that these cells can be potently killed by berberine through direct, effective targeting of MDM2 suggests that this naturally-derived agent may have a highly useful role in the treatment of patients with refractory ALL.

POSTER SESSION 2

POSTER 80

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Poster Session 2
Sunday, October 10
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Penn Museum
Upper Egyptian Gallery

Cancer-associated p53 tetramerization domain mutants: quantative analysis reveals a low threshold for tumor suppressor inactivation

Many genetic abnormalities are frequently observed in human tumors. Approximately half of human tumors have TP53 gene mutations, and, in many cases, tumor suppressor protein p53 is inactivated by missense mutations. The tumor suppressor protein p53 is a 393 amino acid transcription factor that induces cell cycle arrest and apoptosis in response to genotoxic stress. Tetramer formation is critical for its activity. Inactivation of p53 via the mutation of its gene is a key step in tumor progression. In this study, to clarify the mechanism of dysfunction of tumor suppressor activity of p53 with tetramerization domain mutations, we comprehensively and quantitatively analyzed the stability of the p53 tetramerization domain peptide containing the 39 reported amino acid changes in human cancers. Their effects on tetrameric structure were broad, and the stability of the mutant peptides varied widely. At an endogenous concentration of p53 in nucleus, the fraction of tetramer was significantly decreased even in the case of a slightly destabilized mutant. Because formation of a tetrameric structure is critical for protein-protein interactions, DNA binding, and the post-translational modification of p53, a small destabilization of the tetrameric structure could result in dysfunction of tumor suppressor activity. We suggest that the threshold for loss of tumor suppressor activity in terms of the disruption of p53's tetrameric structure could be extremely low. However, other properties of the tetramerization domain, such as electrostatic surface potential and its ability to bind partner proteins, also may be important.

POSTER 81

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Cancer-derived p53 C-terminal mutations alter the proteins' ability to be post translationally modified

Post translational modifications (PTM's) of p53 are a key regulatory mechanism of the protein's stability, and transcriptional activity. Lysine residues in the C-terminus of p53 are sites of ubiquitylation, acetylation and methylation in a mutually exclusive manner. In particular, two key "modification cassettes" comprise lysines 370, 372 and 373 (cassette A) and lysines 381, 382 and 386 (cassette B). Each cassette is capable of interacting productively with multiple enzymes including several protein lysine methyltransferases and acetyltransferases. Although most mutation analyses of cancers have not sequenced exon 11 encoding these cassettes, work from others (as denoted in the p53 database) and our own work suggests that residues can be mutated in the cassette regions. We hypothesized that C-terminal mutations may affect interactions with proteins that carry out or bind to C-terminal p53 PTM's. Using a series of mutant and wild type p53 peptides encompassing unmodified cassettes A, B or both, and those with various PTMs, we identified several cancer derived mutations that differentially effect the p53 binding and catalytic efficiency of methylation by SETD7, G9a and SETD8. Our data are consistent with a delicate interplay between C-terminal posttranslational modifications that may influence the development of cancer when disrupted. We suggest the routine sequencing of exon-11 should be included in all p53 genotyping in order to further assess the potential of mutations in this region for their role in cancer.

POSTER 82

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Poster Session 2
Sunday, October 10
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The Lats2 tumor suppressor augments p53-mediated apoptosis by promoting the nuclear proapoptotic function of ASPP1

Apoptosis is an important mechanism to eliminate potentially tumorigenic cells. The tumor suppressor p53 plays an important role in this process. Many tumors harbor mutant p53, but others evade its tumor suppressive effects by altering the expression of proteins that regulate the p53 pathway. ASPP1 is a key mediator of the nuclear p53 apoptotic response. Under basal conditions, ASPP1 is cytoplasmic. We report that in response to oncogenic stress, the tumor suppressor Lats2 phosphorylates ASPP1 and drives its translocation into the nucleus. Together, Lats2 and ASPP1 shunt p53 to proapoptotic promoters and promote death of polyploid cells. These effects are overridden by the Yap1 oncoprotein, which disrupts Lats2-ASPP1 binding and antagonizes their tumor suppressing function.

POSTER 83

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Single nucleotide polymorphisms in intron 9 and 10 of the MDMX gene are correlated with age of diagnosis and survival in ovarian carcinoma patients in an estrogen-receptor-dependent manner

The tumor suppressor p53 is inactivated in the vast majority of cancers, either by mutations of the p53 gene itself or by alterations of p53-regulating factors. The MDM2 homologue, MDMX, is another critical negative regulator of p53. Only a few data exist so far, demonstrating the clinical importance of SNPs in the MDMX gene. The aim of our study was therefore to perform analyze the impact of SNPs in the MDMX gene. Previously, we showed that a single nucleotide polymorphism (A/C) in the 3'-UTR (SNP34091; rs4245739) is strongly correlated with disease-free and overall survival in ovarian cancer patients with estrogen-receptor (ER) negative tumors. In this study, we extended our analysis to two SNPs in intron 9 (T>C, rs:2290855, SNP31112) and in intron 10 (G>A, rs2290854, SNP31274). The distribution of the genotypes of the respective SNPs in ovarian carcinoma patients was comparable to age-matched healthy volunteers. The minor allele of both SNPs was correlated with earlier age of diagnosis (SNP31112: 9 yrs; SNP31274: 10 yrs) in ER-negative patients compared with ER-positive patients. In patients homozygous for the major allele we did not observe a difference in the age of diagnosis regardless of the ER-status of the tumor. Interestingly, despite being diagnosed later, patients who were homozygous for the major allele and ER-negative tumors had a decreased overall survival time (SNP31112: $p=0.12$; SNP31274: $p=0.048$) and a significantly increased risk of tumor-related death (SNP31112: 3.7-fold, $p=0.049$; SNP31274: 4.5-fold, $p=0.038$). The Cox-regression analysis was adjusted to the residual tumor. In summary, our results show that specific allelic variants of SNPs in the MDMX gene are an independent prognostic factor in ER-negative human ovarian carcinomas.

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Identification of novel p63 interacting proteins

The p53 homologue p63 regulates multiple biological processes in developing and mature organisms including epithelial differentiation, cell cycle control, and apoptotic signaling pathways. p63 primarily functions by transcriptionally controlling downstream target genes that encode proteins involved in many diverse cellular functions. Relative to our understanding of downstream signaling, the upstream regulatory mechanisms that control p63 stability and activity are, for the most part, poorly understood. To identify potential upstream regulators, we used a mass spectrometry-based approach to detect p63 protein interactions in epithelial tumor-derived cell lines as well as primary cultures of epithelial cells. From replicate experiments, we identified numerous proteins in complex with p63, two of which are the known p63 binding partners, p73, and the E3 ubiquitin ligase, ITCH. In addition to these known interactors, we reproducibly detected kinases and ubiquitin-conjugating enzymes that have not previously been linked to p63 regulation. We further characterized an E3 ubiquitin ligase, and show that expression of this protein results in increased p63 polyubiquitination after cisplatin treatment. Additionally, depletion of the E3 ligase perturbs keratinocyte differentiation, suggesting that its regulation of p63 is an important signaling cue during epithelial development. Our discoveries provide insight to the multiple upstream regulatory mechanisms that govern the stability and activity of p63.

POSTER 85

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ΔNp63α Regulates Erk1/2 Signaling To Inhibit Cancer Cell Invasion

The p53 homologue p63 is of crucial importance for maintaining replicative potential in stratified epithelial stem cells. Emerging evidence indicates that deregulation of p63 may play a causative role in human cancer progression and metastasis, as evidenced by an inverse correlation between p63 expression and cancer tumor grade. In order to identify downstream targets of ΔNp63α , the predominant p63 isoform, that are associated with the maintenance of a non-invasive phenotype, we profiled gene expression in human breast cancer Hs578T cells stably expressing wild type ΔNp63α , or a mutant derivative defective in DNA binding or protein-protein interaction. Our microarray data show that ΔNp63α significantly (> 2-fold; $p < 0.05$) up-regulates 160 genes and down-regulates 81 genes. Mitogen Activated Protein Kinase Phosphatase 3 (MKP3), a negative regulator of MAP Kinase signaling whose expression is also decreased at advanced cancer stages, is up-regulated by wild type ΔNp63α , but not by the mutant derivatives. While exogenous ΔNp63α expression in MDA-MB-231 breast cancer cells renders cells less invasive, subsequent MKP3 ablation significantly recovers their invasive phenotype, suggesting that ΔNp63α requires MKP3 induction in order to repress invasion. Conversely, p63 knockdown in mammary epithelial MCF-10A cells and in esophageal squamous cell carcinoma FaDu cells results in decreased MKP3 expression and concomitant higher levels of Erk1/2 phosphorylation. Consistently, p63 knockdown leads to increased cell motility, which is reversed by inhibition of Erk1/2 signaling. Taken together, these data suggest an important role for p63 in preventing cancer progression and metastasis by inhibition of Erk1/2 signaling.

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Identification of novel putative mRNA targets of the p53-induced Wig-1 protein

Wig-1 is a bona fide p53 target gene involved in regulation of mRNA stability. We previously found that the Wig-1 protein is an AU-rich element binding protein (ARE-BP) that stabilizes p53 mRNA by binding to its 3' UTR and preventing its deadenylation (Vilborg et al., 2009). AREs present in the 3'UTR of many early-response and inflammatory genes are important for regulating mRNA turnover and translation. Several ARE-BPs exert posttranscriptional control through binding to such regions. Most ARE-BPs have multiple mRNA targets, and our data indicate that Wig-1 can regulate other ARE-containing mRNAs apart from p53. The aim of this study was to analyze the effect of Wig-1 on global mRNA expression and to identify novel Wig-1 regulated mRNAs. Microarray analysis of total RNA isolated from Wig-1 knock-down HCT116 cells revealed significantly altered expression (fold change > 2) of 2448 gene transcripts. Interestingly, we observed an upregulation of several p53 target mRNAs coding for pro-apoptotic proteins and downregulation of p53 target mRNAs coding for proteins involved in cell cycle arrest. We have also performed RNA immunoprecipitation assays (RIP) followed by deep sequencing to identify putative endogenous mRNA targets bound by Wig-1. This analysis revealed a list of Wig-1 associated mRNAs. The integration of the data obtained from these two approaches will allow us to identify and validate novel Wig-1 targets and the biological effect of their regulation. Moreover, we will be able to understand whether the p53 transcription factor has a role in global mRNA regulation through its target Wig-1, and if Wig-1 itself is a player in p53-dependent decisions of cell fate.

Vilborg A, Glahder JA, Wilhelm MT, Bersani C, Corcoran M, Mahmoudi S, Rosenstierne M, Grander D, Farnebo M, Norrild B, et al. 2009. The p53 target Wig-1 regulates p53 mRNA stability through and AU-rich element. PNAS 106: 15756-15761.

POSTER 87

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Involvement of p53 and pRB/E2F1 pathways in resistance to ABT 737 treatment

With increasing evidence of secondary effects and possible acquisition of resistance to ABT-737, predictive markers of sensitivity and resistance to this compound are crucial. We previously demonstrated that Puma is involved in the apoptotic response of human colorectal cancer cells to the Bcl-2/Bcl-xL inhibitor ABT-737 suggesting that pathways regulating the expression of this BH3-only protein might impact on sensitivity (Gallenne T et al, 2009). As Puma is a direct transcription target of p53, p73 and E2F-1, we focused on the effects of the p53 and pRB/E2F1 pathways on the cellular response to ABT-737. Here we show that silencing of p53 protected cells from apoptosis in response to ABT-737, as well as knock down of E2F1. Our data indicate that knock down of Bcl-2/Bcl-xL and ABT-737 treatment both trigger pRB cleavages leading to displace pRB from E2F1. These cleavages are not only a consequence of cell death, as they were not promoted by a knock down of Mcl-1 or cisplatin treatment that induced similar level of cell death than ABT 737 treatment or either knock down of Bcl-2/Bcl-xL. We currently investigate whether the transcriptional activity of p53 and of E2F1 play a role in ABT 737 treatment. However, our preliminary data suggest that the implication of p53 and E2F1 in apoptosis induced by inhibition of Bcl-2/Bcl-xL might involve post-transcriptional modifications of these two proteins that control their interactions with regulators and thereby their apoptotic functions.

POSTER 88

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Functional dissection of the role of CtBPs in the regulation of mitotic fidelity, p53 pathway homeostasis, and cell survival

C-terminal binding proteins (CtBPs) are multifunctional proteins that can act in the nucleus as transcriptional corepressors and in the cytoplasm as regulators of Golgi fission. CtBP knockout studies show increased sensitivity to apoptotic stimuli, thus making them potential candidates for novel therapeutic targets against cancer. We have shown that ablation of CtBPs in breast cancer derived cell lines using siRNA results in the suppression of cell proliferation through a combination of apoptosis, reduction in cell cycle progression and aberrations in transit through mitosis. Also, CtBP knockdown decreased expression of aurora B and resulted in a marked up-regulation of the p53 protein. Loss of p53 function, either through siRNA or in p53 mutant breast cancer cell lines, rendered cells more susceptible to CtBP siRNA-induced apoptosis. Cell cycle analysis suggested that up-regulation of p53 in CtBP depleted cells is providing a protective role by arresting aberrant cells in G1. We are therefore interested in identifying which functions of CtBP are required for both the maintenance of mitotic fidelity and p53 inactivation. We made a series of dominant negative mutants to disrupt the formation of CtBP chromatin modifying complexes. Using microinjection, mutants were targeted to either the cytoplasm or nucleus of breast cancer cells. Injected cells were analysed by live cell imaging and immunofluorescence analysis. These studies demonstrated that an intact CtBP chromatin modifying complex in the cell nucleus is required for mitotic fidelity, as well as for the maintenance of the low levels of inactive p53 that are a feature of proliferating cells. CtBP repression complex formation is increased in cancer cells due to both tumour hypoxia and the "Warburg effect." Our studies suggest that manipulation of CtBP function may be a potential therapeutic strategy in cancer, particularly those tumours in which the p53 gene is mutated.

POSTER 89

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Selective inhibition of stress-induced p53 accumulation and apoptosis by cAMP signalling in BCP-ALL is promoted by the interaction between p53 and Hdm2

As p53 provides an important barrier to the initiation and maintenance of cancers, it is presumed that p53 function must be inactivated for a tumour to develop. This is achieved by mutation in about 50 % of cases and probably by functional inactivation in the remaining cases. The objective of the present study was to provide mechanistic insight into how DNA damage-induced accumulation of wild type p53 can be prevented by elevation of cAMP in B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) cells. We show that increased levels of cAMP augments the binding of p53 to its negative regulator HDM2, overriding the DNA damage-induced dissociation of p53 from HDM2. This results in maintained levels of p53 ubiquitination and proteasomal degradation, which in turn counteracts the DNA damage-induced stabilization of the p53 protein. These findings potentially implicate deregulation of cAMP signalling as a candidate mechanism utilized by transformed cells to quench the p53 response while retaining wild type p53. Despite the relatively good prognosis of paediatric BCP-ALL, there is a steady search for strategies to improve treatment of this disease, and to prevent the undesired side effects on normal cells. In the present study we also indicate that the inhibitory effect of cAMP on DNA damage-induced cell death in primary BCP-ALL cells could be an acquired trait associated with the malignant transformation, allowing for manipulation of cAMP signalling for selective killing of the malignant cells.

