8TH P53
MUTANT WORKSHOP
LYON - MAY 2019

8th INTERNATIONAL MUTANT P53 WORKSHOP & P53 ISOFORMS

40TH ANNIVERSARY

LYON, 15-18 MAY 2019
LYON CATHOLIC UNIVERSITY

8TH P53 MUTANT WORKSHOP

LYON - MAY 2019

Dear Friends and Colleagues,

On behalf of the scientific and organizing committees, it is my pleasure to welcome you in Lyon for the 8th Mutant p53 Workshop.

18 years after the first workshop in 2001, it is great to bring this meeting back to Lyon. Since then, the workshop has become a major event for the p53 community, building up on a series of successful editions such as the most recent one held in Melbourne in October 2016.

This workshop comes at a very special time for p53 community. Indeed, the Li-Fraumeni syndrome was first described in 1969, p53 itself came into being in 1979 and mutant p53 was recognized as a majorevent in cancer in 1989, followed by the discovery of germline mutations in LFS. We are thus celebrating a triple anniversary highlighting several decades of huge progress in understanding the multiple facets of this fascinating protein.

The workshop is a great opportunity to look forward into the promises of the next decades. We are now at a stage where several drugs targeting p53 are entering the clinics. Mutant p53 is also revealing itself as a potential neoantigen for targeted immunotherapies. Much knowledge still needs to be gained but the road lies wide open for decades of successful translation to cancer care.

Much of these advances owe to the beautiful work of the mutant p53 community – Your work. Over theses decades, we have been competitors, collaborators and we have become friends. I hope that this meeting will be not only a celebration of great science but also of the strong ties what we have built over these years.

Wishing you a wonderful meeting!

Pierre Hainaut

An "outsider's" view of p53 - by Sue Armstrong

Five years ago, my book "p53: the gene that cracked the cancer code" was published by Bloomsbury – one of two books to launch the publisher's new popular science imprint Sigma. As a journalist specialising in science and health, I first heard of p53 in 1996 when I was newly returned to Scotland from seven years in South Africa, where I had been reporting for New Scientist magazine and BBC Radio, and chronicling the ravages of AIDS across the continent for the World Health Organization. Looking for good science stories in Scotland, I found myself in the Dundee lab of David Lane. Intrigued by what I learnt from David, I got a commission from the BBC to make a radio documentary and, in 1998, flew off to Crete to join the p53 community at their biannual workshop.

As I followed the story intermittently over the years, I realised this was too good a story to leave hidden in the passionless pages of academic journals, and the idea for a book aimed at a general audience took root. On the wise advice of Pierre Hainaut, I started my research with a visit to Brazil and the inspiring programmes for Li Fraumeni families run by Maria Isabel Achatz in Sao Paulo and Patricia Prolla in Porto Alegre.
Here was the human face of mutant p53 -- the people whose lives were blighted by the faulty mechanism of tumour-suppression and those caring for them. My visit to Brazil gave me the wide view, a context for the infinitely painstaking research at the molecular level going on in labs around the world that I was to hear about subsequently.

Besides learning of the different elements of the p53 saga – the discovery of the gene, its cloning and identification, the stories of apoptosis, "knock out" mice, tobacco and other "fingerprint" mutations, and Knudson's two-hit hypothesis to name just a few – I gained fascinating insights into how science actually works; and how knowledge advances as much through negative results and thwarted hypotheses as it does by theories that prove to be correct. The intriguing evidence that tumour suppression and ageing are two sides of the same coin through the common mechanism of cell senescence is one I have pursued in much greater detail for my latest book from Bloomsbury Sigma, "Borrowed Time: the science of how and why we age", which came out in January 2019.

"Science without storytelling," wrote the American astrophysicist Janna Levin, "collapses to a set of equations or a ledger full of data." As a science communicator, my aim in all that I write is to stand clear of those dusty ledgers as far as possible and tell the stories of some remarkable people and the fascinating and vital work that they do.

Program

DAY 1 – 15 MAY 2019

Keynote lectures

Chairman: Galina SELIVANOVA

- **3 – 5 pm**
  Welcome & Coffee
- **5 - 6 pm: KEYNOTE LECTURE**
  Varda ROTTER
  Weizmann Institute of Science - Israel
  Establishment of cancer stem cells is mutant p53 dependent
- **6 – 7 pm: KEYNOTE LECTURE**
  David MALKIN
  The Hospital for Sick Children – Canada
  Is Predicting and Preventing Cancer Possible in Li-Fraumeni Syndrome?

DAY 2 – 16 MAY 2019

Understanding mutations: mutant P53 regulatory network

Chairmen: Giannino DEL SAL & Daniel MENENDEZ

- **8 am – 8.30 am**
  Registration & Coffee
- **8.30 am – 9 am**
  Moshe OREN
  Weizmann Institute of Science - Israel
  Pseudomutant wild type p53 – fact or fiction?
- **9 am – 9.30 am**
  Ute MOLL
  Stony Brook Medicine, USA
  Therapeutic Ablation of Gain-of-Function Mutant p53 in Colorectal Cancer Inhibits Stat3-Mediated Tumor Growth and Invasion
- **9.30 am – 10 am**
  Ygal HAUPT
  Peter Mac Callum center - Australia
  MDM4 in hormone related cancers with mutant p53
- **10 am – 10.15 am**
  Chen KATZ
  Columbia University - USA
  Dimer-forming mutant p53 proteins display a distinct metabolic phenotype
- **10.15 am – 10.30 am**
  Chantal THIBERT
  Institute for Advanced Biosciences - France
  Metabolic regulations by LKB1 and p53 in development and cancer
- **10.30 am – 11 am**
  Coffee break
- **11 am – 11.30 am**
  Galina SELIVANOVA
  Karolinska Institute - Sweden
  Mutant p53 controls epigenetic program promoting cancer
- **11.30 am – 11.45 am**
  Jerson SILVA
  National Institute of Science and Technology for Structural Biology and Bioimaging - Brazil
  Aggregation of Mutant p53 is a Promising Therapeutic Target against Cancer
- **11.45 pm – 12 pm**
  Chit Fang CHEOK
  A*STAR Lab - Singapore
  Reprogramming of metabolic and DNA damage response networks in the absence of p53
- **12 am – 12.15 am**
  Magali OLIVIER
  International Agency for Research on Cancer - France
  Revisiting the origin and consequence of TP53 mutations in human cancer
- **12.15 pm – 12.30 pm**
  Andrew GIACOMELLI
  Dana-Farber Cancer Institute, USA
  Decoding the TP53 mutational spectrum
- **12.30 pm – 2.30 pm**
  Lunch & Poster session

Chairmen: Ygal HAUPT & Moshe OREN

- **2.30 pm – 3 pm**
  Guillermina LOZANO
  MD Anderson Cancer Center
  Mutant p53 activities in somatic mouse tumor models
- **3 pm – 3.30 pm**
  Daniel MENENDEZ
  National Institute of Environmental Health Sciences - USA
  siRNA-based synthetic lethal screening to target mutant p53 in response to chemotherapeutic drugs
- **3.30 pm – 4 pm**
  Giannino DEL SAL
  Universitá degli Studi di Trieste - Italy
  Mutant p53 shapes the Golgi structure and drives a prometastatic secretome
- **4 pm – 4.30 pm**
  Coffee break
- **4.30 pm – 5 pm**
  Kanaga SABAPATHY
  Institute of Molecular & Cell Biology - Singapore
  Multiple approaches to targeting p53
- **5 pm – 5.15 pm**
  Wenwei HU
  Rutgers Cancer Institute of New Jersey - USA
  Mutant p53 activates small GTPase Rac1 as a novel GOF mechanism to promote tumorigenesis
- **5.15 pm – 5.30 pm**
  Steven PILLEY
  The Crick Institute - UK
  Selective retention of wild-type function by mutant p53
- **5.30 pm – 6.30 pm: KEYNOTE LECTURE**
  David LANE
  *A*Star Lab - Singapore*
  On the expression of mutant p53 in normal tissues

DAY 3 – 17 MAY 2019

P53 isoforms

Chairmen: Ute MOLL & Magali OLIVIER

8 am – 8.30 am
Registration & Coffee

8.30 am – 9 am
Curtis HARRIS
National Cancer Institute - USA
Aging and Cancer: p53 Isoforms

9 am – 9.30 am
Jean-Christophe BOURDON
Dundee University - UK
Do mutant p53 isoforms mediate cell response to cancer treatment?