POSTER 90

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Transactivation Domain Mutant Mice Reveal Context-Specific Differences in p53's Mechanism of Action

The p53 protein suppresses tumorigenesis by inducing cell cycle arrest, apoptosis, or cellular senescence. At the molecular level p53 plays a central role as a transcriptional activator, and p53 has two amino-terminal transactivation domains important for this role. However, the contribution of each of these domains to p53 activity has not been determined in vivo. To analyze transactivation domain requirements for p53 function, we have generated a series of p53 knock-in mouse models in which we replaced the endogenous p53 gene with transactivation mutants containing point mutations in the first (p53-25,26), second (p53-53,54), or both (p53-25,26,53,54) p53 transactivation domains. These experiments are the first to address the unique contribution of each of the p53 transactivation domains in vivo. By gene expression microarray, we determined that these mutants represent an allelic series of p53 transactivation mutants, whereby p53-53,54 largely retains wild-type p53 transactivation potential, p53-25,26,53,54 loses p53-dependent transactivation, and p53-25,26 exhibits a severely compromised transactivation phenotype. Experiments comparing all three transactivation domain mutants in cell culture and mouse models have unveiled important differences in the function of the two transactivation domains. Apoptosis or cell-cycle arrest downstream of acute DNA damage rely on the first transactivation domain and robust p53 transactivation, while senescence and tumor suppression in response to oncogene activation can occur without the full transactivation function of p53. These results implicate unique p53 transcriptional programs in acute DNA damage responses and tumor suppression, and mechanistically distinguish these two p53 response pathways.

POSTER 91

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p53-repressed miRNAs are involved with E2F in a feed-forward loop promoting proliferation

Normal cell growth is governed by a complicated biological system, featuring multiple levels of control, often deregulated in cancers. The role of microRNAs (miRNAs) in the control of gene expression is now increasingly appreciated, yet their involvement in controlling cell proliferation is still not well understood. Here we investigated the mammalian cell proliferation control network consisting of transcriptional regulators, E2F and p53, their targets and a family of 15 miRNAs. Indicative of their significance, expression of these miRNAs is downregulated in senescent cells and in breast cancers harboring wild-type p53. These miRNAs are repressed by p53 in an E2F1-mediated manner. Furthermore, we show that these miRNAs silence antiproliferative genes, which themselves are E2F1 targets. Thus, miRNAs and transcriptional regulators appear to cooperate in the framework of a multi-gene transcriptional and post-transcriptional feed-forward loop. Finally, we show that, similarly to p53 inactivation, overexpression of representative miRNAs promotes proliferation and delays senescence, manifesting the detrimental phenotypic consequence of perturbations in this circuit. Taken together, these findings position miRNAs as novel key players in the mammalian cellular proliferation network.

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Selective targeting of p53 deficient cells: Nutlin-3 protects normal cells from endoreduplication and cell death induced by aurora kinase inhibition

Aurora kinase inhibitors are being developed as anticancer therapeutics, some of which has entered the clinical trials. VX-680, a specific inhibitor of Aurora A and B, inhibits tumor growth in vivo but also resulted in normal tissue toxicity (neutropenia). The observed cellular phenotypic consequences of aurora kinase inhibition include cell cycle arrest, apoptosis, polyploidy and senescence. We demonstrate that VX-680 induces endoreduplication and apoptosis in both p53-positive and p53-mutated tumor cell lines suggesting that normal cells are also susceptible to the effects of VX-680. Checkpoint activation is evident in p53-positive A549 and HCT116 cells and coincided with the suppression of cyclin A, cyclin B, Cdk1/2 activities, downregulation of Emi1 and hypophosporylation of Rb. Normal human primary keratinocytes undergo extensive endoreduplication suggesting that the p53-dependent postmitotic checkpoint is not sufficient to protect normal cells from endoreduplication and tetraploidy. Pre-treating normal primary keratinocytes and p53-positive tumor cells with a potent and specific MDM2-antagonist, nutlin-3, activates p53-dependent arrest and suppresses VX-680 induced endoreduplication. Reversing the order (VX-680 followed by nutlin-3 addition) results in an increase in the bax/bcl-2 ratio and synergistic activation of apoptosis instead. The correct sequential combination of nutlin-3 and VX-680 may selectively protect p53-positive cells from endoreduplication and apoptosis and therefore discriminates between normal cells and p53-compromised tumor cells in anticancer therapy.

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p53 Regulation by Phosphorylation

Post-translational modifications may be important regulators of p53 stabilisation and activation in response to a wide range of stresses. The objective of this study is to understand the effects that differences in p53 phosphorylation have on p53 by looking at sites that are regarded as important for p53 activities. Ser392 is reported to be a major p53 phosphorylation site that is modified only in response to UV by CK2 or the p38 MAP Kinase pathway. Here we show that phosphorylation of Ser392 is an integral event that occurs not only in response to UV, but also during the induction of p53 by a range of stimuli including Nutlin 3a. Strikingly, phosphorylation of serines 33 and 392 was also observed following induction of the p53 pathway by ARF, which has previously been thought to induce p53 in a phosphorylation-independent manner. The induction of Ser392 phosphorylation by diverse stimuli can be explained by a common mechanism: if the rate of phosphorylation is slow; rapid turnover of p53 under unstressed conditions would limit the accumulation of Ser392-phosphorylated p53 until a stimulus stabilises p53 and allows the Ser392-phosphorylated p53 to accumulate. We also provide evidence that the constitutively active kinase CK2 is the sole Ser392 kinase - and that p38 MAPK does not modify the site directly.

POSTER 94

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Analysis of (–)-Nutlin-3-mediated activation of the p53 signaling pathway using both in vitro and in vivo model systems

The p53 signaling pathway is an attractive therapeutic target for activation in human cancers due to its important role in the regulation of cell cycle, senescence, and apoptosis after exposure to many types of genotoxic stress. MDM2 and MDM4, potent negative regulators of p53 transcriptional activity, are often overexpressed in many types of cancers that retain wild-type p53. Nutlin-3 (Hoffmann-La Roche) is a small-molecule inhibitor which acts to abrogate MDM2 and MDM4 binding and subsequent degradation of p53, allowing for activation of downstream transcriptional targets. One challenge for clinical investigation using Nutlin-3 is the ability to selectively prepare large quantities of the more active enantiomer ('Nutlin-3a'). Further, there are limited analyses examining organ specificity of Nutlin activity in vivo. In the present study we describe the use of the more potent enantiomer of Nutlin-3 resulting from the first enantioselective chemical synthesis of (–)-Nutlin-3, in both in vitro and in vivo model systems. Colorectal cancer-derived cell lines (HCT116 p53 +/+, HCT116 p53 -/-, RKO-NEO, and RKO-E6) were treated 8 h with 8uM (–)-Nutlin-3, leading to the rapid elevation of downstream p53 target genes. Cell viability was measured 72 h after treatment, resulting in IC50 values comparable to published reports. Mice harboring homozygous wild-type or null alleles of TP53 were treated with 200 mg/kg Nutlin-3 to determine its in vivo activity. A panel of tissues was harvested for examination of increased p53 stability and downstream signaling activity. Select tissues showed increased p53 nuclear localization and differential downstream target gene expression. These results indicate that (–)-Nutlin-3 is capable of p53 activation in vitro and in vivo. A more thorough characterization of (–)-Nutlin-3 activity both in vitro and in vivo will allow for a more broadly applicable activation of the p53 signaling pathway for use in clinical cancer therapy.

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Inhibition of p53 DNA binding function by the MDM2 acidic domain

MDM2 is an important regulator of p53 that acts in part by promoting p53 ubiquitination. Previous studies also implicated the presence of degradation-independent mechanisms of p53 regulation. Here we show that the MDM2-p53 complex lost DNA binding activity in vitro and in vivo. P53, in complex with MDM2, adopts a mutant-like conformation, losing reactivity to Pab1620, while exposing the Pab240 epitope. The acidic domain of MDM2 is required to induce p53 conformational change and inhibit p53 DNA binding. Biochemical analysis suggests that MDM2 acidic domain acts by interacting with the p53 DNA binding domain, causing p53 to shift to the mutant-like conformation. Introduction of ARF into the MDM2-p53 complex restores p53 wild type conformation and rescues DNA binding activity, allowing the p53-MDM2-ARF complex to bind to DNA. Furthermore, the histone methyl transferase SUV39H1 also interacts with MDM2 acidic domain, restores p53 wild type conformation and allowing p53-MDM2-SUV39H1 complex to bind DNA. These results reveal an ubiquitination-independent mechanism of p53 regulation by MDM2, and provide a mechanism by which MDM2-interacting transcription repressors gain access to p53 target promoters and directly repress transcription.

POSTER 96

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Differential effects of AMPK activation on apoptosis in human breast cancer cells

The tumour suppressor protein, p53 is a crucial regulator of cellular proliferation and survival, controlling both cell cycle phase progression and apoptosis in order to eliminate DNA damaged cells. TP53 is mutated in at least 50% of human cancers and in approximately 30% of sporadic breast carcinomas and this impacts on the therapeutic response. AMP activated kinase (AMPK) is "fuel sensing," responding to low energy (ATP) status in the cell. Also, AMPK may be a bona fide target for cancer therapy. To study the effect of TP53 status on breast cancer response to activated AMPK, MCF-7 (p53+), T47D (p53 mutant) and MDA-MB-231 (p53 mutant) breast cancer cell lines were treated with the AMPK activators, AICAR and phenformin, and assessed for proliferation, mitochondrial membrane potential, apoptosis (annexin V and morphology) and cell cycle analysis. AMPK activation in treated cells was confirmed by Western blotting. Activated AMPK produced a pro-apoptotic effect in MCF-7 and MDA-MB-231 as confirmed by all methods. In contrast, T47D were the most sensitive cells, activation of AMPKcausing cell cycle arrest, but a delayed pro-apoptotic effect. Interestingly, inhibition of caspases 3/7, 6, 8 & 9 blocked the apoptotic effect of AICAR in MCF-7 cells but not in T47D cells. In conclusion, the different modes of action of activated AMPK could be attributed at least in part to the p53 status of these cell lines. This confirms that the genetic background could have an impact on the responses of patients to a given treatment regimen. These results highlight the potential impact of individual variation in the response to activated AMPK, which could be a novel therapeutic target for some breast cancers.

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Requirement of ATM for Rapid p53 Phosphorylation at Ser46 without Ser/Thr-Gln Sequences

The tumor suppressor protein p53 activates the transcription of numerous target genes involved in cell cycle arrest, apoptosis, and DNA repair. p53 phosphorylation at Ser46 following DNA damage is important for preferential transactivation of proapoptotic genes. Here, we report that ataxia-telangiectasia mutated (ATM) kinase is responsible for Ser46 phosphorylation of p53 during early phase response to DNA damage. To elucidate the direct phosphorylation of p53 at Ser46 by ATM, an ATM mutant (ATM-AS) sensitive to ATP analogues was engineered. In vitro kinase assays revealed that p53 was phosphorylated at Ser46 by ATM-AS, even when ATP analogues were used as phosphate donors, although this phosphorylation site is not in an SQ motif, a consensus ATM site. Furthermore, Ser46 phosphorylation by ATM was dependent on the N- and C-terminal domains of p53, unlike Ser15 phosphorylation. Immunofluorescence analyses showed that Ser46 phosphorylated p53 was observed as foci in response to DNA damage and colocalized with γ-H2AX or Ser1981-phosphorylated ATM. These results suggest that ATM phosphorylates a noncanonical serine residue on p53 by mechanisms different from those for the phosphorylation of Ser15.

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HDBR1 Maintains Transcriptome Integrity and Contributes to P53 Tumor Suppressor Activity

Coding and noncoding RNAs of a given transcriptome associate in a dynamic manner with RNA biogenesis and function, and affect cellular events and, ultimately, cell fates. The proper content of transcriptome or "transcriptome integrity" is therefore crucial for normal cellular function. However, the essence of transcriptome integrity is poorly known. Here we show that human housekeeping RNA debranching enzyme hDBR1 can maintain a right context of transcriptome and contribute to p53 tumor suppressor activity. We found that compromised expression of hDBR1 causes aberrant assembly in exons and insufficient degradation of nonsense transcripts. Subsequently, the disintegrated transcriptome resulting from a lower level of hDBR1 expression was found to confer an elevated-oncogenic potential, promoting the development of xenograft tumors. More importantly, hDBR1 can function as a direct target of p53. Unlike many of p53 targets known, hDBR1 can be upregulated by wtp53 under hypoxia that solid tumor cells mostly encounter. Furthermore, the compromised expression of hDBR1 was found to be directly associated with deficient p53 in human solid tumor tissues, suggesting tumorigenicity of malfunctioned p53 is at least partly attributed to a low expression level of hDBR, which is failed in maintaining proper RNA populations. These results build up a framework for understanding transcriptome integrity, and reveal a hidden role of p53 tumor suppressor in guarding the transcriptome integrity. We anticipate this study is the beginning of appreciation for the integrity of transcriptome, and our ongoing studies and those of others will continue refining "transcriptome integrity maintenance" showing it to be of no less importance than that of the DNA integrity checkpoint.

POSTER 99

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Targeting of DR5/TRAIL-R2 in combination with Irinotecan trigger depletion of Lgr5+ stem cells and chk2-p53-dependent gastrointestinal toxicity

Targeting the TRAIL-death receptor DR5/TRAIL-R2 with agonistic antibodies in combination with chemotherapy may offer more efficient killing of cancer cells compared to either therapy alone. Clinical trials indicate that antibodies targeting TRAIL-death receptors as monotherapy appears safe, however the safety of combination therapy is less well investigated. Wild-type, trail-r-/- (mice lacking the mouse orthologue to human DR5/TRAIL-R2), chk2-/- and Trp53-/- mice were subjected to treatment with a TRAIL-R-agonistic antibody; MD5-1 (1.2 mg/kg bw) alone or in combination with either Irinotecan (280 mg/kg bw) or 5-fluorouracil (5-FU; 150 mg/kg bw). No toxicity was observed up to a minimum of 1 month following monotherapy with either MD5-1, 5-FU or Irinotecan. However, when MD5-1 was combined with Irinotecan, wild-type but not trail-r-/-, chk2-/- nor Trp53-/- mice showed significant weight loss and increased lethality within 7 days following treatment. Weight loss and lethality was associated with increased acute epithelial crypt apoptosis and loss of Lgr5-GFP+ stem cells in Lgr5-EGFP-ires-CreERT2 mice that preceded subsequent (4 days) epithelial atrophy and inflammation in the gastrointestinal (GI) tract. In order to address the relevance of our findings to normal human cells, we also treated the GI-epithelial cell line FHs 74-Int with Irinotecan and 5-FU in combination with a DR5/TRAIL-R2 agonistic antibody (mAb631). A small increase of cell death (sub-G1 content) relative to untreated control was detected following treatment with mAb631 alone. However, when mAb631 was combined with either of the chemotherapeutics a clear synergistic increase in sub-G1 content was observed. Interestingly, although genetic loss of trail-r and p53 in mice did provide complete protection from GI-toxicity when 5-FU or Irinotecan were combined with MD5-1, loss of chk2 only conferred significant protection following Irinotecan and MD5-1, suggesting that loss of chk2 may offer an Irinotecan-specific protection from GI-toxicity when Irinotecan is combined with TRAIL-R-targeting antibodies. Indeed, co-treatment of animals with Chk2 inhibitor II or the p53-inhibitor Cyclic pifithrin-α significantly reduced the manifestation of GI-toxicity following MD5-1 in combination with Irinotecan suggesting that the observed GI-toxicity can be pharmacologically modulated. In conclusion, our findings suggest that Irinotecan sensitizes stem cells within the GI-epithelium to TRAIL-R-mediated apoptosis and this precedes chk2- and p53-dependent GI-toxicity. Combined pharmacologic inhibition of p53 and chk2 may reduce GI-toxicity and subsequently improve the therapeutic window of TRAIL-R2/DR5-agonists and chemotherapy.

POSTER 100

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Gain-of-Function Effects of Mutant p53 in a 3D Model of Breast Cancer

p53 is the most frequent target for mutation in human tumors and mutation at this locus is a common and early event in breast carcinogenesis. Breast tumors with mutated p53 often contain abundant levels of this mutant protein, which has been postulated to actively contribute to tumorigenesis by acquiring pro-oncogenic ("gain-of-function") properties. To examine the gain-of-function hypothesis, we employed a three-dimensional (3D) culture model, in which mammary epithelial cells are cultured in a laminin-rich extracellular matrix and form structures highly reminiscent for many aspects of acinar structures found in vivo. We found that exogenous expression of tumor-derived mutants of p53 in normal mammary epithelial cells profoundly disrupts acinar morphogenesis, an event which invariably occurs during breast tumor progression. We also engineered breast cancer cell lines to inducibly knockdown endogenous mutant p53 and demonstrated that mutant p53 depletion is sufficient to induce a phenotypic reversion in 3D culture. To investigate the molecular effects of mutant p53 in 3D culture, we performed genome-wide expression analysis following shRNA-mediated depletion of mutant p53 from breast cancer cell lines cultured in a 3D microenvironment. We identified the sterol biosynthesis pathway as being upregulated by oncogenic mutant p53 and preliminary evidence suggests that this pathway may account for the phenotypic effects of mutant p53 in 3D culture. Elucidating the oncogenic functions of mutant p53 on mammary tissue architecture and the molecular mechanisms by which these occur will not only greatly contribute to our understanding of early breast carcinogenesis, but may also offer novel therapeutic targets.

POSTER 101

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Genetically Modified Human Embryonic Stem Cells: A Tool for Studying Human p53

Mouse genetics has been used extensively to study the function and regulation of p53. While the function of p53 is highly conserved evolutionarily, the physiological and cellular differences between mice and humans have made it apparent that some aspects of p53 biology are different between the two systems. For example, while p53-deficiency primarily leads to lymphomas and sarcomas in mice, carcinomas are the primary tumor type in humans. Additionally, certain p53 targets such as AIP1 are only expressed in human cells. To further investigate the physiological function and regulation of p53 in human cells, we decided to genetically manipulate human embryonic stem cells (hESCs). Somatic human cells are not fruitful targets for genetic manipulation due to their limited proliferative potential. Established cell lines are likely to harbor genetic lesions and epigenetic modifications that could make them unsuitable for studying the effects of specific genetic alterations. hESCs cells have an unlimited proliferative potential and are capable of generating all cell types of the human body. Thus, genetically modified hESCs provide a consistent supply of study material which can be differentiated into various cell types in order to study the effects of those genetic alterations in different tissues. Our study aims to employ genetically modified hESCs to improve our understanding of the roles of p53 in suppressing human tumorigenesis, the importance of post-translational modifications of p53 in human cells, and the mechanisms of cancer promotion by p53 gain-of-function mutants. Our laboratory has recently developed the bacterial artificial chromosome (BAC)-based strategy for high efficiency gene targeting in hESCs. Prior to this advance, it has been technologically challenging to genetically modify hESCs. Several hESC lines with genetically altered p53, including p53-deficient hESCs, have been generated, and will provide additional insight into the function and regulation of wild type and mutant p53 in human cells.

POSTER 102

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Positive feedback between p53 and TRF2 in telomere damage signaling and cellular senescence

p53 plays critical roles in sensing and signaling cellular stresses (e.g., telomere dysfunction) that induce cellular senescence as a tumor suppressor mechanism in vivo. p53 is also involved in stem cell functions and aging in vivo. A multi-protein complex (shelterin) containing the single-stranded and double-stranded telomere binding proteins, including TRF2, protects functional telomeres from initiating unwanted DNA damage response. Uncapped, dysfunctional telomeres at the end of cellular replicative lifespan lose this protective mechanism and trigger telomere-initiated DNA damage signaling to activate p53 and thereby induce replicative senescence. Here we identify a signaling pathway involving p53, Siah-1 (a p53-inducible E3 ubiquitin ligase) and TRF2. Endogenous TRF2 and Siah-1 were down- and up-regulated, respectively, at replicative senescence when p53 was physiologically activated. Allelic loss, shRNA knockdown, dominant-negative inhibition, nutlin-3a activation and overexpression of p53 all showed that p53 induced Siah-1 and repressed TRF2. TRF2 was stabilized by knockdown or dominant-negative inhibition of endogenous Siah-1, as well as by a proteasome inhibitor. TRF2 was found to be ubiquitinated in p53- and Siah-1-dependent manners in vivo. Our in vitro and in vivo ubiquitination experiments identified Siah-1 as the E3 ubiquitin ligase responsible for TRF2 ubiquitination. Mass spectrometry analysis identified the lysine residues at positions 173, 180 and 184 as the ubiquitination sites of TRF2. siRNA knockdown of Siah-1, as well as TRF2 overexpression, extended the cellular replicative lifespan, suggesting that the proteolytic control of TRF2 is a novel telomere-mediated mechanism by which p53 regulates cellular senescence. This study reveals that p53, a downstream effector of the damage signaling from uncapped telomeres, also functions upstream of the telomere-capping protein complex, and suggests that the p53-Siah-1-TRF2 pathway orchestrates the telomere-initiated damage signaling to replicative senescence.

POSTER 103

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p53-miR-dependent post-transcriptional circuits: mechanisms, targets and inter-individual variation.

Using bioinformatics approaches, we identified a group of candidate microRNAs (miRs) for direct p53 transcriptional control. To validate p53 family-mediated control of the newly predicted target miRs we evaluated the potential for wild type p53, p63β and p73β to transactivate from p53 response elements (Res) mapped at the miR promoters. Results obtained with an established yeast-based assay indicated that the Res found at miR-10b, -23b, -106a, -151, -191, -198, -202, -221, -320, -1204, -1026 genes were responsive to p53 of which 8 were also responsive to p63β or p73β. ChIP assays in doxorubicin-treated HCT116 cells -p53+/+ vs p53-/-- revealed moderate p53 occupancy at the miR-202, -1206 sites, and weak occupancy at miR-10b, -191. RT-qPCR analyses in HCT116 and MCF7 cells showed modest doxorubicin- and/or p53-dependent regulation for miR-23b, -151, -191. We then focused on miR-191 and its potential impact on MDM4, an important modulator of p53. The A>C SNP (rs4245739) in the proximal portion of the MDM4 3'UTR occurs in a predicted binding site for miR-191, with the A allele resulting in a mismatch for miR-191 binding. This SNP is in strong association with the tagSNP (rs2369244) within the MDM4 haplotype recently reported to impact on cancer risk. Using MCF7 cells that are heterozygous for rs4245739, we examined at the endogenous MDM4 gene level the impact of miR-191. A slight allelic imbalance was observed by allele-specific qPCR when miR-191 was transfected, and the co-transfection of miR-34a, for which seed sequences are predicted in the distal portion of the MDM4-3'UTR, had a more than additive effect, leading to ~30% reduction of the C-allele. Our study reveals additional miRs that could be directly regulated by the p53-family of transcription factors and could contribute to the tuning of p53-induced responses with the possibility of inter-individual variations due to functional SNPs.

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Loss of TP53 is the predominant prognostic factor in relapsed myeloma and a marker of progression

Multiple myeloma (MM) is an incurable plasma-cell proliferative disorder. Many stages are described ranging from indolent monoclonal gammopathy of undetermined significance (MGUS), smoldering myeloma (SMM), to MM and finally plasma cell leukemia (PCL). Among the principal genetic events associated with disease progression are alterations affecting TP53. We analyzed results from gene expression profiling (GEP), array-based comparative genomic hybridization (aCGH), interphase fluorescent in situ hybridization (FISH) and mutational data on the largest cohort published to date of 834 patients from all MM stages and 48 human cell lines (HMCLs). The prevalence of monoallelic TP53 deletions goes from 1.4%, 3.7%, to 8.9% in MGUS, SMM and MM, respectively. In relapsed MM, the prevalence of monoallelic TP53 deletions increases from 9/49 patients (18%) at first relapse, to 8/27 patients (30%) in second relapse and 3/4 patients (75%) in third relapse. We detected by FISH monoallelic TP53 deletion in 15/141 patients progressing from indolent to relapsed MM, and confirmed that in 10/15, deletion has been acquired during disease progression. The presence of TP53 deletion was associated with poor overall survival in relapsed/refractory MM patients (4.2 vs. 37.8 months, $p = 0.015$). Deletion of TP53 was observed in 26/48 (53%) HMCLs, 4/26 (15%) were biallelic deletions. The mutational status of TP53 was investigated in 59 relapsed MM patients, 5/59 samples (8.5%) had TP53 mutations. Four mutations were missense and one nonsense, and all were localized in exons 5 to 9. Four out of five mutations were located in the DNA binding domain. Two of those mutations were not previously reported in MM patients, one localized at the DNA binding domain (Cys135Ser) and another in the nuclear localization signal (NLS). Emergence of TP53 deletion/mutations in MM remains the overriding genetic factor for poor prognosis determination and denotes progressive genetic events.

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POSTER 105

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The expanding universe of p53: Identification of new active mouse p53 isoforms

Our laboratory has previously identified p53 isoforms in human and drosophila cells and highlighted an association of some p53 isoforms with survival in cancer patients. We have also shown that human p53 isoforms modulate p53 transcriptional and tumour suppressor activities. In order to determine the physiological relevance of the p53 isoforms during embryonic development, ageing and carcinogenesis, it is required to develop a mouse genetic model. As the mouse p53 isoforms were not completely explored, we set out to identify and characterise them. Here we report that the mouse p53 gene expresses 6 different p53 isoforms (p53, p53AS, D40p53, D40p53AS, D157p53 and D157p53AS), confirming the previously described alternative splicing of intron 10 leading to p53AS expression[1]. Interestingly, we determine that D40p53 isoform can also be obtained in mouse by alternative splicing of intron 2. Moreover, we demonstrate that intron 4 of the mouse p53 gene contains a promoter region that leads to the expression of D157p53 isoforms. Additionally, we show that p53 isoforms are differentially expressed in normal mouse tissues. With the aim to investigate the biological activities of the mouse p53 isoforms, scientific tools were developed. We show that some p53 isoforms have p53-dependent and -independent transcriptional activities and can modulate p53 transcriptional activity in a promoter-dependent manner.

POSTER 106

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Regulation of p53 polyubiquitylation by CBP

The stability of the p53 tumor suppressor protein is tightly controlled via ubiquitin-dependent proteasomal degradation. While multiple monoubiquitylation of p53 is mediated by the E3 ubiquitin ligase Mdm2, an additional factor, called an E4 ubiquitin ligase, is required to extend ubiquitin chains. Two E4 ubiquitin ligases, CBP and p300, were recently identified for p53. Both CBP and p300 are required for polyubiquitylation and rapid turnover of endogenous p53. The molecular mechanism of the E4 ligase activity of CBP is not yet understood. We have used a p53-ubiquitin fusion protein as a model substrate for studying p53 polyubiquitylation by CBP, as it was previously reported to mimic monoubiquitylated p53. We have identified a novel ubiquitin binding domain in CBP that can bind to both K48- and K63-ubiquitin chains, but not monomeric ubiquitin. The CBP ubiquitin binding domain was also required for CBP's association with ubiquitylated p53. Mutations within this domain were sufficient to block the ability of CBP to polyubiquitylate p53, but did not diminish CBP's intrinsic E3 activity, as measured by its ability to assemble free ubiquitin chains. Moreover, a point mutation of the ubiquitin binding domain also blocked the ability of CBP to target p53 for degradation. We show that consistent with its ability to bind K63-ubiquitin chains, CBP could also target K63-ubiquitylated p53 for degradation. We conclude that the E4 ligase activity of CBP requires a novel ubiquitin binding domain that facilitates the association of CBP with with mono-, K48- or K63-ubiquitylated p53. This domain is also required for CBP-dependent proteasomal degradation of p53, presumably through its contribution to CBP E4 activity. Therefore, CBP appears to play a broad role in p53 homeostasis, contributing to canonical Mdm2-dependent polyubiquitylation and degradation of p53, but also regulating the abundance and stability of K63-ubiquitylated p53 species, whose function(s) remains yet to be defined.