9.30 am – 10 am
Anthony BRAITHWAITE
University of Otago - New Zealand
Modulation of Antigen Presenting Cell function by the Delta 133 isoforms

10 am – 10.15 am
Olivier TERRIER
Centre International de Recherche en Infectiologie - France
p53 isoforms as key regulators in the front line in viral infections: the example of influenza viruses

10.15 am – 10.30 am
Izumi HORIKAWA
National Cancer Institute, USA
Δ133p53: Senescence-selective dominant-negative isoform of p53

10.30 am – 11 am
Coffee break

11 am – 11.30 am
Xin LU
Ludwig Institute for Cancer Research - UK
Structural insights into p53-iASPP interactions: target selectivity, mutations and the oncoprotein HPV E6 connection

11.30 am – 12 pm
Zhaohui FENG
University of New Jersey, USA
Mutant p53 protein accumulation promoted by MDM2 isoforms and BAG proteins in cancer

11.45 pm – 12 pm
James MANFREDI
Icahn School of Medicine at Mount Sinai - USA
Wild-type and tumor-derived mutant p53 are distinctly regulated by the C-terminal domain in vivo

12 pm – 2.15 pm
Lunch & Poster session

Constitutive P53 mutant & Li-Fraumeni syndrome

Chairmen: Giovanni BLANDINO & Klas WIMAN

2.15 pm – 2.45 pm
Sharon SAVAGE
National Cancer Institute - USA
Clinical Challenges in Li-Fraumeni Syndrome: Cancer and Beyond

2.45 pm – 3 pm
Amr GHALEB
Stony Brook University - USA
Molecular mechanisms of p53 loss of heterozygosity in breast cancer in response to irradiation

3 pm – 3.30 pm
Thierry FREBOURG
Rouen University Hospital - France
Towards a personalized and appropriate medical management of TP53 variation carriers

3.30 pm – 3.45 pm
Thibaut BARNOUD
The Wistar Institute, USA
Targeted Therapy for the African-Centric S47 Variant of p53

3.45 pm – 4 pm
Chang CHAN
Rutgers Robert Wood Johnson Medical School, USA
Spectrum and mutational landscape of LFS tumors

4 pm – 4.30 pm
Coffee break

4.30 pm – 4.45 pm
Paul HWANG
National Heart, Lung, and Blood Institute - USA
Mutant p53 protects against doxorubicin cardiomyopathy

4.45 pm – 5 pm
Maria Isabel ACHATZ
Hospital Sirio Libanês - Brazil
Atypical phenotypes in Li-Fraumeni syndrome, TP53 germline pathogenic variants and cancer risk

5 pm – 6 pm: KEYNOTE LECTURE
Arnold LEVINE
Institute for Advanced Studies - USA
The Roles of Initiating p53 Mutations in Human Cancers: The Order of Mutations and Tumor Cell Type Matters

7.30 pm
Workshop Dinner

DAY 4 – 18 MAY 2019

Targeting mutant P53 in cancer

Chairmen: Jean-Christophe BOURDON & Guillermina LOZANO

8 am – 8.15 am
Registration & Coffee

8.15 am – 8.45 am
Giovanni BLANDINO
IRCCS Regina Elena National Cancer Institute - Italy
Tracing and targeting TP53/PI3K mutations in HNSCC patients

8.45 am – 9 pm
Michael DUFFY
St Vincent's University Hospital - Ireland
Mutant p53 as a target for breast cancer treatment

9 am – 9.15 am
Min LU
Shanghai Jiao Tong University School of Medicine - China
Small-molecule compounds fully rescue a batch of structural mutant p53 with solved atom-level mechanism

9.15 am – 9.30 am
Tomer COOKS
Ben-Gurion University, Israel
Mutant p53 as a master regulator of immune dynamics driving tumorigenesis via exosomes mechanism

9.30 am – 9.45 am
Chandra VERMA
A*Star Lab - Singapore
Probing the dynamics of wild type and mutant p53: novel routes to restabilization

9.45 am – 10 am
Luis MARTINEZ
Stony Brook University - USA
Mutant p53 interacts with TANK-binding kinase 1 (TBK1) to inhibit activation of cancer cell-autonomous innate immune response

10 am – 10.15 am
Daniel ABERDAM
Saint Louis Hospital - France
TP53-reactivating drug PRIMA-1MET repurposing to treat severe skin involvement in p63-related ectodermal dysplasia syndromes

10.15 am – 10.30 am
Nicholas CLEMONS
Peter MacCallum Cancer Centre - Australia
Genome wide CRISPR knockout and activation screens provide novel insights into the mechanism of action of APR-246 in mutant p53 cancer cells

10.30 am – 11 am
Coffee break

11 am – 11.30 am
Daniela KANDIOLER
Medical University of Vienna - Austria
p53 in the clinic – still a challenge

11.30 am – 12 am
Lars ABRAHMSEN
Aprea Therapeutics AB - Sweden
APR-246 for treatment of TP53 mutated tumors and hematological malignancies - clinical status and mode-of-action

12 am – 12.30 am
Klas WIMAN
Karolinska Institute - Sweden
Missense and nonsense mutant TP53 as targets for novel cancer therapy

12.30 pm – 12.45 pm
Conclusions & Remarks

Practical informations

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p53

Oral communications abstracts

ESTABLISHMENT OF CANCER STEM CELLS IS MUTANT P53 DEPENDENT

Varda Rotter

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

Mutations in the tumor suppressor p53 are the most frequent alterations in human cancer. These mutations include p53-inactivating mutations as well as oncogenic gain-of- function (GOF) mutations that endow p53 with capabilities to promote tumor progression.

A primary challenge in cancer therapy is targeting stemness features and cancer stem cells (CSC) that account for tumor initiation, metastasis, and cancer relapse. Here we show that in vitro cultivation of tumors derived from mutant p53 murine bone marrow (BM) mesenchymal stem cells (MSC) gives rise to aggressive tumor lines (TL). These MSC-TL exhibited CSC features as displayed by their augmented oncogenicity and high expression of CSC markers.
Comparative analyses between MSC-TL with their parental mutant p53 MSC allowed for identification of the molecular events underlying their tumorigenic properties, including an embryonic stem cell (ESC) gene signature specifically expressed in MSC-TL.

Knockout of mutant p53 led to a reduction in tumor development and tumorigenic cell frequency, which was accompanied by reduced expression of CSC markers and the ESC MSC-TL signature. In human cancer, MSC-TL ESC signature-derived genes correlated with poor patient survival and were highly expressed in human tumors harboring p53 hotspot mutations. These data indicate that the ESC gene signature-derived genes may serve as new stemness-based prognostic biomarkers as well as novel cancer therapeutic targets.

LI-FRAUMENI SYNDROME: IS EARLY CANCER DETECTION AND PREVENTION POSSIBLE?

David Malkin

The Hospital for Sick Children – Canada

More than 85% of patients with Li-Fraumeni syndrome harbour germline TP53 mutations. The spectrum of mutations and the heterogeneity of tumor presentation (age of onset and type) within and between families is remarkable, and indicates that modifiers must play an important role in defining the phenotype of each patient and each family. We recently reported the feasibility and utility of a clinical surveillance protocol for early detection of tumours in TP53 mutation carriers (Villani et al Lancet Oncol 2016).