POSTER 107

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Effect of a Loss of Function Mutation in p53 on Gene Expression in the Mitochondria of ENU-exposed Murine Hearts

It is well known that p53 plays a crucial role in the maintenance of genomic stability and that loss of p53 function leads to an impaired DNA damage response. Further, p53 mutational status may affect the integrity of mitochondria which, in addition to their role in cellular energy metabolism, are recognized as players in p53-mediated apoptosis. In order to provide insight into the role of p53 in function of the mitochondria after exposure to DNA damaging agents, we exposed 6 C57Bl/6 p53-/- and 6 C57Bl/6 p53+/+ mice to 150 mg/kg of ENU. Equal numbers of mice were exposed to an equal volume of saline. Three mice from each genotype were sacrificed at 4 and 24 hours after exposure. Differential gene expression was determined using quantitative PCR and a targeted array with 84 genes known to be involved in the mitochondrial pathway. Multi-variate analysis of variance (MANOVA) of the 10 mitochondrial gene functional groups was used to compare the effect of time and genotype on the expression of each functional group. No differences in expression of the functional groups were found in the p53+/+ mice between 4 and 24 hours or between the ENU and Control groups. In contrast, differences in the level of expression between 4 and 24 hours of the small molecule transport, mitochondrion protein import, inner membrane translocation, mitochondrial localization and apoptotic genes functional groups were detected in the p53-/- mice. Finally, a significant effect of genotype on the level of expression of the following functional groups was found 24 hours after ENU exposure: targeting proteins to mitochondria, mitochondrion protein import, outer membrane translocation, inner membrane translocation, mitochondrial fission and fusion and mitochondrial localization. Our data may be interpreted that loss-of-function mutations in p53 affect the expression of mitochondrial gene functional groups after exposure to DNA damaging agents.

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A role for the codon 72 polymorphism in the p53 response to metabolic stress

The p53 tumor suppressor gene has been extensively studied for its functions in response to genotoxic stress and oncogene activation. In contrast, the role for this protein in response to metabolic stress is only now becoming clear. Recent reports have identified a role for p53 in metabolic stress-mediated cell cycle arrest and autophagy through AMPK and mTOR signaling pathways, respectively. Several genetic polymorphisms of p53 have been found to alter its functions, including Proline and Arginine variants at residue 72. Our work and others have found that Pro72 induces a higher level of cell cycle arrest, whereas Arg72 shows a significantly stronger ability to induce apoptosis upon DNA damage. However, the effect of this polymorphism on p53's functions in metabolic stress response and autophagy is not known. The effect of metabolic stress on the functions of p53 was investigated in this study using normal human fibroblast cells (NHFs) that are homozygous for either Pro72 or Arg72. Pro72 and Arg72 NHFs showed indistinguishable response to the DNA damaging agent etoposide, as judged by phosphorylation of amino terminal residues of p53 and the transactivation of p53 target genes such as p21 and MDM2. In contrast Pro72 and Arg72 NHFs responded quite differently to metabolic stress induced by either pharmacological activation of AMPK or by glucose deprivation. Specifically, the Arg72 variant was seen to respond more efficiently to metabolic stress, with increased stabilization and amino-terminal phosphorylation. QRT-PCR analysis was conducted to identify differences in the transcriptional profiles between Arg72 and Pro72 variants of p53 in response to metabolic stress. These studies should contribute to our further understanding of the impact of the Arg72/Pro72 polymorphism on p53 function.

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Nucleosome binding by p53 within the p21 promoter in vivo precedes nucleosome loss and transcriptional activation

It is well established that p53 contacts DNA in a sequence-dependent manner in order to transactivate its myriad target genes. Yet only little is known about how p53 interacts with its binding site/response element (RE) within such genes in vivo in the context of nucleosomal DNA. In this study we demonstrate that both distal (5') and proximal (3') p53 REs within the promoter of the p21 gene in unstressed HCT116 colon carcinoma cells are localized within a region of relatively high nucleosome occupancy. In the absence of cellular stress, p53 is pre-bound to both p21 REs within nucleosomal DNA in HCT116 cells. Treatment of cells with the DNA-damaging drug doxorubicin or the p53 stabilizing agent Nutlin-3, however, is accompanied by p53-dependent but transcription-independent subsequent loss of nucleosomes associated with such p53 REs. We show that in vitro p53 can bind to mononucleosomal DNA containing the distal p21 RE, provided the binding site is not close to the diad center of the nucleosome. In line with this, our data indicate that the p53 distal RE within the p21 gene is located close to the end of the nucleosome (which shows diverse positioning). Thus, low- and high-resolution mapping of nucleosome boundaries around p53 REs within the p21 promoter have provided insight into the mechanism of p53 binding to its sites in cells and the consequent changes in nucleosome occupancy at such sites.

POSTER 110

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A small molecule inhibitor of p53 stimulates amplification of hematopoietic stem cells but does not promote tumor development in mice

It has been shown that genetic inhibition of p53 leads to enhanced proliferation of hematopoietic stem cells (HSCs). This could, in theory, contribute to the increased frequency of tumor development observed in p53-deficient mice and humans. In our previous work, we identified chemical p53 inhibitors (PFTs) that suppress the transactivation function of p53 and protect cultured cells and mice from death induced by γ irradiation (IR). Here we found that when applied to bone marrow cells in vitro or injected into mice, PFTβ impeded IR-induced reduction of hematopoietic stem cell (HSC) and hematopoietic progenitor cell (HPC) population sizes. In addition, we showed that PFTβ stimulated HSC and HPC proliferation in the absence of IR in vitro and in vivo and mobilized HSCs to the peripheral blood. Importantly, however, PFTβ treatment did not affect the timing or frequency of tumor development in irradiated p53 heterozygous mice used as a model for determination of carcinogenicity. Thus, although PFTβ administration led to increased numbers of HSCs and HPCs, it was not carcinogenic in mice. These findings suggest that chemical p53 inhibitors may be clinically useful as safe and effective stimulators of hematopoiesis.

POSTER 111

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New insights into the mechanism of stabilization of mutant p53 proteins

Human tumors and tumor cell lines exhibit a profound hyperstabilization of missense mutant p53 protein (mutp53) for reasons that are poorly understood. Here we report that tumor-derived mutp53 proteins show a profound lack of ubiquitination, leading to their severe degradation defects. We provide evidence that in addition to the expected lower levels of MDM2 due to impaired transcriptional activation, functional inhibition of the remaining MDM2 activity is a major determinant of the profound hyperstabilization of endogenous mutp53. Moreover, functional MDM2 inhibition is specific to mutp53 cancer cells, consistent with the notion that mutp53 proteins confer a positive gain-of-function to tumor cells that depends on its hyperstability. Biochemical evidence identifies the heat shock HSP90 multi-chaperone machinery - which is frequently activated and upregulated in cancer - as responsible for mutant p53 hyperstability. Our data indicate that specific pharmacologic inhibition of HSP90 by 17AAG accelerates ubiquitination and degradation of endogenous mutp53 in cancer cells, and implicate functional reactivation of MDM2. Furthermore, the only cytoplasmic histone deacetylase, HDAC6, positively regulates HSP90 binding to its client proteins. Conversely, hyperacetylation of HSP90 at K294 results in its dissociation from clients and their subsequent degradation. We found that similar to HSP90 inhibition by 17AAG, HDAC6 knockdown by siRNA also destabilizes mutp53. Moreover, HDAC6- specific inhibitor Sulfarafane (SFN) exhibits MDM2-mediated destabilization of endogenous mutp53. In support, the histone deacetylase SAHA also significantly reduces mutp53 levels and synergizes with 17AAG. Importantly, drug-mediated destabilization of mutp53 inhibits the malignant phenotype of human cancer cells. In sum, we show for the first time that a HSP90 inhibitor together with a specific (HDAC6) or broad HDAC inhibitor block HSP90's "cage" activity towards mutp53 and synergize towards dramatically destabilizing mutp53. Interestingly, SAHA (Vorinostat) was recently approved by the FDA for cancer therapy and 17AAG is in late phase (III) clinical trials.

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Restoration of p53 in the Tumors Overexpressing Mdm2

Under homeostasis, p53 activity is dampened by the murine double minute 2 (Mdm2). Overexpression of Mdm2 has been reported in a variety of human cancers with wild type p53, suggesting that elevating Mdm2 level is an alternative way to inactivate p53 during tumorigenesis. And yet, tumors exist with concomitant p53 mutation and Mdm2 overexpression, and although not frequent, have earlier onset and poorer prognosis. Mouse models also support the more malignant nature of tumors with both alterations. The onset of tumorigenesis in p53-deficient Mdm2 transgenic (Mdm2Tg) mice is much earlier than that in Mdm2Tg mice with a p53 wild type background. These observations suggest p53 loss imposes selection advantage in oncogenesis even if there is an increase in Mdm2 level. However, it is still not clear whether loss of p53 is required for maintaining the established tumors overexpressing Mdm2. Our group has established a mouse model, in which restoration of a hypomorphic p53 latent allele, p53neo, to a functional p53-neo allele by DNA recombination leads to remission or stasis of spontaneous tumors. Here, we hypothesize that p53 restoration in spontaneous tumors with simultaneous loss of p53 and overexpression of Mdm2 will lead to tumor growth suppression. To test this hypothesis, we are currently examining the effects of p53 restoration on spontaneous tumors and the underlying mechanisms by which tumors respond to p53 restoration in the p53neo/neo Mdm2Tg Cre-ERTM study mouse cohort and the p53neo/neo Mdm2Tg control cohort. Our in vitro data have indicated that moderate increase in Mdm2 levels (~4-fold) attenuates p53-dependent DNA damage response in mouse embryonic fibroblasts (MEFs), while restoring p53 expression in p53neo/neo Mdm2Tg Cre-ERTM MEFs alleviates the inhibition by Mdm2. Preliminary in vivo data suggest that restoration of p53 expression results in growth suppression of two tumors examined thus far in p53neo/neo Mdm2Tg Cre-ERTM mice.

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ARF promotes efficient MDMX degradation by MDM2

MDMX is a critical regulator of p53 activity following stresses imposed by DNA damage, loss of ribosomal integrity and aberrant mitogenic signaling. MDMX overexpression contributes to p53 functional inactivation in cancer. Elimination of MDMX is a potentially useful approach to activate p53 and sensitize tumors to chemotherapy drugs. MDM2 is the major cellular ubiquitin E3 ligase that mediates MDMX degradation, which can be accelerated by stress such as DNA damage or ribosomal stress. Here we show that induction of high-level MDM2 using Nutlin alone does not cause efficient MDMX degradation in certain tumor cells such as U2OS or HCT116, but can efficiently degrade MDMX in non-transformed human fibroblasts. Both increased MDMX mRNA level and inefficient degradation of MDMX in tumor cells after Nutlin prevent MDMX elimination in the presence of high-level MDM2. Restoration of ARF expression in tumor cells enables MDM2 to degrade MDMX in a dose-dependent fashion due to increased ubiquitination mediated by MDM2. ARF binds specifically to MDM2 but not MDMX, increases MDMX-MDM2 binding efficiency and promotes MDMX ubiquitination. These results show that ARF cooperates with MDM2 to regulate MDMX in normal cells and loss of ARF expression during tumor genesis leads to a defect in MDMX degradation that further compromises p53 function.

POSTER 114

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Negative Regulation of the Tumor Suppressor p53 Gene by MicroRNAs

The tumor suppressor p53, encoded by the TP53 gene, is recognized as the guardian of the human genome because it regulates many downstream genes to exercise its function in cell cycle and cell death. Recent reports have revealed that several microRNAs (miRNAs) are important components of the p53 tumor suppressor network with miR-125b and miR-504 directly targeting TP53. In this report, we use a screening method to identify that two miRNAs (miR-25 and miR-30d) directly target the 3'UTR of TP53 to down-regulate p53 protein levels and reduce the expression of genes that are transcriptionally activated by p53. Correspondingly, both miR-25 and miR-30d adversely affect apoptotic cell death, cell cycle arrest, and cellular senescence. Inhibition of either miR-25 or miR-30d expression increases endogenous p53 expression and elevates cellular apoptosis in several cell lines, including one from multiple myeloma that has little TP53 mutations. Thus, beyond miR-125b and miR-504, the human TP53 gene is negatively regulated by two more miRNAs: miR-25 and miR-30d. We will report that all four miRNA binding sites on the TP53 3'UTR have somatic mutations in a sequencing project with ~1,200 tumor specimens and that we have knockouted out a allele of a miRNA that targets the p53 pathway.

POSTER 115

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Regulation of Nucleostemin

Nucleostemin (NS) is a nucleolar GTP-binding protein that is probably involved in rRNA processing, essential for embryonic stem cell proliferation, and highly expressed in several human cancers. Previously our lab and others have shown that NS can act a key regulator of stem cell proliferation and cell cycle regulation through p53. We have identified a novel mechanism by which both overexpression and depletion of NS can abrogate MDM2 induced degradation of p53, leading to cell cycle arrest. These results suggest that not only are the levels of NS finely monitored, but also aberrant NS expression is detrimental to cells. However, how NS itself is regulated still remains unknown. GTP binding is necessary for NS localization in the nucleolus and the disruption of GTP binding can not only trigger NS relocation into the nucleoplasm, but also reduce NS protein levels. Furthermore, depletion of GTP in culture can induce NS depletion, which is rescued by treatment with proteasome inhibitor MG132, indicating NS is regulated by a proteasome-dependent pathway. Here, our preliminary studies show that NS is ubiquitinated in vivo. Interestingly, the polyubiquitination chains marking NS are linked by ubiquitin lysine residue 63, which is associated with signaling function rather than proteasomal degradation. Based on these observations, we hypothesize is that upon GTP depletion, NS is relocated from the nucleolus to the nucleoplasm where it is degraded by an ubiquitin-independent mechanism. This hypothesis will be tested by the following specific aims: 1) To determine the role of GTP binding in regulating by comparing the GTP binding mutant NS and wild type NS and monitoring changes in protein stability; 2) To identify and purify the NS E3 ligase from cell culture using affinity purification and mass spectrometry; 3) To identify the functional role of the NS E3 ligase.

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POSTER 116

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Roles for the P/CAF Acetyltransferase in p53 Stabilization and p21 Induction

Oncogene activation is countered in mammalian cells in part through ARF-mediated p53 stabilization and activation. Though ARF is well known to stabilize p53 through inhibition of MDM2 E3 ubiquitin ligase activity, human p14ARF additionally promotes the acetylation of both the p53 C-terminus and DNA binding domain. To date, the HAT responsible for ARF-induced p53 acetylation has not been identified. To determine which HAT is responsible for this activity, and to define the importance of p53 acetylation in ARF signaling, we expressed siRNAs against the known p53 HATs p300, CBP, and P/CAF in the presence or absence of exogenous p14ARF. In line with previous reports characterizing p300/CBP as E4 enzymes for p53, p300 or CBP knockdown stabilized p53; however, knockdown of P/CAF completely abrogated p53 stabilization as well as p21 induction in response to p14ARF, suggesting that P/CAF plays a critical role in p14ARF signaling to p53. In addition to its role in ARF signalling to p53, P/CAF was also required for the transactivation of p21 expression by exogenous p53 in p53-null cells. Surprisingly, transcriptional activation of p21 by the p53 K320R mutant was also dependent on P/CAF, suggesting that the role for P/CAF in p21 induction is independent of K320 acetylation, a previously described P/CAF target site. ChIP analysis of the p21 promoter also indicated that promoter occupancy by p53 was not altered by P/CAF knockdown. Together our experiments indicate that P/CAF is necessary for ARF-mediated stabilization of p53, as well as transactivation of the p21 promoter by exogenous p53.