Results from deep sequencing of both the genome and epigenome of mutation carriers provide exciting insight into the role of modifiers on both the functional activity of p53 in the germline as well as their influence on tumor onset, tumor spectrum and clinical outcome/response to therapy. Data will be presented to support the creation of molecular algorithms that may be used in a precise manner that reflects the unique genetic and epigenetic signature of LFS patients. It is anticipated that these algorithms can then be used to more effectively refine and guide the creation of personalized surveillance strategies for these patients.

PSEUDOMUTANT WILD TYPE P53 – FACT OR FICTION?

Gal Benor, Sharathchandra Arandkar, Noa Furth, Yael Aylon, Eytan Domany and Moshe Oren

Weizmann Inst. of Science, Rehovot, Israel

While the TP53 gene is mutated in about half of all human cancer cases, it remains wild type (wt) in the other half, suggesting that its tumor suppressor activities are either abrogated or circumvented in the latter cases. What happens to p53 in tumors where the TP53 gene is not mutated?
The two most sensible scenarios are: 1. p53 remains functional, but is unable to exert tumor suppressive effects in particular contexts or particular cell types; 2. The protein loses its functionality, approximating a p53-null situation. In both cases, depletion of the retained wtp53 is not expected to have significant biological consequences.

Recent work from our lab supports a third possibility, wherein the retained wtp53 protein acquires properties that are usually ascribed to cancer-associated p53 mutants, namely oncogenic gain-of-function properties. This has been demonstrated in human mammary epithelial cells upon silencing of the Hippo tumor suppressor pathway, as well as in cancer-associated fibroblasts. In both situations, depletion of the endogenous wtp53 protein leads to attenuation of cancer-associated features, such as cell migration and invasion, implying that the wtp53 of those cells actually contributes to those "gain of function" features. We thus proposed that such cells actually harbor "pseudomutant" p53.

To evaluate whether pseudomutant wtp53 can be observed in actual human tumors, we interrogated gene expression profiles and pathway deregulation patterns in the METABRIC breast cancer dataset as a function of TP53 mutation status. As expected, a gene expression signature that distinguishes between tumors that carry TP53 mutations and those that retain wt TP53 could be defined and validated. Interestingly, a small subset of wt TP53 tumors nevertheless displayed gene expression and pathway deregulation patterns markedly similar to those observed in TP53-mutated tumors, and distinct from the major group of wt TP53 breast tumors. At least in several of those cases, p53 expression could be observed by immunohistochemistry, suggesting that the wtp53 protein is rewired rather than absent. Analysis of the transcriptional profile of this "pseudomutant" subset of wt TP53 tumors identified a small number of pathways that are preferentially deregulated in those tumors as compared to bone fide TP53-mutated tumors, raising the possibility that their deregulation may play a role in the rewiring of wt p53 into a "pseudomutant" state.

THERAPEUTIC ABLATION OF GAIN-OF-FUNCTION MUTANT P53 IN COLORECTAL CANCER INHIBITS STAT3-MEDIATED TUMOR GROWTH AND INVASION

Ute M. Moll

Department of Pathology, Stony Brook University, Stony Brook, NY 11794, USA

Over half of colorectal cancers harbor TP53 missense mutations ('mutp53'). We show that the most common mutp53 allele R248Q (p53Q) exerts gain-of-function (GOF) and creates tumor dependence in mouse colorectal cancer models. mutp53 protein binds Stat3 and enhances activating Stat3 phosphorylation by displacing the phosphatase SHP2. Ablation of the p53Q allele suppressed Jak2/Stat3 signaling, growth, and invasiveness of established, mutp53-driven tumors.

Treating tumor-bearing mice with an HSP90 inhibitor suppressed mutp53 levels and tumor growth. Importantly, human colorectal cancers with stabilized mutp53 exhibit enhanced Jak2/Stat3 signaling and are associated with poorer patient survival. Cancers with TP53R248Q/W are associated with a higher patient death risk than are those having nonR248 mutp53. These findings identify GOF mutp53 as a therapeutic target in colorectal cancers.

I will also briefly discuss data from our ongoing studies on therapeutic ablation of mutp53 in an autochthonous hepatocellular carcinoma model.

Reference: Schulz-Heddergott R, Stark N, Edmunds SJ, Li J, Conradi LC, Bohnenberger H, Ceteci F, Greten FR, Dobbelstein M, Moll UM (2018). Cancer Cell 34(2):298-314.e7. PMID: 30107178

TARGETING MDM4 ALONE, OR IN COMBINATION WITH P53 REACTIVATION, IN HORMONE-RELATED CANCERS

Sue Haupt[1], Octavio Mejia[1], Simon Keam[1], Jeffreena Panimaya[1], Dinesh Raghu[1], Aart Jochemsen[2], and Ygal Haupt[1]

[1]Peter MacCallum Cancer Centre, Melbourne, Victoria Australia.
[2]Department of Molecular Cell Biology, University Medical Centre, Leiden, The Netherlands

The tumour suppressive functions of p53 are universally compromised in cancers. In over half the cancer cases this is achieved by direct mutations of the TP53 gene. However, in the remainder, alternative regulatory pathways inactivate its tumour suppressive functions, and/or reduce its expression. This is primarily achieved through elevation in the expression of the key inhibitors of p53: MDM2 or MDM4(X). In breast cancer (BrCa), the frequency of p53 mutations varies markedly across the different sub-types, with basal-like BrCa bearing a high frequency of p53 mutations, while luminal BrCas generally express wild type (wt) p53.

We have recently shown that Inducible knockdown (KD) of MDM4 in luminal BrCa MCF-7 cells impedes growth of cultured cells, and this effect is p53-dependent. Here we show that MDM4 is also elevated in basal-like BrCa samples. Conditional KD of MDM4 provokes growth inhibition in a range of breast cancer sub-types with mutant p53, in vitro and in vivo. MDM4 was shown to be crucial to the establishment and progression of tumours. We have performed RNA and protein analysis to explore the downstream targets. Our preliminary analysis will be presented.

We have recently explored the involvement of MDM4 in prostate cancer, and tested its efficacy as a therapeutic target, which will be presented. Overall, our study supports MDM4 as an attractive therapeutic target for hormone related cancers expressing either wt p53 or mutant p53. MDM4 targeting enhances the efficacy of p53 reactivation in these mutant p53 expressing cancers.

DIMER-FORMING MUTANT P53 PROTEINS DISPLAY A DISTINCT METABOLIC PHENOTYPE T

Chen Katz, Joshua Choe, David Tong and Carol Prives

Department of Biological Sciences, Columbia University, 10027

The tetramerization domain (TD) of the p53 protein facilitates its oligomerization, which is essential for efficient DNA binding, protein-protein interactions, and transactivation of downstream targets. Mutations in the TD may result in p53 forming dimers or monomers, instead of tetramers and p53 TD mutations occur in both sporadic tumors and Li-Fraumeni Syndrome (LFS) patients. Dimer-forming mutations in human cells remain poorly characterized, especially in the context of transcriptional activity and chemotherapeutic sensitivity.

Here, we characterized dimer-forming mutations using three different systems; (i) U2OS cells transiently expressing dimer-forming mutant p53 proteins, (ii) fibroblasts endogenously expressing a heterozygous LFS dimer-forming mutation of p53 (WT/A347D), and (iii) a set of isogenic CRISPR/Cas9-derived U2OS clones endogenously expressing wild-type p53, no p53, or dimeric mutations (either heterozygous or homozygous). In all three systems dimeric mutant p53 was found to be hyper-stable and defective in trans-activating traditional p53 target genes (Mdm2, p21, etc.).

Unexpectedly the CRISPR-dimeric-p53 U2OS clones exhibit a distinct glycolytic phenotype marked by enhanced glucose influx and antioxidant generation. Despite elevated antioxidant pools, dimeric mutant cells display increased basal ROS and significantly morphologically aberrant mitochondria characteristic of stressed cells. Consistent with this finding, these mutant cells are highly vulnerable to DNA damage response inducers. We hypothesize that dimer-forming mutant p53 may exert a GOF activity to induce mitochondrial stress, eliciting an upregulation of glycolysis as a survival adaption to fuel antioxidant production.

These results implicate metabolic reprogramming in overcoming mutant p53-imposed stress during carcinogenesis and suggest a targetable vulnerability in p53 TD-mutant cancers such as in LFS patients.

METABOLIC REGULATIONS BY LKB1 AND P53 IN DEVELOPMENT AND CANCER

Anca Radu, Sakina Torch, Anthony Lucas, Marie Mével, Florence Fauvelle, Lionel Larue, Marc Billaud, Pierre Hainaut, Chantal Thibert

IAB, Inserm U1209, CNRS UMR5309, UGA, Grenoble, France

Cells adjust constantly their metabolism to their environment through signaling pathways that act as biosensors of nutrient availability. The tumor suppressor kinase LKB1 is essential to adapt cell fate to nutrient availability and control various cellular processes such as stem cell quiescence, proliferation or differentiation mainly through its substrate AMPK, a nutrient sensor of energy levels.

The tumor suppressor and transcription factor p53 is also a key metabolic regulator essential to determine cellular outcome. Using genetically engineered mouse models of spatio-temporal inactivation of Lkb1, we have recently uncovered that LKB1/AMPK governs cell behavior by connecting mTOR activity to pyruvate-alanine cycling and glutamate-glutamine conversion. We also highlighted that LKB1 prevents oxidative DNA damages and p53 activation, a necessary process for correct cell fate both in vitro and in vivo.
We are currently exploring the underlying molecular mechanisms of LKB1-p53 crosstalk to better understand how LKB1 signaling under metabolic stress impacts p53 non-transcriptional, transcriptional and epigenetics activities in normal and pathological conditions.

MUTANT P53 CONTROLS EPIGENETIC PROGRAM PROMOTING CANCER

Gema Sanz[1], Sylvain Peuget[1], Madhurendra Singh[1], Henrik Johansson[2], Laxmi Silwal-Pandir[3], Janne Lehtio[2], Kristine Sahlberg[4], Nardin Samuel[5], David Malkin[6] and Galina Selivanova[1]

[1]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden, [2]Science for Life Laboratory, 17177 Stockholm, Sweden, [3]Department of Cancer Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway, [4]Department of Research, Vestre Viken, Drammen, Norway, [5]MD/PhD Program, Faculty of Medicine, University of Toronto, Toronto ON, [6]Division of Hematology/Oncology, Department of Pediatrics, The Hospital for Sick Children, Toronto ON

p53 missense mutations are present in nearly 40% of all human tumors. Mutant p53 proteins not only lose the tumor suppression function, but also frequently act as driver oncogenes, which promote invasion, metastasis, and chemoresistance, leading to reduced survival in patients.
Notably, the molecular mechanisms of this oncogenic gain-of-function (GOF) are yet to be elucidated. Here, we show that mutant p53 regulates a set of oncogenic epigenetic factors. Acute mutant p53 deletion resulted in decreased expression of several histone modifiers and chromatin remodellers, implicated in cancer, including Brd4, DNMT3a, NCOA3, SETD2, SETD8 and others.

Inhibition of these epigenetic factors contributed to tumour suppression by mutant p53 reactivating compound APR-246 in vitro, ex vivo and in vivo. Elevated protein and mRNA of mutant p53 epigenetic targets in breast cancer patients is associated with p53 mutations and poor survival.
Our findings uncover mutant p53-mediated regulation of epigenome as a new mode of its gain-of-function activity. These can help to rationally design most efficient drug combinations for treatment of cancers with mutant p53.

AGGREGATION OF MUTANT P53 IS A PROMISING THERAPEUTIC TARGET AGAINST CANCER

Jerson L. Silva

Instituto de Bioquímica Médica Leopoldo de Meis, Instituto Nacional de Biologia Estrutural e Bioimagem, Centro Nacional de Ressonância Magnética Nuclear Jiri Jonas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Protein misfolding results in grave degenerative diseases and cancer. Among the culprits involved in these illnesses are amyloids and prion-like proteins, which can propagate by converting normal proteins to the wrong conformation. p53 mutations are the most common genetic alterations found in cancers and are observed in more than 50% of all tumors. Mutant p53 not only undergoes misfolding but also aggregation, similar to that observed with amyloids, playing a crucial role in the development of cancer through loss of function, negative dominance and gain of function (1-3).

Studies from our laboratory and others have demonstrated that the formation of prion-like aggregates of mutant p53 is associated with loss-of-function, dominant-negative and gain-of-function (GoF) effects (1,4). Compounds and peptides that have been described to inhibit mutant p53 aggregation also lead to a decline of tumor proliferation and migration (4-7). Thus, the misfolded and aggregated states of mutant p53 are formidable targets for the development of novel therapeutic strategies against cancer (6). (This work was supported by CNPq, FAPERJ, FINEP and CAPES).

References:

(1) Silva JL et al. Trends Biochem. Sci. 2014, 39, 260-267.
(2) Ishimaru D et al. Biochemistry 2003, 42, 9022-9027.
(3) Ano Bom AP et al. J. Biol. Chem. 2012, 287, 28152-28162.
(4) Silva JL et al. Acc Chem Res. 2018 51(1):181-190.
(5) Soragni A et al. Cancer Cell 2016, 29, 90-103.
(6) Ferraz da Costa DC et al. Oncotarget. 2018 Jun26;9(49):29112-29122
(7) Rangel LP et al. J Biol Chem. 2019 294(10):3670-3682.

REPROGRAMMING OF METABOLIC AND DNA DAMAGE RESPONSE NETWORKS IN THE ABSENCE OF P53

Chit Fang Cheok

A*STAR Lab - Singapore

DNA repair and metabolic networks are comprised of compensatory and redundant pathways that underlie the resiliency of cells to multiple perturbations.
p53 lie at the centre of these adaptive changes in response to cellular stresses to promote cell survival and to ensure the fidelity of replication processes. Impairing p53 function in cells therefore alters pathway choices, thus making them highly susceptible to certain metabolic stresses or challenges arising from DNA transactions and damage. We recently identified a new function of p53 in the regulation of fatty acids through lipid oxygenation pathways and showed that the impairment of this pathway in p53 mutant cells thwarted their growth and represents a unique vulnerability for exploitation against p53 mutant tumors.

In a systematic screen against DNA damaging agents, we discovered an exquisite sensitivity of p53 mutant cells towards replication and ATR inhibitors. Here, we further outlined the mechanism underlying the increased sensitivity to replication inhibitors. We showed that deficiency in p53 function renders increased fork stalling induced by hydroxyurea and ATR inhibition. Analysis of fork stability at a single molecule resolution further showed a progressive degradation of stalled forks in p53-deficient cells that was dependent on MRE11 nuclease activity, as well as increased fork reversal activity. Finally, we demonstrated that increased fork breakage in p53-defective cells depends on the coordinated activity of MRE11 and structure specific endonuclease SLX4.

The results shed exciting new insights on the role of p53 in maintaining genomic stability during replication, that is clearly distinct from its role in cell cycle che points previously thought to protect against replication-induced damage.