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D-peptide inhibitors of the p53-MDM2 interaction for targeted molecular therapy of malignant neoplasms

MDM2 negatively regulates the activity and stability of the tumor suppressor protein p53, conferring tumor development and survival. Small molecule inhibitors that target the p53-binding domain of MDM2 activate the p53 pathway, validating a new therapeutic paradigm for cancer treatment. Using mirror image phage display, we have identified several high-affinity D-peptide inhibitors of the p53-MDM2 interaction, including DPMI-α (TNWYANLEKLLR, 219 nM), DPMI-β (TAWYANFEKLLR, 34 nM), and DPMI-γ (DWWPLAFEALLR, 53 nM). Crystallographic studies coupled with systematic mutational analysis structurally and biochemically verified the mode of action of these D-peptide antagonists of MDM2. Despite being resistant to proteolysis, the D-peptide inhibitors failed to actively traverse the cell membrane and, when conjugated to cationic cell-penetrating peptides, indiscriminately caused necrotic cell death independently of p53 status. When encapsulated in liposomes, however, DPMI-α exhibited potent p53-dependent growth inhibitory activity against glioma tumor cells in culture. Liposome-encapsulated DPMI-α effectively inhibited tumor growth in a subcutaneous-transplant model of U87 human glioblastoma xenografts, and prolonged the survival of nude mice bearing intracranial glioblastoma. Our findings validate D-peptide antagonists of MDM2 as a novel class of p53 activators for targeted molecular therapy of malignant neoplasms harboring wild type p53 and elevated levels of MDM2.

POSTER 118

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Genotoxic exposure: novel cause of selection for a functional dN-p53 isoform

The p53 gene is frequently mutated in cancers and it is vital for cell cycle control, homeostasis and carcinogenesis. We describe a novel p53 mutational spectrum, different to those generally observed in human and murine tumors. Our study shows a high prevalence of nonsense mutations in the p53 N-terminus of 2-AAF induced urinary bladder tumors. These nonsense mutations forced downstream translation initiation at codon 41 of Trp53, resulting in the aberrant expression of the p53 isoform dN-p53 (or p44). Genotoxic 2-AAF exposure resulted in a significant selection ($p = 0.0011$) of nonsense mutations upstream of the alternative start site 41 when compared to the rest of the gene. Previously, it has been reported that the dN-p53 isoform occurred naturally or as a splice variant. We show that chemical exposure can act as novel mechanism for the origination and the selection for this isoform and that codon 41 is used as an alternative start site. Additionally, our data suggest that the occurrence of dN-p53 accounts, at least in mice, for a cancer phenotype. We also show that microarray profiles of ES cells carrying the dN-p53 isoform in a p53 null background are divergent from p53 knock-out ES cells, and therefore postulate that dN-p53 itself has functional transcriptional properties. We are currently creating mouse models expressing the isoform in different genotypes (in the absence and presence of full length p53) to further investigate this functional isoform.

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Deacetylation of the DNA-binding domain regulates p53-mediated apoptosis

In unstressed cells, the p53 tumor suppressor is highly unstable. DNA damage and other forms of cellular stress rapidly stabilize and activate p53. This process is regulated by a complex array of post-translational modifications that are dynamically deposited onto p53. Recent studies show that these modifications orchestrate p53-mediated processes such as cell cycle arrest and apoptosis. Cancer cells carry inherent genetic damage, but avoid arrest and apoptosis by inactivating p53. Defining the enzymatic machinery that regulates the stress-induced modification of p53 at single-residue resolution is critical to our understanding of the biochemical mechanisms that control this critical tumor suppressor. Specifically, acetylation of p53 at lysine 120 (K120), a DNA-binding domain residue mutated in human cancer, is essential for triggering apoptosis. Given the oncogenic properties of deacetylases and the success of deacetylase inhibitors as anti-cancer agents, we investigated the nature of K120 deacetylation using pharmacologic and genetic approaches. This analysis revealed that HDAC1 is the enzyme predominantly responsible for the deacetylation of K120 and that this event requires KAP1 and the NuRD co-repressor complex. Furthermore, treatment with clinically-relevant HDAC inhibitors enhances K120 acetylation, an event that is mechanistically linked to their apoptotic effects. These data expand our understanding of the mechanisms controlling p53 function and suggest that regulation of p53 modification status at single residue resolution by targeted therapeutics, can selectively alter p53 pathway function. This knowledge may impact the rational application of deacetylase inhibitors in the treatment of human cancer.

POSTER 120

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Poster Session 2
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The human Toll-like receptor family is integrated into the DNA damage and p53 response network

Human Toll-like receptors (TLRs) is a family of membrane receptors that play a key role in inflammatory responses against pathogen infection. Recognition of pathogen-associated molecules present on bacteria, viruses, fungi and protozoa by TLRs, results in activation of both innate and adaptive immune responses. Our studies of promoter sequences targeted by the p53 master regulatory transcription factor suggested a general role for this stress-responsive tumor suppressor in human TLR expression. We find that expression of all TLRs tested (TLR1-TLR10) in primary human blood lymphocytes and alveolar macrophages, is inducible by DNA metabolic stressors although there is considerable inter-individual variability. Most TLRs respond to p53 via canonical and noncanonical target binding sites in their promoters. Furthermore, a polymorphism in a TLR8 response element provides the first human example of a p53 target sequence specifically responsible for endogenous gene induction. Surprisingly, p53 regulated TLR expression appears unique to primates suggesting that evolutionary expansion of the p53 network to TLRs provided greater integrated responsiveness to environmental threats. Our findings demonstrate that regulation of the human innate immunity system is responsive to DNA metabolic stresses and has many implications for human health and disease.

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Poster Session 2
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Mutant p53 drives an invasive program that involves multiple RTKs

p53 is mutated in about half of all tumours. Most of these mutations lead to the expression of a mutant p53 protein that has not only lost its tumour suppressive function, but has also acquired tumour promoter capabilities in driving invasion and metastasis. Previously, we showed that mutant p53 expressing cells invade better in vivo and in vitro than p53 null cells. Mutant p53 was shown to drive enhanced signalling through the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (RTK) that can drive invasion via signalling to AKT. This activity appears to reflect the ability of mutant p53 to inhibit p63, resulting in enhanced Rab Coupling Protein (RCP) and integrin facilitated recycling of the EGFR. As integrins have been reported to interact with multiple RTKs, we investigated the possibility that mutant p53 might regulate other RTKs. Using inducible mutant p53 cells, we now observed mutant p53 dependent dispersion of cells. Dispersion is frequently observed after hepatocyte growth factor (HGF) stimulation, the ligand for another RTK, c-Met. A c-Met inhibitor completely blocked mutant p53 dependent dispersion, implying that mutant p53 also regulated c-Met. c-Met dependent dispersion is caused by loss of cell-cell junction proteins and we detected a loss of tight junction proteins after mutant p53 induction. Furthermore, mutant p53 induced a potent c-Met dependent invasion towards HGF. Using siRNA against specific tight junction proteins we induced invasion of control cells to levels observed after mutant p53 expression, indicating the importance of loss of cell-cell junctions in invasion. Both invasion towards HGF and dispersion were dependent on integrins, RCP and p63, suggesting that mutant p53 might regulate c-Met in a similar fashion as EGFR. These data suggest that mutant p53 drives metastasis by regulating multiple RTKs that cause dissociation of cells from the tumour mass and promote invasion.

POSTER 122

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Mutant p53 drives invasion through induction of an oncomiR (miR-155) and silencing of ZNF652 expression

Mutations in the TP53 gene occur in approximately half of all cancers. Not only do these mutations ablate the tumour suppressor functions of p53, but they also acquire a gain-of-function which endows an enhanced invasive and metastatic potential to cancer cells expressing mutant p53. We have identified an oncogenic microRNA (miR-155) that is specifically activated by mutant p53. This oncomiR is associated with invasion and metastasis of breast tumours in vivo. Activation of miR-155 results from the gain-of-function properties of mutant p53, as miR-155 is constitutively repressed by wild-type p53 in a physiological setting[1]. To our knowledge, miR-155 is the first microRNA identified as a transcriptional target of p53 gain-of-function mutants. A search for cancer-related target genes of miR-155 revealed ZNF652, a novel zinc finger transcriptional repressor. ZNF652 was demonstrated to directly repress the expression of several genes involved in invasion and metastasis, including EGFR. Silencing of ZNF652 in an epithelial cancer cell line promoted invasion into matrigel. Importantly, loss of ZNF652 expression in primary breast tumours was significantly correlated with increased local invasion. Taken together, these findings define a novel pathway whereby mutant p53 drives invasion through indirect silencing of ZNF652 due to stimulation of miR-155 in breast tumours.

[1]Brosh et al. p53-repressed miRNAs are involved with E2F in a feed-forward loop promoting proliferation. Mol Sys Biol (2008) 4:229.

POSTER 123

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The critical role of Lysine120 in specific p53-DNA recognition and selective gene transactivation

p53 can serve as a paradigm in studies of how allosteric perturbations in transcription factors triggered by small changes in DNA response element (RE) sequences, can spell selectivity in co-factor recruitment. p53-REs are 20-base pair DNA segments specifying diverse functions while they share a common motif. A key question is then how does a particular p53-RE sequence perturb the p53 structure and conformation? Here, representative p53-REs regulating diverse functions including cell cycle arrest, DNA repair, and apoptosis were simulated in explicit solvent. Among the major interactions between p53 and its REs involving Lys120, Arg280 and Arg248, the base pairs interacting with Lys120 vary, while the interacting partners of other residues are less so. We observe that each p53-RE quarter site sequence has a unique pattern of interactions with p53 Lys120. The allosteric, DNA sequence-induced conformational and dynamic changes at the altered Lys120 interactions are amplified by the perturbation of other p53-DNA interactions such as Arg280-G base hydrogen bonding. The combined subtle RE sequence-specific allosteric effects propagate in the p53 and in the DNA. The resulting amplified allosteric effects far-away are reflected in changes in the overall p53 organization and in the p53 surface topology and residue fluctuations which are known to play key roles in selective co-factor recruitment. As such, these observations suggest how similar p53-RE sequences can engage the preferred co-factor binding, a key to the selective gene transactivation.

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The p53-Mdm2 autoregulatory loop is essential for hematopoiesis but not p53 degradation after DNA damage

The p53 tumor suppressor regulates itself by binding the promoter and transactivating Mdm2, which encodes an inhibitor. To examine the significance of this autoregulation in vivo, we generated a knock-in mouse with mutated p53 response elements in the Mdm2 promoter. Surprisingly, autoregulation deficient Mdm2P2/P2 mice were viable with no phenotypic abnormalities, and after irradiation most tissues showed normal kinetics of p53 downregulation. However, in contrast to Mdm2+/+, Mdm2P2/P2 mice were extremely sensitive to sub-lethal irradiation and succumbed to drastic bone marrow aplasia. Post-irradiation, the hematopoietic stem cell pool was depleted and exhibited impaired repopulation capacity. These results demonstrate that the p53-Mdm2 autoregulatory loop is dispensable for embryonic development and p53 degradation, but is critical for maintaining the integrity of the hematopoietic compartment after DNA damage.

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POSTER 125

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Impact of TP53 Mutations on Outcome: Results from the CLL8 Trial (FC vs. R-FC) of the GCLLSG

There is growing evidence that TP53 mutations are associated with poor survival in CLL. There are limited prospective trial data on treatment effects of chemoimmunotherapy in CLL with and without TP53 mutation. The current study set out to define the impact of TP53 mutation in patients treated with FC or R-FC in the CLL8 trial.

Analysis of TP53 mutation status by microarray-based re-sequencing assay (Amplichip p53 Research Test in development by Roche Molecular Systems) and confirmatory direct DNA sequencing were performed in a central reference laboratory. Samples were available for 628 (76.9%) patients at study entry and this cohort was representative of the full trial population regarding other baseline prognostic factors and demographics. In addition, 94 follow-up samples of 89 patients at relapse were available.

The incidence of the TP53 mutations was 11.9% (71/628; 41 in FC arm, 30 in R-FC arm). Forty-two of 51 patients (82.4%) with 17p deletion had a TP53 mutation. 5% of patients without 17p deletion (28/553) had a TP53 mutation.

Patients with TP53 mutation showed lower complete response (CR) and overall response (OR) rates as compared to the group without TP53 mutation (6.9 vs. 36.4% and 62.1% vs. 95.3% ($p<0.001$).

In conclusion, 17p deletion and TP53 mutation (independent of 17p deletion) are powerful and independent prognostic markers after 1st line FC and R-FC treatment in CLL. When considering the genetic profile in a multivariate model including these, R-FC improves PFS and OS compared to FC in CLL

POSTER 126

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Treatment of Ewing Sarcomas through the pharmacological activation of p53

Patients diagnosed with Ewing Sarcoma have the most unfavorable prognosis of all primary musculoskeletal tumors, strongly suggesting the urgent need of systemic and targeted therapies to treat this aggressive pediatric malignancy. Ewing Sarcomas are associated with a remarkably low frequency of p53 mutations (< 10%), making this tumor type an ideal candidate for p53-targeted therapies. Thus, we have investigated the therapeutic potential of molecules that activate wildtype p53 to treat this aggressive pediatric malignancy.

We have examined the molecular and cellular responses associated with activation of the p53 pathway in Ewing Sarcoma cell lines through treatment with Nutlin-3a or 'low dose' Actinomycin D; two MDM2 antagonists. Our findings suggest that p53-dependent apoptosis is the primary cellular response to p53 activation in Ewing Sarcoma cell lines following exposure to Nutlin-3a and 'low dose' Actinomycin D. Sensitivity to these agents was restricted to cell lines that retain wildtype p53 status. Interestingly, Nutlin-3a and 'low dose' Actinomycin D were synergistic, suggesting that these compounds may have subtle differences in their mechanism of p53 activation. Furthermore, the antitumour activity of Nutlin-3a was also found to be synergistic with the chemotherapeutic agents Doxorubicin and Etoposide, two compounds currently used Ewing sarcoma chemotherapy protocols, and we have defined the dosages of these agents necessary to achieve the most potent synergistic effects. Due to the central role of p53 in oncogenesis and therapy response, findings from this study will provide comprehensive evidence to support the rational use of p53 activators as a novel systemic therapeutic approach for Ewing Sarcomas retaining wildtype p53.