REVISITING THE ORIGIN AND CONSEQUENCE OF TP53 MUTATIONS IN HUMAN CANCER

Magali Olivier

International Agency for Research on Cancer - France

The IARC TP53 Database has been providing the largest set of data and annotations on the prevalence, type, phenotype, and functional impacts of TP53 germline and somatic mutations, with clinical and epidemiological correlates. In the last 10 years, the deluge of data coming from genome-wide screens of tumour samples and human populations produced the same amount of data that hundreds of Sanger sequencing studies produced over 40 years.

Large systematic experimental data on the functional impact of TP53 mutations have also been recently produced for more than 8000 mutations. These new data offer a unique opportunity to revisit our knowledge on the origin and consequences of TP53 mutations in human cancers.
I will show how we are using these data and mutational signatures derived from genome-wide data to derive new hypotheses on the forces that shape TP53 mutation patterns in different types of cancer.

DECODING THE TP53 MUTATIONAL SPECTRUM

Andrew O. Giacomelli

Dana-Farber Cancer Institute, Boston, Massachusetts, USA. Broad Institute or MIT and Harvard, Cambridge, Massachusetts, USA. Princess Margaret Cancer Centre, Toronto, Ontario, Canada

Most TP53 mutations found in human tumors are missense mutations. Mutant p53 protein is often highly expressed in tumor cells and some variants display dominant-negative or gain-of-function activities in model systems. However, it remains unclear why some TP53 mutations are observed more frequently than others in sporadic human tumors. Through interrogation of large-scale loss-of-function screens conducted in hundreds of human cancer cell lines and saturation mutagenesis screens carried out in an isogenic pair of TP53-wild-type and-null cell lines, we found that the selective advantage associated with TP53 mutation results from loss-of-function and dominant-negative activity.

By integrating our screening data with the COSMIC mutational signatures database, we developed a statistical model that describes the TP53 mutational spectrum as a function of the baseline probabilityof acquiring each mutation and the cellular fitness advantage conferred by attenuation of p53 activity. These observations suggest that mutational processes and phenotypic selection play important roles in shaping the landscape of recurrent TP53 alterations seen in human cancer.

MUTANT P53 ACTIVITIES IN A SOMATIC MODEL OF BREAST CANCER

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Disruption of the p53 tumor suppressor pathway commonly occurs via missense mutations, many of which exhibit gain-of-function activities such as increased tumor aggressiveness and metastasis. We have developed novel conditional mutant p53 alleles that switch wild type p53 to mutant in a Cre-specific manner to explore the role of the microenvironment in tumor development and progression. A somatic model of breast cancer with metastases will be discussed. These models most closely simulate the genesis of somatic cancers and will thus be invaluable in testing novel therapeutic combinations.

SIRNA-BASED SYNTHETIC LETHAL SCREENING TO TARGET MUTANT P53 IN RESPONSE TO CHEMOTHERAPEUTIC DRUGS

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Screens for synthetic lethality provide important approaches for identifying targets in anticancer therapies as well as for understanding gene functions and interdependence between pathways and signaling networks in tumor cells. Since TP53 gene mutations occur in more than half of human cancers, it is important to identify anticancer drugs that specifically target p53 mutant tumor cells. We hypothesized target genes that when reduced in expression employing genome-wide siRNA-based screens would enhance sensitivity to chemotherapeutic drugs and radiotherapy in p53 deficient/mutant cell lines, resulting in synthetic enhanced lethality (SEL).

We identified several potential p53 SEL target genes that increase the lethal responses of WT, mutant (R175H and R273H) or p53 null HCT116 human cancer cell lines to the commonly used topoisomerase II inhibitor etoposide. The lethal response often depended on the specific p53 status. Among the SEL genes were those that had an impact only on p53 mutants and in some cases they were specific to the particular mutant. We found that a defect in p53 could result in significantly more Top2-DNA complexes in cells treated with etoposide. Importantly, the amount of complex could be influenced by the SEL gene product.

Overall, the identification of genes that when silenced selectively enhance the chemosensitivity of TP53 mutant cancer cells but not TP53 wild-type cells provides opportunities to identify drug targets for anticancer drug development. These SEL genes provide insight into opportunities for individualized cancer treatments, where the nature of the p53 cancer-associated mutation might dictate the combination therapy agents/protocols.

MUTANT P53 SHAPES THE GOLGI STRUCTURE AND DRIVES A PROMETASTATIC SECRETOME

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Tumors evolve through genetic and epigenetic changes that modify fundamental cellular programs of growth and proliferation. This is followed by selection of reprogrammed cells that best adapt to a variety of suboptimal conditions they encounter, either transiently or durably, during progression and alterations in TP53 represent a driving force in these processes.

The majority of TP53 mutations are missense and occur within the DNA binding domain of p53, leading to expression of mutant p53 (mut-p53) proteins that not only lose the tumor suppressive functions of the wild-type form, but may also acquire novel oncogenic features, generally referred to as gain of function (GOF). mut-p53 activities subvert the nature of the p53 pathway by promoting invasion, metastasis and chemoresistance and are associated with adverse prognosis mut-p53 exerts its oncogenic functions through different mechanisms, a major one being the alteration of gene expression profiles, including both coding and non-coding RNAs.
Here we describe the impact of a novel small miRNA induced by mutant p53 on the structural organization of Golgi apparatus and on the secretory machinery. The increased rate of trafficking sustained by mut p53 via this small non coding RNA causes the release of a pro-malignant secretome increasing extracellular stiffness, remodeling the tumor microenvironment and enhancing metastatic colonization.

This study provides new insights into the mechanisms by which mut-p53, through induction of non coding RNAs, can exert pro-tumorigenic functions in a non cell-autonomous fashion, and highlights potential non-invasive biomarkers and therapeutic targets to treat tumors harboring mut-p53.

MULTIPLE APPRACHES TO TARGETING P53

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Mutations in the tumor suppressor gene p53 are synonymous with tumorigenesis. The presence of mutant p53 predisposes to cancer development; promotes the survival of cancer cells; and prevents effective therapeutic response.

However, there are no drugs approved that can abrogate mutant p53's oncogenic functions. We will present data from multiple approaches undertaken in our laboratory to target p53 mutants, using a variety of technologies including mutation-specific antibodies, mutation-specific siRNAs and antisense-oligonucleotides. We have also been exploring the possibility of activating alternate isoforms of p53 to improve therapeutic efficacy. Details and implications will be discussed.

MUTANT P53 ACTIVATES SMALL GTPASE RAC1 AS A NOVEL GOF MECHANISM TO PROMOTE TUMORIGENESIS

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Tumor suppressor p53 is the most frequently mutated gene in human cancer. Mutant p53 (mutp53) not only loses the tumor suppressive activity of wild type p53, but also often gains new oncogenic activities to promote tumorigenesis, which is defined as mutp53 gain of function (GOF). While the concept of mutp53 GOF is now established, its underlying mechanism is not well-understood.

We found that mutp53 activates small GTPase Rac1 as a critical mechanism for mutp53 GOF to promote tumorigenesis. Mutp53 activates Rac1 through enhancing Rac1 SUMOylation, a modification that is critical to maintain the active Rac1 form and enhance its activity in cells. Mechanistically, mutp53 interacts with Rac1, and mutp53-Rac1 interaction inhibits Rac1 to interact with SUMO-specific protease 1 (SENP1), which in turn inhibits SENP1-mediated de-SUMOylation of Rac1. Furthermore, mutp53 expression is associated with enhancedRac1 activity in clinical tumor samples.
Targeting Rac1 signaling by RNAi or the pharmacological Rac1 inhibitor can effectively block mutp53 GOF in promoting tumor growth and metastasis. These results uncover a new mechanism for Rac1 activation in tumors, and reveal that activation of Rac1 and its signaling pathway is an unidentified and critical mechanism for mutp53 GOF to promote tumorigenesis and metastasis.

These results strongly suggest that targeting Rac1 and its signaling could be a feasible therapeutic strategy for cancer cells containing mutp53.