POSTER 127

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Mdm2 Lysine 454 is Required for MdmX Interaction and Protein Stability but not p53 Degradation

One of the central roles of the Mdm2 oncoprotein is to regulate itself, the p53 tumor suppressor, and other protein targets via ubiquitination and the proteasomal degradation pathway. The C-terminal RING domain of Mdm2, which confers E3 ubiquitin ligase activity to the protein, is unique in that it contains a conserved P-loop motif characteristic of nucleotide binding proteins. We show that mutation of this region, specifically of lysine residue 454, alters the stability of Mdm2 in transfected H1299 cells, suggesting an important role for this residue in regulation of Mdm2 and its ubiquitination targets. We have found that both Mdm2 K454D as well as a previously investigated K454A mutant (Poyurovsky et al., Mol. Cell 2003) are significantly less stable in cells than wild type protein and are each deficient at binding to MdmX, a binding partner and homologue of Mdm2. This suggests that the source of the instability of these mutant versions of Mdm2 is their inability to form heteromeric complexes with MdmX. Interestingly, Mdm2 K454D, unlike Mdm2 K454A, is completely unable to degrade p53 in cells but is able to ubiquitinate p53 both in vivo and in vitro, indicating a defect in post-ubiquitination activities of Mdm2. Taken together, our data indicate that strong MdmX binding is required to stabilize Mdm2 in vivo but is not required for p53 degradation by Mdm2. Ultimately, investigation of this region of Mdm2 will help elucidate the mechanism of Mdm2 substrate specificity as well as the post-ubiquitination role of Mdm2 in degradation of p53, and may lead to therapies that redirect the ubiquitin ligase activity of overexpressed Mdm2 in human tumors.

POSTER 128

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DNA damage downregulates the transcription factor Sp1 leading to enhanced p21 protein stability and reinforcement of p53-dependent cell cycle checkpoints

The gene encoding the cyclin-dependent kinase inhibitor p21 contains response elements for both p53 and the transcription factor Sp1. Previous studies have suggested an obligate role for Sp1 in transcriptional upregulation of p21. To directly test this, an RNAi approach was used to ablate expression of Sp1. Surprisingly, such knockdown of Sp1 expression led to an increase in p21 expression at the protein but not the messenger RNA level. This was shown to be due to a prolonged half-life of the p21 protein in the basal setting that is Sp1-dependent. Interestingly, under these experimental conditions, the level of p21 approached that which is observed after DNA damage, yet this did not result in detectable cell cycle arrest. Mdm2 has previously been shown to target p21 for proteasome-mediated degradation and to be a target itself for Sp1-dependent transcriptional regulation. However, Mdm2 levels did not change as a result of Sp1 knockdown and cannot account for the increased levels of p21. This suggests that the observed Sp1-dependent alterations in p21 protein stability are due to a functional effect on Mdm2 or are, in fact, Mdm2-independent. Lack of Sp1 did not influence the p53-dependent increases in p21 mRNA levels after DNA damage. This finding suggests that p21 is not a transcriptional target of Sp1. However, the possibility of functional redundancy among Sp family members has not been fully addressed as yet. Thus, DNA damage causes a decrease in Sp1 protein levels that in turn upregulates p21 and augments the p53-dependent transcriptional effects on p21. This is consistent with the idea that Sp1 antagonizes p53-dependent cellular outcomes and may provide functional significance for high levels of Sp1 that are found in a subset of human tumors.

POSTER 129

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APC/C plays a role in ubiquitin-mediated turnover of ΔNp63α

p63 plays a pivotal role in epithelial development and morphogenesis. The ΔN isoforms of p63 are thought to be involved in both basal-epidermal gene expression and maintenance of epithelial progenitor proliferative potential. It is also thought that ΔN versions of p63 and p73 can serve to inhibit the abilities of their TA isoform counterparts to induce cell cycle arrest and apoptosis. Consistent with this, after DNA damage, while levels of p53 and TAp73 are increased, ΔNp63 proteins are quickly downregulated. To investigate how ΔNp63α is regulated in both normal and stress conditions, we sought to identify novel ΔNp63α interacting proteins. We partially purified ΔNp63α associated proteins from lysates of retrovirally infected FLAG- and HA-tagged ΔNp63α-expressing human keratinocyte HaCaT cells using sequential immunopurification with anti-Flag followed by anti-HA antibodies linked to beads. Using MALDI-TOF mass spectrometry analysis 20 polypeptides were identified which specifically associated with ΔNp63α protein. Among these were three components of the APC/C ubiquitin ligase complex. We confirmed that these subunits and other APC-associated proteins could interact with ΔNp63α in HaCaT cells using immunoprecipitation-immunoblotting analysis. Ectopic expression of the APC/C activators Cdc20 and Cdh1 led to reduced half-life of ΔNp63α when compared to ΔNp63α expressed alone. Further, the proteasome inhibitor MG132 suppressed degradation of ΔNp63 mediated by either Cdc20 or Cdh1. Importantly, siRNAs directed against Cdc20 increased levels of endogenously expressed ΔNp63α but not mutant p53 in HaCat cells. Taken together, our data suggest that the APC/C-Cdc20 complex plays a role in ubiquitin-mediated turnover of ΔNp63α.

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POSTER 130

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Expression of mitochondrial protein import machinery components in muscle of p53 knockout (KO) mice

We and others have previously demonstrated that p53 is important for maintaining skeletal muscle mitochondrial content and metabolism. Lack of p53 expression results in reduced mitochondrial biogenesis and impaired endurance capacity. Mitochondrial biogenesis is the result of the coordinated interplay between nuclear and mitochondrial genomes. Since the majority of mitochondrial proteins are imported into the mitochondria via the protein import machinery (PIM), we sought to elucidate whether the reduced mitochondrial content in muscle of p53 KO animals could be attributed to a decrease in the expression of PIM components. Subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria were isolated from mixed hindlimb muscle from p53 wildtype (WT) and knockout (KO) mice. Cytochrome c levels were reduced by 48% ($p<0.05$) in SS mitochondria from KO mice. The PIM receptor protein Tom20 was decreased by 35% ($p<0.05$) in the SS mitochondrial fraction from the KO animals. The chaperone protein mitochondrial heat shock protein70 (mtHSP70), crucial for import of proteins into the mitochondria, was depleted by ~60% in SS ($p<0.05$) and 54% in IMF ($p<0.05$) mitochondria from KO mice when compared to WT counterparts. Furthermore, mtHSP60 was reduced by ~50% in IMF mitochondria from KO. In contrast, another mitochondrial chaperone cpn10, did not change between the two genotypes. These data suggest that absence of p53 culminates in lower protein expression of important components of the PIM. Future experiments will be conducted to assess whether these decrements affect the rate of protein import in p53 KO mice and whether exercise, which promotes mitochondrial biogenesis, can rescue this defect. These findings may help define an important facet of the p53-mediated regulation of mitochondrial biogenesis and homeostatic metabolism.

POSTER 131

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Tim50, a Component of the Mitochondrial Translocator, Contributes to Cell Growth and Survival in Mutant p53 Expressing Cells

Mutations in the tumor suppressor protein, p53 are some of the most common mutations in human cancers. Some p53 mutations, in addition to eliminating the normal functions of p53, impart additional oncogenic properties to cells and have therefore been deemed p53 gain of function mutations. To investigate how gain of function p53 mutants exert their oncogenic effects, protein expression was compared between a p53 null cell line (H1299) stably expressing vector alone or the p53 gain of function mutants p53-R175H and -R273H. One protein that was upregulated 2-3 fold in H1299 cells expressing the p53 gain of function mutant p53-R175H and -R273H compared to the vector control cell line was identified by mass spectrometry as translocator of the mitochondrial membrane 50 (Tim50). Analysis of a panel of breast cancer cell lines indicated that Tim50 protein expression was approximately 15 and 50 fold higher in MDA-MB-468 and SK-BR-3 cells respectively which harbor the p53 mutants -R175H and -R273H compared to MCF-10A cells that express wild-type (WT) p53. In addition, p53-R175H and -R273H, but not WT p53, upregulated the luciferase activity of a Tim50 promoter construct. Tim50 siRNA reduced the growth rate and survival of cells from treatment with paclitaxel in H1299 cells expressing p53-R175H but had no significant effect on vector transfected cells. Taken together, this data suggests that one pathway by which mutant p53 may upregulate cell growth and chemoresistance is through induction of Tim50 protein expression, a protein critical for mitochondrial protein import and maintenance of mitochondrial membrane potential.

POSTER 132

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Mutant p53 facilitates somatic cells reprogramming and augments the malignant potential of reprogrammed cells

p53 deficiency was recently demonstrated to enhance the efficiency of somatic cells reprogramming. As p53 is usually mutated in human tumors and many mutated forms were shown to gain novel activities, we aimed to study the function of mutant-p53 in reprogramming. Our data indicate a novel gain-of-function property for mutant-p53, which dramatically enhanced the efficiency of this process compared to cells lacking p53. Importantly, this novel activity of mutant-p53 was accompanied with alterations in the characteristics of the reprogrammed cells; while p53-knockout cells, reprogrammed with only Oct4 and Sox2, maintained their pluripotent capacity in vivo, reprogrammed cells expressing mutant-p53 lost this capability, and gave rise to malignant tumors. This novel gain-of-function of mutant-p53 is not attributed to its effect on proliferation, as both p53-knockout and mutant-p53 cells displayed similar proliferation rates. In addition, we demonstrate an oncogenic activity of Klf4, as its overexpression in either p53-knockout or mutant-p53 cells induced aggressive tumors. Overall, our data highlight the notion that reprogrammed cells with the capacity to differentiate into the three germ layers in vitro can form malignant tumors, suggesting that in genetic instable cells, reprogramming may result in the formation of cells with cancer forming potential.

POSTER 133

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Concurrent activation of the p53 pathway and inhibition of the Bcl-2/Bcl-xL proteins as a cancer treatment strategy

Genetic mouse models and pharmacological studies have shown the tremendous potential of p53 activation as a cancer therapeutic strategy. Small-molecule inhibitors of the MDM2-p53 interaction activate p53 in cancers retaining wild-type (wt) p53, which constitute nearly half of all solid human tumors. Objective and Approach: p53 function remains suppressed in tumors with wtp53. MDM2 directly binds to and inhibits p53, while Bcl-2 and Bcl-xL negatively modulate p53-induced apoptosis. The objective of the study is to evaluate whether the concurrent targeting of MDM2 and Bcl-2/Bcl-xL will sensitize tumor cells to apoptosis. We have designed MI-219, a high affinity selective small-molecule MDM2 inhibitor. ABT-737, a potent and selective small-molecule inhibitor of Bcl-2/Bcl-xL proteins designed by Abbott Laboratories, was used in this study. Results and conclusions: MI-219 induces selective apoptosis in prostate tumor cells in vitro and in vivo and completely inhibits the growth prostate tumor xenografts. MI-219 induces neither apoptosis in melanoma cancer cell lines nor does it inhibit melanoma tumor xenograft growth. RNA interference (RNAi) studies revealed that apoptosis induction by MI-219 is p53 and Bax dependent. These data suggested that the Bcl-2/Bcl-xL proteins will inhibit MI-219-induced apoptosis and treatment with Bcl-2/Bcl-xL inhibitor will sensitize the cells to apoptosis by MI-219. We validated this combination therapeutic approach using RNAi strategy. Silencing Bcl-2 and Bcl-xL gene expresssion in tumor cells enhanced MI-219 induced apoptosis. Importantly, we found that the combination of MI-219/ABT-737 combination induces rapid and robust apoptosis in human melanoma and prostate cancer cell lines. Cellular apoptosis data were supported by biochemical apoptosis markers. Furthermore, apoptosis by MI-219/ABT-737 was found to be p53 and Bax dependent. In conclusion, MI-219/ABT-737 combination accelerates and enhances tumor cell apoptosis. Significance: Our data suggest that concurrent activation of p53 and inhibition of the Bcl-2/Bcl-xL is a promising strategy for cancer treatment.

POSTER 134

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PRIMA-1MET targets mutant p53 family members p63 and p73

The low molecular weight compound PRIMA-1 and its structural analog PRIMA-1MET /APR-246 reactivate mutant p53 through covalent binding to the core domain, trigger apoptosis in cultured tumor cells, and inhibit xenograft tumor growth in vivo. We have tested the effect of PRIMA-1MET/ APR-246 on mutant forms of the p53 family members p63 and p73 that share high homology with p53. We found that PRIMA-1MET stimulated DNA binding of mutant TAp63γ and induced expression of the p53/p63/p73 downstream targets p21 and Noxa. PRIMA-1MET/ APR-246 restores the pro-apoptotic activities to mutant TAp63γ and TAp73β in tumor cells. Furthermore, PRIMA-1MET/ APR-246 reactivates mutant p63α for induction of differentiation in human keratinocytes derived from patients carrying endogenous mutant p63. These results indicate that PRIMA-1MET/ APR-246 interacts with the homologous structures in point mutant p53 family proteins and thus restores common functional defects.

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Structural and Functional Comparison of the RING Domains of the p53 E3 ligases, Mdm2 and Pirh2

The tumor suppressor p53 maintains genome stability and prevents malignant transformation by promoting cell cycle arrest and apoptosis. Both Mdm2 and Pirh2 have been shown to ubiquitylate p53 through their RING domains, thereby targeting p53 for proteasomal degradation. Through structural and functional analyses, we show that the Pirh2 RING domain differs from the Mdm2 RING domain in its oligomeric state, surface charge distribution and zinc coordination scheme. Pirh2 possesses weaker E3 ligase activity towards p53 and directs ubiquitin to different residues on p53. NMR and mutagenesis studies demonstrate that while Pirh2 and Mdm2 share a conserved E2 binding site, the seven C-terminal residues of the Mdm2 RING form an extended E2 binding surface, a feature unique to Mdm2 and absent in the Pirh2 RING domain. This comprehensive analysis of the Pirh2 and Mdm2 RING domains provides structural and mechanistic insight into p53 regulation by its E3 ligases.

POSTER 136

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The role of the candidate tumor suppressor BTG3 at the crossroad of cellular senescence and neoplastic transition

The B-cell translocation gene 3 (BTG3) is a member of the antiproliferative BTG gene family and a downstream target of p53. BTG3 also binds and inhibits E2F1, a transcription factor required for cell proliferation. Although it connects functionally two major growth regulatory pathways, the physiological role of BTG3 remains largely uncharacterized. Furthermore whether and how BTG3 is related to the tumorigenic process is also unclear. In this study, we present evidence that loss of BTG3 in normal cells mimics oncogenic stress and induces hallmarks of cellular senescence such as SAHFs, DNA damage foci and expression of p16INK4a that correlates with enhanced ERK-AP1 signaling. In addition, BTG3 binds and inhibits AKT activation. BTG3 ablation in cancerous cells resulted in increased AKT-GSK3β signaling, elevated beta-catenin activity, and enhanced cell migration. Importantly, BTG3 expression is specifically downregulated and inversely correlated with AKT phosphorylation in human prostate cancer. Collectively, our study provides mechanistic insights on the biological consequences of BTG3 loss and unveils a potential role for this protein in the tumorigenic process of prostate cancer.