SELECTIVE RETENTION OF WILD-TYPE FUNCTION BY MUTANT P53

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Much success has been found in showing how mutant p53 proteins promote tumourigenesis through novel oncogenic gains of function and the loss of wild-type activities which inhibit proliferation.
However, for a tumour cell, the retention of wild-type p53 activities which maintain cell survival are just as beneficial as the loss of activities which promote cell death. Our lab showed in a mouse xenograft model that whilst tumours expressing wild-type p53 or the R248W mutant can survive nutrient stress in the form of serine starvation, tumours with the R175H mutant or no p53 cannot.
This was found to be because of a wild-type activity retained by the R248W mutant to induce p21 and MDM2 during nutrient stress. The expression of these canonical p53 targets allow for the induction of serine synthesis pathway enzymes and protection from oxidative stress which ultimately lowers cell viability in p53-null and R175H cells.

Perhaps unsurprisingly given these observations, human data reveals that patients with cancers expressing the R248W mutant show reduced survival compared to patents with R175H.
Recently, we have found further initial data to account for this. It was previously shown that malignant cells within a tumour are able to interact with each other, and it appears that these interactions may be affected by their p53 status. Cells with wild-type p53 or the R248W mutant are able to 'outcompete' cells with the R175H mutant or no p53.
This interaction could represent another retention of wild-type function which allows for cell survival and a consequently more resilient and aggressive tumour.
Understanding the mechanism behind this interaction would allow for a better appreciation of the intercellular dynamics within the tumour microenvironment and perhaps the development of better strategies for therapeutic interventions.

ON THE EXPRESSION OF MUTANT P53 IN NORMAL TISSUES

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Individuals born with Li-Fraumeni syndrome express both mutant and wild type p53 in their normal tissues. The growing evidence that individual point mutant alleles of p53 differ in their oncogenic function both as dominate negatives (DN) and in gain of function (GOF) contexts poses many questions about Li-Fraumeni families.
This is further exacerbated by new sequencing diagnostic tests that are identifying many new mutations in p53 in individuals and families that fall outside earlier definitions of Li-Fraumeni syndrome and are often given a report stating that they have a variant of unknown significance (VUS) in p53.

The expression of both DN and GOF phenotypes must depend on levels of p53 protein expression and we have been studying this in both fish and mouse models of Li-Fraumeni syndrome examining whole tissues for p53 expression at both the RNA level and the protein level. We have discovered that in addition to Mdm2 many other factors regulate p53 levels. These include the highly tissue and cell type restricted expression of p53 mRNA which is tightly confined in epithelial tissues to the proliferating compartment and also the clear effect of LOH and gene copy number. Mutant p53 expression levels are induced by radiation and chemotherapy and show surprising kinetics of delayed clearance when compared to that of the wild type protein. We are trying to develop using the mouse model simple non-invasive ways to measure quantitative p53 activity in human material to aid clinical diagnosis and better understand human variation.

We hope in the end by this research to develop tools to allow the community to be able resolve issues of VUS for affected individuals.

AGING AND CANCER: P53 ISOFORMS

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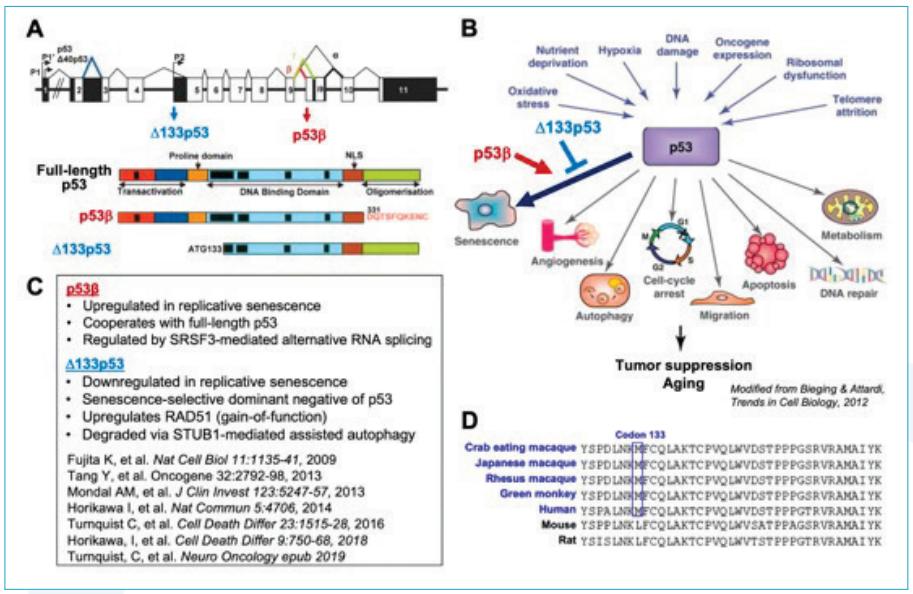
The p53 network is an intrinsic monitoring and responsive pathway of telomeric attrition and chronic stressinvolved in cellular aging and senescence (1). Cellular senescence, in cancer cells, can be a tumor suppressive mechanism that can be activated by p53. Cellular senescence of tumor stromal ceils can enhance carcinogenesis and tumor progression.

We and others are currently studying the molecular mechanisms of cellular senescence in normal human cells and the role of p53 and its isoforms in aging and cancer (2-4). Our research focuses on the functional role of p53 isoforms. e.g., "dominant negative» 133p53 and "co-transactivator» of wild-type p53, p53, both as natural regulators of cellular senescence. DNA repair and stem cell biology and are dysregulated in cancer, including damaging side effects of radiation and chemotherapy, and aging diseases, such as Hutchinson-Gilford Progeria Syndrome and Alzheimer's Disease (A-C).

Because of molecular evolution, 133p53 is specifically present in humans and primates. but not in other organisms, including mice, because of lack of an initiating methionine codon corresponding to the human codon 133 at exon 5 of TP53 (D)



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DO MUTANT P53 ISOFORMS MEDIATE CELL RESPONSE TO CANCER TREATMENT?

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Wild-Type TP53 is conventionally thought to prevent cancer formation and progression to metastasis, while mutant TP53 would be inactive or would have transforming activities. However, in the clinic, it is not so clear-cut. Somatic hot-spot mutations of TP53 are not reliable to define the most efficient cancer treatment for a patient and do not predict the clinical outcome of the patient (precision medicine).

Recent publications indicate that the differential co-expression of p53 protein isoforms in tumours is associated to patients' clinical outcome, regardless of TP53 gene mutation status (1-11). Therefore, do the p53 isoforms play an active role in inducing the cell response to cancer treatments whether tumour cells express WT or mutant TP53 ?
Here, we report the generation of novel antibodies specific of different p53 protein isoforms. We then determined the endogenous expression of p53 protein isoforms in normal human tissue and tumours of diverse tissue origins in response to cancer treatments and we investigated whether cell responses to cancer treatments are p53 isoform dependent in WT and mutant TP53 cell lines.

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5-The presence of the intron-3 16 bp duplication polymorphism of p53 (rs17878362) in breast cancer is associated with a low Δ40p53:p53 ratio and better outcome. Morten BC, et al., Carcinogenesis. 2016 Jan;37(1):81-86. 6-TP53 drives invasion through expression of its Δ133p53 variant.Gadea G, Arsic N, Fernandes et al., Elife. 2016 Sep 15;5. pii: e14734. 7-Δ133p53 isoform promotes tumour invasion and metastasis via interleukin-6 activation of JAK-STAT and RhoA-ROCK signalling. Campbell H, et al., Nat Commun. 2018 Jan 17;9(1):254.
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MODULATION OF ANTIGEN PRESENTING CELL FUNCTION BY DELTA 133 ISOFORMS.