POSTER 137

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Differential regulation of p53 and p21 by MKRN1 E3 ligase controls cell cycle arrest and apoptosis

Makorin Ring Finger Protein 1 (MKRN1) is a transcriptional co-regulator and an E3 ligase. Here, we show that MKRN1 simultaneously functions as a differentially negative regulator of p53 and p21. In normal conditions, MKRN1 could destabilize both p53 and p21 through ubiquitination and proteasome-dependent degradation. As a result, depletion of MKRN1 induced growth arrest via activation of p53 and p21. Interestingly, MKRN1 utilized previously unknown sites, K291 and K292, for p53 ubiquitination and subsequent degradation. Under severe stress conditions, however, MKRN1 primarily induced the efficient degradation of p21. This regulatory process contributed to the acceleration of DNA damage-induced apoptosis by eliminating p21. MKRN1 depletion diminished adriamycin or ultraviolet-induced cell death, while ectopic expression of MKRN1 facilitated apoptosis. Furthermore, MKRN1 stable cell lines that constantly produced low levels of p53 and p21 exhibited stabilization of p53 but not p21 with increased cell death upon DNA damage. Our results indicate that MKRN1 exhibits dual functions of keeping cells alive by suppressing p53 under normal conditions, and stimulating cell death by repressing p21 under stress conditions.

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Repression of Wip1 and induction of HdmX degradation upon reactivation of p53 by RITA contribute to robust apoptosis induction

The p53 tumour suppressor is frequently inactivated in human tumours, either by genetic mutation or by over-expression of its negative regulators, such as Hdm2 and HdmX (Mdm2 and Mdm4 in mice, respectively). Reactivation of p53 by targeting Hdm2 and HdmX is therefore a promising strategy for cancer treatment. However, Hdm2 inhibitors are not able to prevent inhibition of p53 by HdmX. Thus, blocking HdmX might be an important strategy for p53 reactivation. We have previously demonstrated that the small molecule RITA binds p53 thereby preventing p53/Hdm2 interaction. Here we show that p53 reactivation by RITA leads to efficient HdmX degradation in a number of tumour cell lines of different origin, as well as in xenographt tumours in vivo. Notably, HdmX degradation specifically occurs in cancer cells, but not in non-transformed cells. Our study reveals that the depletion of HdmX protein contributes to the robust induction of apoptosis by reactivated p53 in human tumour cell lines. We identified ATM-mediated destabilization and inhibition of stabilizing factors, namely Wip-1 and the PI3K/Akt as the major mechanisms of HdmX depletion upon RITA treatment. In contrast to previously reported transcriptional induction of its own inhibitor Wip1 by p53, we observed p53-dependent repression of Wip-1 expression, suggesting the existence of a molecular mechanism which prevents induction of the negative feedback loop conferred by Wip1, thus irreversibly shifting the survival-death balance towards elimination of tumour cells.

POSTER 139

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Modulation of the Vitamin D3 Response by Cancer-Associated Mutant p53

The p53 gene is mutated in many human tumors. Cells of such tumors often contain abundant mutant p53(mutp53) protein, which may contribute actively to tumor progression via a gain-of-function mechanism. We applied ChIP-on-chip analysis and identified the vitamin D receptor (VDR) response element as overrepresented in promoter sequences bound by mutp53. We report that mutp53 can interact functionally and physically with VDR. Mutp53 is recruited to VDR-regulated genes and modulates their expression, augmenting the transactivation of some genes and relieving the repression of others. Furthermore, mutp53 increases the nuclear accumulation of VDR. Importantly, mutp53 converts vitamin D into an antiapoptotic agent. Thus, p53 status can determine the biological impact of vitamin D on tumor cells.

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TAp63 controls energy metabolism through transcriptional regulation of SIRT1

SIRT1 (silent information regulator T1) and AMPK-activated protein kinase (AMPK) are two key metabolic regulators that reduce lipogenesis and increase fatty acid oxidation by sensing energy stresses like exercise, fasting, and caloric restriction (CR). Importantly, SIRT1 is also a critical regulator of longevity, and we have shown previously that TAp63 plays a similar role in preventing premature aging suggesting a possible relationship between these genes. Interestingly, we have found that the majority of TAp63-/- mice become obese and develop insulin resistance by 4 months of age, similar to what is seen in mouse models with tissue specific deletion of SIRT1. To determine whether the TAp63-/- mice have a metabolic disorder and whether this is through dysregulation of the SIRT1/AMPK pathway, we used mouse embryonic fibroblasts (MEFs) and liver tissues to show that TAp63 is indeed important in regulating energy metabolism. Specifically, we found that levels of fatty acid synthase (FAS) are high and carnitine palmitoyltransferase-I (CPT-I) are low in the livers of TAp63 deficient mice. Furthermore, loss of TAp63 abolished upregulation of phosphoenolpyruvate carboxykinase (PEPCK) and glutaminase 2 (GLS2) during caloric restriction indicating that TAp63 is required for the response to caloric restriction. To determine whether TAp63 directly regulates the SIRT1 through transcriptional regulation, we identified p63 binding sites within the SIRT1 promoter by chromatin immunoprecipitation. Our study defines a novel role of TAp63 in regulating energy metabolism and weight control in mice through regulation of SIRT1. Thus, TAp63 is a potential therapeutic target not only for cancer, but also for obesity and metabolic disorders.

POSTER 141

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In vitro targeting of p53 tumor suppressor and its homolog, p73 by protoporphyrin IX

Photodynamic therapy of cancer (PDT) requires phototosensitizer excited by the light to annihilate tumor cells mainly by the reactive oxygen species generation. We have previously shown that protoporphyrin IX (PpIX), the widely used photosensitizer in PDT, is able to induce apoptosis of cancer cells prior to light excitation which might be explained by the direct interaction between PpIX and p53 as estimated by the fluorescence correlation spectroscopy. This interaction probably rescues p53 from the binding to HDM2 and therefore activates p53-dependant pathway. Though, the exact mechanism remains undiscovered. The next unanswered question is, through what pathway PpIX induces apoptosis of p53-deficient cells in the dark. Here we show, with the use of the several biophysical techniques, that PpIX directly targets N-terminal transactivation domains of p53 and p73 in vitro. Firstly, we visualised the p53-PpIX and p73-PpIX complexes in a fluorescent band shift assay and further the results were confirmed by the size exclusion chromatography. Finally, by the means of fluorescence polarization measurements we indicated the formation of p53-PpIX and p73-PpIX complexes observed as an increase of PpIX fluorescence polarization in comparison to the unbound photosensitizer. Our data provide strong evidence that PpIX targets N-terminal domains of p53 and p73 in vitro. We propose the hypothesis where PpIX by direct binding to transactivation domains, disrupts p53/MDM2 or MDMX and p73/MDM2 and therefore activates the apoptotic response driven by the described tumor suppressor proteins. Of notice, further in vitro analysis is needed to confirm our findings.

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Pro-apoptotic Puma, and to a lesser extent Noxa, are critical for the therapeutic effects of the p53-activating compound Nutlin3a.

The transcription factor p53 is a tumour suppressor, mutated in ~50% of human cancers. Activated by a range of stress stimuli, including DNA damage or oncogene activation, p53 triggers cell cycle arrest, DNA repair and/or apoptotic cell death, depending on cell type and extent of insult. Several processes, including ubiquitination by the E3 ubiquitin ligase, MDM2, regulate p53's activity. MDM2 is over-expressed in certain cancers with resultant decreased cellular levels and consequently reduced activity of p53, facilitating resistance to apoptotic/growth arrest stimuli. Nutlin3a, a novel non-genotoxic anti-cancer therapeutic, represents a new approach to treat tumours retaining potentially functional p53. Binding specifically to MDM2 and blocking its interaction with p53, Nutlin3a restores/increases functional p53 levels. We investigated which downstream p53 pathways are activated by Nutlin3a to promote tumour cell killing and which ones facilitate collateral killing of normal cells, such as lymphoid ones. Specifically, we examined whether the reported Nutlin3a-induced decreases in leukocytes require Puma and/or Noxa-dependant apoptosis or p21-dependant cell cycle arrest. Utilising in vitro cell-survival assays, with lymphocytes from gene-targeted mice lacking p53 downstream effectors, we have shown that loss of Puma causes resistance to Nutlin3a-induced apoptosis. The additional loss of Noxa further enhances this resistance. Using in vivo drug trials we have shown that Nutlin3a reduces thymocyte numbers by ~85% and mature lymphocyte numbers in spleen, lymph nodes and blood by ~50-85%. This loss is largely abrogated in mice lacking Puma and almost completely abrogated by combined loss of Puma and Noxa. Furthermore through use of in vitro cell-survival assays we have shown that Eμ-myc lymphomas lacking Puma are resistant to Nutlin3a-induced cell death. These results show transcriptional induction of pro-apoptotic BH3-only Bcl-2 family members Puma and to a lesser extent Noxa, but not the cell-cycle inhibitor p21, is critical for the therapeutic effects of the p53-activating compound, Nutlin3a.

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Mitochondrial p53 Regulates Mitochondrial Permeability Transition During Oxidative Stress-Induced Programmed Necrosis

It is well established that besides being a transcriptional regulator, p53 exerts numerous transcription-independent functions. One of the latter is the direct apoptotic mitochondrial p53 program. Upon stress, a cytoplasmic pool of p53 rapidly translocates to mitochondria, where it physically interacts with both anti- and pro-apoptotic Bcl-2 family members to inhibit or activate their functions, respectively, inducing mitochondrial outer membrane permeabilization (MOMP) and apoptosis. Whether p53 has a role in programmed necrosis, a pathologically very important form of cell death of normal and tumor tissues is unclear. Here we provide evidence that during oxidative stress mitochondrial p53 plays an important role in regulating the mitochondrial Permeability Transition (mPT), an event that precedes programmed cell necrosis. In contrast to the prototypical MOMP-inducer tBid, purified p53 protein regulates the PTP pore independently of Bax and Bak in isolated mitochondria. Treatment of primary and cancer cell lines with hydrogen peroxide (H2O2), the classic oxidant to study cellular response to oxidative stress, induces rapid translocation of p53 to mitochondria. p53 interacts directly with the obligatory PTP-regulatory protein cyclophilin D, but not with VDAC and ANT which are structural PTP components. Both p53 -/- and cyclophilin D-deficient cells show reduced sensitivity to H2O2-induced PTP opening and cell death. Conversely, in p53-proficient cells and mitochondria, PTP opening is inhibited by the cyclophilin D inhibitor cyclosporine A. Blocking p53 transcriptional activity does not significantly prevent mPT, indicating direct involvement of mitochondrial p53 in regulating PTP in vivo. Mitochondrial targeting of p53 increases mPT and cell death caused by H2O2, independently of Bax and Bak and in a cyclophilin D-dependent manner. Necrotic rather than apoptotic cell death was evident, as measured by viability assays, electron microscopy, HMGB1 release and TUNEL. In sum, we describe a novel function of mitochondrial p53 in regulating PTP and programmed necrosis during oxidative stress.

POSTER 144

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p53 mutants induce oncogenic functions in cancer cells by forming acetylated histones on chromatin of induced oncogenic genes.

Cancer cells with p53 mutations show higher oncogenic activity measured by aggressive growth rate in tumor and in cell culture, and demonstrate reduced sensitivity towards chemotherapeutic drugs. Here, using a number of breast and lung cancer cell lines with mutated p53, we show that lowering the level of p53 lowers oncogenicity. Using RNAi against p53 family members, p73 and p63, we demonstrate that the gain of function observed by the expression of mutant p53 in reducing chemosensitivity cannot be explained by the ability of mutant p53 to neutralize p73 and p63 alone. Tumor-derived mutant p53 expression up-regulates p52/p100, Axl, and other oncogenic genes. RNAi against mutant p53 targets lowers oncogenicity suggesting that mutant p53 induces increased oncogenicity by up-regulating expression of growth promoting genes. Chromatin immunoprecipitation (ChIP) assays indicate that histones H3 and H4 get specifically acetylated by mutant p53 expression on the regulatory regions of mutant p53 target genes at specific sites. Bio-informatic analysis of mutant p53 targets indicates one of the common target sites for mutant p53 on promoters induced by p53 mutants - CREB indicating involvement of CREB-binding protein (CBP) and it family members. Thus, our work has uncovered a mechanism used by mutant p53 to induce oncogenic changes in cancer cells.

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Fine-tuning p53 activity through C-terminal modification significantly contributes to HSC homeostasis and mouse radiosensitivity

Cell cycle regulation in hematopoietic stem cells (HSCs) is tightly controlled during homeostasis and in response to extrinsic stress. p53, a well known tumor suppressor and transducer of diverse stress signals, has been implicated in maintaining HSCs quiescence and self-renewal. However, the mechanisms that control its activity in HSCs, and how it in turn contributes to HSC cell cycle control, are poorly understood. Here, we use a genetically engineered mouse to show that p53 C-terminal modification is critical for controlling HSC abundance during homeostasis and HSC proliferation after irradiation. We previously generated a mouse p53 mutant, in which 7 C-terminal conserved lysines were replaced with arginines (7KR) to prevent ubiquitinylation or acetylation, to demonstrate that p53 function is fine tuned in vivo (Krummel, 2005). In this study, we showed that when 7KR mice were subjected to a low dose of whole body ionic irradiation that has little effect on wild type mice, half of them died of heart failure, which is a secondary effect from severe anemia. CBC and colony formation analysis revealed that irradiation caused pancytopenia and defects in hemapotoietic progenitors in 7KR mice. When transplanted into lethal irradiated recipient mice, 7KR bone marrows exhibited severe defects in long term repopulation even in the absence of irradiation, indicating that the hematopoietic defects of 7KR mice reside in the HSC compartment. Consistently, FACS analysis indicated that even before exposed to the irradiation, HSC in 7KR BMCs was 2 fold lower than the one in the wild type. We further showed that the mechanism involves fine-tuning the expression levels of the cyclin dependent kinase inhibitor p21, a p53 target gene. In summary, our data suggest that preventing p53 C-terminal modification renders mice exquisitely radiosensitive due to defects in HSC proliferation, a critical determinant for restoring hematopoiesis after irradiation. Our study has implications for cancer therapy and for radioprotection strategies.