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Our mouse model of the delta133p53 isoforms (designated delta122p53), as well as studies on several human cancers, have shown that one or more of the delta133p53 family regulate genes involved in immune regulation which result in a pro-inflammatory environment. Inflammation can be a double edge sword - addressing transient pathologies such as infection is beneficial, but long term can drive pathology, including cancers. Using the pro-inflammatory phenotype of the delta122p53 mice we asked whether Antigen Presenting Cells (APC) from delta122p53 mice could have prophylactic vaccine capacity.

To explore this possibility we have phenotypically characterised delta122p53 APCs. The data show they express surface activation markers and secrete multiple cytokines even in the absence of antigen exposure. Next we used an adoptive transfer model using the B16 melanoma cells expressing the ovalbumin transgene (B16-OVA) to test whether delta122p53 APCs could provoke an anti-tumour response. To do this mice were injected with APCs from delta122p53 or wild type (WT) p53 mice pulsed with OVA or left unpulsed, and subsequently challenged with B16-OVA cells. Results showed that delta122p53 APCs could elicit anti-tumour response that was not significantly different from WT APCs after exposure to OVA.

However, delta122p53 APCs could elicit a partial anti-tumour response without exposure to OVA. Given this, we suggest that incorporating human delta133p53 isoform in anti-tumour vaccines may improve their efficacy.

P53 ISOFORMS AS KEY REGULATORS IN THE FRONT LINE IN VIRAL INFECTIONS: THE EXAMPLE OF INFLUENZA VIRUSES

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Influenza A viruses (IAV), the causative agents of Flu, constitute a major public health issue, causing illness and death in high-risk populations during seasonal epidemics or pandemics. IAV are known to modulate cellular pathways to promote their replication and avoid immune restriction via the targeting of several cellular protein, including the master regulator p53.

Through its multiple roles in the regulation of a panoply of biological processes, including cell cycle, apoptosis, senescence or immune response, p53 is also involved in the control of viral infections. Viruses have developed a wide diversity of mechanisms to modulate/hijack p53 functions to achieve an optimal replication in their hosts. Our group and others have previously shown that p53 activity was finely modulated by different multi-level mechanisms during IAV infection.

Our work has notably highlighted a major regulatory role of p53 isoforms during the time course of infection1,2. More recently, we characterized the IAV non-structural protein NS1 and the cellular factor CPSF4 as major partners involved in the IAV-induced modulation of the TP53 alternative splicing that was associated with a strong modulation of p53 transcriptional activity and notably the p53-mediated antiviral response3. More particularly, our results indicate that spliced p53 and p53 isoforms are major contributors to the global p53-mediated regulation of type I IFN, through a common pathway with CPSF4. Future works will explore the specific role of p53 isoforms in the context of other viral infections and their interplay with the immune and inflammatory responses.

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Δ133P53: SENESCENCE-SELECTIVE DOMINANT-NEGATIVE ISOFORM OF P53

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D133p53 is an N-terminally truncated isoform of p53, which is translated from the human-specific methionine codon 133. D133p53 inhibits p53-mediated senescence and extends cellular replicative lifespan. D133p53 also rescues functional decline in senescent cells and restores normal cell functions: i) conversion of neurotoxic, senescent astrocytes to neuroprotective ones; ii) regeneration from senescent/exhausted to stem cell/central memory CD8+ T cells; and iii) restoration of DNA double-strand break (DSB) repair in progeria cells. These findings support therapeutic applications of D133p53 in: i) aging- and cancer treatment-associated neurodegeneration; ii) cancer immunotherapy such as CAR-T; and iii) senescence/DSB-based therapy in progeria. While the dominant-negative (DN) activity of D133p53 against p53 may raise safety concern, we have accumulated data that D133p53 is non-oncogenic and non-mutagenic:
1) D133p53 overexpression in normal human cells does not cause immortalization or transformation;
2) D133p53 overexpression does not induce chromosome instability or somatic mutations;
3) D133p53 overexpression would rather enhance DSB repair in part via RAD51;
4) human ES and iPS cells, with endogenously abundant or overexpressed D133p53, maintain chromosome stability and baseline somatic mutation rates and do not show malignant histology in vivo;
5) 133p53 preferentially represses p53-inducible senescence genes (e.g., p21WAF1), but not p53-inducible apoptosis and DNA repair genes (e.g., PUMA and p53R2).
The RNA sequencing data indicate that:
a) p21WAF1 is the major 133p53-repressed gene in various cell types;
b) cellular senescence-related signaling pathways are downregulated by 133p53, while apoptosis- and DNA repair-related pathways are maintained;
c) many of 133p53-induced genes are cell cycle regulators whose repression by p53 requires p21WAF1 (e.g., Cyclins).
This study defines 133p53 as a senescence-selective DN isoform of p53, which contributes to ES/iPS self-renewal and has therapeutic potentials for senescence/aging-associated diseases, while p53-regulated DNA repair and apoptosis are maintained to ensure genome stability and elimination of severely damaged cells. With marked contrast to total inhibition of p53 activities leading to loss of genetic integrity, 133p53 can be a therapeutic target for enhancement with minimal safety concern. We will investigate the molecular mechanism for the p53 target gene selectivity by 133p53.

SMALL-MOLECULE COMPOUNDS FULLY RESCUE A BATCH OF STRUCTURAL MUTANT P53 WITH SOLVED ATOM-LEVEL MECHANISM

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As titled, many small-molecule compounds are either hit in screening or rationally synthesized in our group. In assays quantitively comparing with wild-type p53, these compounds efficiently enhance a batch of structural mutant p53's thermal stability (5-6), protein folding (fully restored PAb1620 epitope), transcriptional activity (fully restored transcriptional activity in luciferase assay, fully restored express profile of p53 targets in RNAseq), tumor-suppressive functions (selective to structural mp53).

Rescue efficiency of these compounds for thousands of mutant p53s (covers >95% p53 mutations deposited in IARC, R18) are quantitively determined, providing the patient recruitment guide in the coming personalized clinical trials (based on individual mutant p53 rather than whether p53 is mutated). The talk with be ended with atom-level rescue mechanism (stapling four key residues that respond for modern-day human p53 thermal instability), progresses in industry, and progresses in clinical trial.

Reference

1.Tumor suppressor TP53 is the most frequently mutated gene in human cancers (Kandoth et al, Nature 2013)
2.TP53 is the most studied gene of all time, much more than the second TNF (Dolgin, Nature 2017).
3.TP53 keeps being the most cited gene every year since 2001, much more than the second APOE (Dolgin, Nature 2017).
4. So far, 415 clinical trials involving p53 have been registered on Clinicaltrials.gov. (https://clinicaltrials.gov)
5. So far, at least 45 groups have worked with mutant p53-based therapies (Sabapathy et al, Nat Rev Clin Oncol 2018).
6. So far, approx. 82 targeted anti-cancer drugs are approved with most of them inhibiting oncogenic proteins (Abramson, www.mycancergenome.org 2018), to our knowledge, none repairing tumor suppressors.
7. Together with Bcl-2, -catenin, c-myc, RAS, NF-kB, and others, p53 is hard to be targeted compared to routinely targeted kinase. It may partially attribute to three facts: 1) p53 is a transcription factor, 2) p53 apparently has 'no' druggable pocket on the protein surface except for a reported pocket unique to p53-Y220C, 3) p53 as a tumor suppressor need to be repaired but not inhibited. While Bcl-2 with an unconventional large pocket (a large BH3 pocket) is now drugged meanwhile KRAS-G12C with an unconventional allosteric pocket (GTP/GDP-binding pocket-neighboring allosteric pocket) is being drugged with substance progresses, we find p53 also harbors some unconventional pockets (largely mutant p53-specific, dynamic, odd-shape, generated by evolution, not overlapping with DNA-binding interface, manipulatable to rescue rather than inhibit p53 transcriptional activity), laying basis for our compounds to drug mutant p53.