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POSTER 146

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Targeted rescue of the destabilized p53 core domain mutant Y220C: From the first discovery of small molecule binders to lead candidates

The large-to-small mutation Y220C, located in the beta-sheet region of the p53 core domain, causes temperature sensitivity and destabilizes the protein by about 4 kcal/mol due to the formation of a surface cleft.[1] As a result, the mutant is almost completely unfolded at body temperature. Y220C is the ninth-most frequent p53 cancer mutant, accounting for about 75,000 new cancer cases per annum. We have proposed that Y220C can be rescued by small drug-like molecules that raise the stability of the mutant by binding to the mutation-induced cavity.[2] By combining in silico screening and biophysical testing, we have discovered PhiKan083, a small molecule binder based on a 9H-carbazole scaffold that binds to T-p53C-Y220C with an affinity of about KD = 150 µM, raises the melting temperature of the Y220C core domain and significantly prolongs its half-life at 37 °C.[3] Based on the carbazole scaffold we have identified molecules with improved binding properties that show specific p53-induced effects in cancer cell lines. In addition, we have developed a new class of compounds that are both non-toxic and easily available in terms of standard medicinal chemistry. These compounds exhibit improved binding properties and thus present promising candidates for lead optimization towards a potential cancer drug that rescues the function of the Y220C mutant in vivo.

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POSTER 147

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An illegitimate microRNA target site within the 3' UTR of MDM4 affects ovarian cancer progression and chemosensitivity

The tumor suppressor p53 is inactivated in the vast majority of cancers, either by mutations of the p53 gene itself or by alterations of p53-regulating factors. Genetic data have highlighted a critical role of mouse Mdmx in the regulation of p53. Even modest decrease in Mdmx levels compromises p53-dependent tumour suppression in mice. There is so far only limited evidence supporting the clinical relevance of MDMX in humans. We sequenced the MDM4 gene in a series of 113 ovarian carcinomas to identify mutations and/or single nucleotide polymorphisms (SNPs). We identified a SNP (SNP34091; A/C) in the 3'-UTR of MDM4 that creates a putative target site for has-miR-191, a microRNA that is highly expressed in normal and tumor tissues. Biochemical evidence supports specific miR-191-dependent regulation of the MDM4-C, but not MDM4-A, variant. Consistently, the A-allele was associated with statistically significant increased expression of MDM4 mRNA and protein levels in ovarian carcinomas. Importantly, the wild-type genotype (A/A) was found at higher frequency (62.5% versus 37.5 for A/C and C/C) in patients with high-grade carcinomas. Moreover, A/A patients that do not express the estrogen receptor had a 8.1-fold (p=0,009) increased risk of recurrence and 8.4-fold (p=0,012) increased risk of tumor-related death. Acquisition of an illegitimate miR-191-target site causes down-regulation of MDM4 expression and hence significantly delays ovarian carcinoma progression.

POSTER 148

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p21 and survivin do not affect the apoptotic outcome of non-genotoxic p53 activation

p21Waf1/Cip1 is a p53 transcription target implicated in both major functions of the tumor suppressor - cell cycle arrest and apoptosis. It is a potent inhibitor of the key cyclin-dependent kinases and has been thought to be the main mediator of p53-dependent G1 and G2 arrest. However, an increasing body of information suggests that in addition to its cell cycle inhibitory activity p21 can affect p53-dependent apoptosis. These data have been obtained from experiments in which p53 is activated primarily by genotoxic stress. Here, we use the selective MDM2 antagonist, nutlin-3a, as a non-genotoxic p53 activator and show that depletion of p21 did not increase the apoptotic response to nutlin-3a in all seven cancer cell lines tested and p21 overexpression did not protect apoptosis-sensitive lines from death. Disruption of the p53-MDM2 interaction by nutlin leads to stabilization and activation of p53 followed by profound changes in gene expression. One of the most prominently downregulated genes was BIRC5 encoding the survivin protein. Survivin is a member of the inhibitors of apoptosis protein (IAP) family frequently found overexpressed in human cancer presumably due to its anti-apoptotic role. We found that the nutlin-induced p53-dependent downregulation of survivin is mediated by elevated p21. However, neither depletion or overexpression of survivin affected the apoptotic outcome of nutlin treatment, suggesting that it does not play an anti-apoptotic role.

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POSTER 149

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The p53-MDM2-Rb network in controlling cell growth, apoptosis and cellular senescence

The tumor suppressor proteins p53 and retinoblastoma protein (Rb) play critical roles in regulation of cell growth, apoptosis and cellular senescence. Abundant evidence indicates that the p53 and Rb pathways crosstalk, which dictates cell fate upon various cellular stresses. The ubiquitin E3 ligase MDM2 is a key negative regulator to promote p53 protein degradation. Our work has demonstrated that MDM2 also binds to and promotes Rb degradation. Small MDM2 antagonist nutlin-3 potently activates p53 by blocking p53-MDM2 complex formation, resulting in cancer cell growth arrest and apoptosis. Nutlin-3 also significantly affects both Rb protein stability and Rb phosphorylation in cancer cells, which has profound impact on cell fate upon chemotherapy. In addition, we present evidence that ΔNp63α regulates metastasis suppressor CD82 and ERK1/2 modulator MKP3 to inhibit cancer cell migration and metastasis.

POSTER 150

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Poster Session 2
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A dinucleotide core within the p53 response element is critical in controlling p53-dependent transcriptional regulation

The tumor suppressor p53 is widely recognized as the "Guardian of the Genome," which functions as a master transcriptional regulator that affects diverse cellular events by regulating transcription of a broad range of target genes. It interacts with DNA containing p53 response element (RE) that was originally defined as 2 decamer motifs RRRCWWGYYY separated by a 0-13bp spacer, where "R" represents purine, "W" stands for adenine or thymine, and "Y" represents pyrimidine. However, even with a number of validated p53 RE sequences available, there are no definite rules to predict the consequence of p53 binding to specific DNA sequence on transcriptional behavior. Here, we report a systematic analysis on the role of specific nucleotides within the p53RE by using p21, a well-known target for p53 activation, and contrasting it with Lasp1 which was recently identified to be transcriptionally repressed by p53. Interestingly, luciferase assays using HCT116 (p53-/-) cells revealed that replacing Lasp1 p53RE with p21 p53RE sequence resulted in a functional conversion from p53-mediated repression to activation. A further nucleotide substitution analysis demonstrated a specific dinucleotide core combination within the p53RE CWWG motif to be the key factor that controls whether p53 transcriptionally activates or represses a target gene. Moreover, the flanking triplet RRR and YYY sequences were also shown to play an important role in modulating the transcriptional behavior of p53. By applying a set of predictive rules raised from the current study, we re-assessed 162 published p53REs and revealed distinct patterns of nucleotide frequency distribution in activating and repressing p53RE groups. From the above results, we were able to generate the consensus sequences for the p53 activating and repressing REs respectively. This clearer definition of the functional outcome of p53 interaction with its RE will provide better insight towards the understanding of its fundamental role in cellular networks.

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Allele Specific p53 Mutant Synthetic Lethality

Rescuing the function of mutant p53 is an attractive cancer therapeutic strategy. The NCI60 database has reported the growth inhibition of about 50,000 compounds in a panel of 60 human tumor cell lines with the p53 status characterized. We developed a new algorithm which could use the NCI60 dataset as an "in silico" screen to identify compounds with enhanced activity in cell lines with p53 mutations, but little activity in cell lines with wildtype p53. Through validation using isogenic mouse embryonic fibrablasts (MEFs) with human mutant p53 proteins, we identified NSC319726, a compound that has specificity to inhibit growth of cells with p53 mutations (175, 248, 273) but not in cells with wildtype p53. In particular, NSC319726 showed the strongest induction of apoptosis in the exogenous p53R175H MEF cells as well as a human tumor cell line with an endogenous p53R175H. Knockdown of mutant p53 by siRNA reduces the apoptotic response suggesting that the mechanism is depdendent on the 175 mutant protein. Immuno-fluorescent staining showed that, upon treatment of NSC319726, the conformation of the p53R175H protein has been changed from mutant to wild type form. These conformation changes are associated with an upregulation of several targets of p53 suggesting that wildtype p53 function could be restored. In conclusion, we have developed a bioinformatic approach to the identification of compounds that demonstrate sensitivity in cell lines with p53 mutations. NSC319726 preferentially induces apoptosis in cells with p53 mutations using a mechanism that involves restoration of the wildtype p53 protein function. Confirmation of this allele specificity in mouse tumor models is under investigation. This study will provide the basis for the therapeutic potential of this compound in tumors with specific p53 mutations.

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Targeting p53 residues S33 and S37 by RITA inhibits p53/MDM2 and p53/MDMX interactions via an allosteric mechanism

Small molecule RITA activates p53 by preventing p53/MDM2 interaction, thus inducing massive tumor cells apoptosis, yet it remained unclear how exactly it acts. Here we addressed the molecular mechanism by which RITA interferes with the binding with MDM2. Deletion and mutation analysis followed by molecular modeling and its thorough validation allowed to identify the key p53 residues targeted by RITA, S33 and S37, outside of the MDM2 binding site. This implies that p53/MDM2 interaction is prevented by RITA via formerly unrecognized allosteric mechanism. We propose that RITA/p53 binding induces a conformational trap locking MDM2-contacting residues in an orientation unfavorable for the interaction. This also prevents the binding of another p53 inhibitor, MDMX. Elucidation of allosteric mechanism regulating p53/MDM2 complex and identification of a structural element involved might facilitate the design of a new class of allosteric modulators of p53. Given the immense relevance of the p53 tumor suppressor for cancer, it is not surprising that several p53 activators which sterically inhibit MDM2 have been identified. Elucidating the mechanisms of action of p53-activating molecules will help to evaluate their safety and efficiency in clinic. Allosteric mechanism of action of p53 activator RITA which we discovered is an unexpected turn in the p53 story. Our findings could provide a basis for generation of p53 activators with the similar mechanism of action, which can facilitate development of novel therapeutics. Allosteric modulators might have great potential as single agents, as well as in combination therapy to decrease toxicity and resistance. Further, p53 modulators could serve as invaluable tools to better understand its biology.

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RNPC1, an RNA-binding protein and a target of the p53 family, regulates p63 expression through mRNA stability

p63, a p53 family tumor suppressor, is involved in many cellular processes, including growth suppression and differentiation. Thus, p63 activity needs to be tightly controlled. Here, we found that RNPC1, a RNA-binding protein and a target of the p53 family, regulates p63 mRNA stability and consequently p63 activity. Specifically, we showed that over-expression of RNPC1 decreases, whereas knockdown of RNPC1 increases, the half-life of p63 transcript, which leads to altered p63 expression. Consistent with this, we showed that RNPC1 binds the AU-/U-rich elements in p63 3' UTR in vitro and in vivo and the RRM domain in RNPC1 is required for binding, and regulating the stability of, p63 transcript. Furthermore, we showed that RNPC1 promotes keratinocyte differentiation by repressing p63 expression. Together, we uncovered a previously undetected mechanism by which p63 expression is regulated via mRNA stability and a novel regulatory feedback loop between RNPC1 and p63.

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POSTER 154

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Inauhzin suppression of tumor growth via activation of the p53 pathway

The tumor suppressor p53 pathway plays a centrally important role in the development and progression of all types of human cancers and thus has been an attractive target for identifying small molecules as potential anti-cancer drugs. In such an attempt, we identified a small molecule named as Inauhzin that possesses an ability to induce p53 level and activity, consequently leading to p53-dependent apoptosis at a digital micromolar concentration. It came out as a surprising finding because although initially we were searching for small molecules that can disrupt the interaction between p53 and MDMX via a computational docking program followed by a cellular assay, our intensive followup studies unraveled its inhibitory effect on the deacetylase activity of SIRT1 likely via an uncompetitive mechanism. More surprisingly, Inauhzin does not appear to inhibit the activity of other Sirtuin family members, such as SIRT2 and SIRT3, and nor HDAC, such as HDAC8 in vitro. In line with these results, this small molecule induced p53 acetylation in cells, but not tubulin acetylation. Further analyses of its chemical analogs defined key chemical moieties essential for p53 activation. Inauhzin induced p53 acetylation, prevented MDM2-mediated ubiquitylation of p53 in cells, though not directly in vitro, and induced p53-dependent apoptosis without apparently causing genotoxic stress. Remarkably, Inauhzin repressed the growth of xenograft tumors derived from human lung (H460) and colon cancer cells (HCT116) by activating p53 in a dose- and p53-dependent fashion. Hence, our study unearths Inauhzin as a novel anti-cancer therapeutic candidate that inhibits SIRT1 activity and activates p53.

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POSTER 155

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p73 Controls the Innate Immune Response of Ciliated Airway Epithelia

Ciliated cells of the airways underlie mucocilliary transport of microorganisms, but their role in the response to pathogens remains more obscure. Here we show that p73, a p53 homolog whose loss in mice results in chronic airway inflammation, is highly expressed in ciliated cells. We also show pathogens trigger the expression of inflammatory cytokine genes in ciliated cells as well as the degradation of p73, suggesting the possibility that p73 normally acts to suppress this inflammatory response. These data implicate the ciliated airway epithelia in the primary innate immune response to pathogens and suggest novel molecular targets for the treatment of acute and chronic airway inflammation. We are developing in vitro models for analysis of the innate immune system of the airways by differentiating p63-expressing nasal airway stem cells in air-liquid interface cultures that give rise to both ciliated cells expressing p73 and goblet cells that do not. We anticipate that this in vitro model will permit a molecular analysis of the innate immune response of ciliated cells and the pathway by which p73 regulates cytokine gene expression.

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Philadelphia, PA

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GENERAL INFORMATION

Important phone numbers, bank locations, and transportation information can be found in the University City map provided in your registration bag.

Registration Table:

The registration table is located in the Reading Room at Houston Hall, 3417 Spruce Street on October 8^{th}, 2010. After this date, a small table will be located in the lobby of Stemmler Hall, Dunlop Auditorium, 3450 Hamilton Walk.

Meeting Message Board:

There is a message board located near the registration table. There will also be one located in the lobby of Stemmler Hall, Dunlop Auditorium, 3450 Hamilton Walk.

Name Tags:

Name tags must be worn to all meeting sessions and events.

Cell phones:

Please be sure cell phones are turned off during all sessions.

Personal belongings:

For your own protection, please be aware of the whereabouts of your personal property and do not leave belongings unattended even for a brief period. To report lost or found items, please see the Registration Desk.

15th International p53 Workshop
October 8—12, 2010
University of Pennsylvania
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Philadelphia, Pennsylvania USA

Hotels

1. **Hilton Inn at Penn**
2. **Sheraton University City**

Workshop Registration

3. **Houston Hall (Reading Room)**
   3417 Spruce St.

Keynote Talks/Opening Reception

4. **Irvine Auditorium**
   3401 Spruce St.

Workshop Programming

5. **Stemmler Hall**
   3450 Hamilton Walk

Poster Session 1

3. **Houston Hall (Bodek Lounge)**
   3417 Spruce St.

Poster Session 2

6. **University Museum**
   3260 South St.

Transportation

7. **Trolley Tour and Closing Banquet Bus Boarding (behind Stemmler Hall)**
8. **SEPTA #42 Bus from Center City**
9. **SEPTA #42 Bus to Center City**
10. **SEPTA Market-Frankford El (34th Street Station)**