MUTANT P53 PROTEIN ACCUMULATION PROMOTED BY MDM2 ISOFORMS AND BAG PROTEINS IN CANCER

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Tumor suppressor p53 is the most frequently mutated gene in human cancer. Many cancer-associated mutant p53 (mutp53) proteins promote tumorigenesis through gain-of-function (GOF) mechanisms. Mutp53 proteins often accumulate to a high level in human cancer, which is crucial for mutp53 to exert their GOFs. The mechanism underlying mutp53 protein accumulation in cancer is not well understood. E3 ubiquitin ligases MDM2 and CHIP have been reported to maintain mutp53 protein levels low in normal tissues but not in cancer, suggesting that some changes in cancer inhibit mutp53 degradation mediated by MDM2 and CHIP and thereby leads to mutp53 accumulation in cancer. Our recent studies revealed that overexpression of small splicing isoforms of MDM2 and Bcl-2 associated athanogene (BAG) family proteins in human cancer play important roles in mutp53 accumulation in cancer.

MDM2 has multiple spliced short isoforms in addition to the full-length MDM2. Some MDM2 spliced short isoforms are frequently overexpressed in human cancer. Majority of MDM2 isoforms lack the central region, including the p53 binding domain and nuclear localization signal, therefore they are localized in the cytoplasm and cannot bind to and degrade mutp53. We found that they can interact with full-length MDM2, which in turn sequester full-length MDM2 in cytoplasm and inhibit MDM2-mediated mutp53 degradation. Therefore, overexpression of MDM2 isoforms in human cancer promotes mutp53 accumulation and GOF in tumorigenesis.

BAG family proteins function as adapter or co-chaperone proteins through the BAG domain which mediates direct interaction with the ATPase domain of Hsp70/Hsc70 molecular chaperones. We found that BAG2 and BAG5, two BAG family proteins that are frequently overexpressed in human cancer, preferentially interact with mutp53 proteins through the BAG domain. This interaction protects mutp53 from ubiquitination and degradation by MDM2 and CHIP, which in turn promotes mutp53 protein accumulation and GOFs in tumorigenesis. Furthermore, BAG2 and BAG5 proteins exhibit a cooperative effect on promoting mutp53 protein accumulation and GOF in cancer cells.

Taken together, results from our studies uncovered novel mechanisms underlying mutp53 protein accumulation and GOFs in cancers containing mutp53.

WILD-TYPE AND TUMOR-DERIVED MUTANT P53 ARE DISTINCTLY REGULATED BY THE C-TERMINAL DOMAIN IN VIVO.

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Mutation of TP53 is frequent in cancer with wild-type p53 implicated as a tumor suppressor. A growing body of evidence supports the notion that tumor-derived mutant p53 has not only lost tumor suppressing activity, but has also gained oncogenic roles. A central and significant challenge is to elucidate the molecular bases both for the tumor suppressing functions of wild-type p53 and the oncogenic activities of mutant p53 as well as deconvoluting the molecular relationship between the two. To address this, mouse models have been engineered in which the endogenous p53 gene expresses either the wild-type or the mutant (R172H) as truncated proteins lacking varying extents of their C-terminal 24 amino acids.

Mice expressing two alleles of a truncated wild-type p53 (amino acids 1-366, Δ24) die within two weeks of birth with anemia due to bone marrow failure, ataxia with impaired cerebellar development, and reduced thymic development with a high incidence of apoptosis in thymocytes. This contrast with other tissues such as liver which appear normal. p53 protein expression is similar between thymus and liver in spite of their phenotypic differences. Gene expression analyses show that Δ24 in the thymus has enhanced basal activity on the Bbc3 (Puma) gene that is not found in liver. Use of an inducible allele for Δ24 give similar results in adult mice. Interestingly, upon X-ray treatment, Δ24 behaves in a distinct manner showing reduced Bbc3 expression in thymus but enhanced levels in the liver. This suggests that the CTD in liver interacts with a repressor in the liver. Chromatin immunoprecipitation analyses indicate that the effect in the liver is likely to be at the level of gene occupancy, These effects can be localized to amino acids 367-371 as additional engineered models lacking either the last 10 (Δ10) or 19 (Δ19) amino acids are phenotypically normal. This points to a key role for lysines 366, 368 and 369.

Corresponding engineered alleles have been made for the tumor-derived mutant R172H. Mice with two endogenous alleles of a truncated mutant p53 (Δ24) are indistinguishable from mice expressing full-length R172H during development. Mice expressing either the full-length or truncated alleles succumb to spontaneous tumors with associated metastasis, but numbers remain too low to have statistical power. Consistent with studies from the Lozano laboratory, R172H is at low levels in normal tissues but shows robust expression in lymphomas. In contrast, the Δ24 allele is expressed at high levels in normal tissues. This shows a key role for the CTD in mutant p53 protein stability, an effect that is not observed with the wild-type protein. Lymphomas from R172HΔ24 mice have similar p53 expression as with the full-length protein.
These finds support an important role for the CTD in regulation of mutant p53 protein stability and begin to provide a molecular explanation for the increased expression of mutant p53 that is found in tumors and not normal tissues.

CLINICAL CHALLENGES IN LI-FRAUMENI SYNDROME: CANCER AND BEYOND

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The highly penetrant nature of cancer in Li-Fraumeni syndrome (LFS) poses complex challenges for patients and their providers. LFS is a family history diagnosis requiring a proband with a sarcoma diagnosed at <45 years of age who has a 1st degree relative with cancer at <45 years and a 1st or 2nd degree relative with cancer <45 years or a sarcoma at any age. About 70% of such families have a germline TP53 mutation. The lifetime risk of cancer in germline TP53 mutation carriers is ~90% by age 60. Intensive cancer surveillance (annual whole body, brain and breast MRI, and abdominal ultrasounds in children, and quarterly blood tests) has been shown to significantly reduce cancer-related mortality. Notably, ~7% of patients have a new, asymptomatic malignancy diagnosed on their first cancer surveillance evaluation.

The IRB-approved longitudinal cohort study of LFS at the National Cancer Institute (www.lfs.cancer.gov, NCT01443468) opened to accrual in 2011 and has enrolled >200 families. In addition to research-based annual cancer screening of 140 individuals at the NIH Clinical Center, we are conducting clinical, genetic, and epidemiologic assessments of patients with LFS and their unaffected family members, including detailed questionnaires, medical record review, and biospecimens collection.

This presentation will describe the latest results from epidemiologic and psychosocial research in the NCI LFS study. In addition to our prior work quantifying the types and risks of first and subsequent cancers in this cohort, we recently completed the first study of reproductive factors and breast cancer risk in women with LFS, in which we observed a protective effect of breastfeeding on breast cancer risk.

The psychosocial component of the study includes quantitative and qualitative studies of families with LFS. For example, we conducted 26 semi-structured interviews with married, heterosexual couples in which one partner had LFS and identified mechanisms by which these couples cope with the intense physical and emotional demands of living with LFS. Similarly, we explored healthcare roles for managing LFS-related cancer risk assumed by parents, adolescents, and adult children in 23 additional families and characterized the family dynamics related to cancer risk.

The incorporation of epidemiologic and psychosocial research with clinical and molecular studies of LFS are essential in advancing our understanding of this complex syndrome and improving patient care.

MOLECULAR MECHANISMS OF P53 LOSS OF HETEROZYGOSITY IN BREAST CANCER IN RESPONSE TO IRRADIATION

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Mutations in the p53 tumor suppressor gene are the most prevalent genetic events in human ErbB2-positive breast cancer and are associated with poor prognosis and survival. In the early stages of cancer, a p53 mutation in one allele is often followed by loss of heterozygosity (LOH) in the second allele at the later stages of tumor progression. Despite a strong notion that p53 mutations with subsequent LOH are driving events in breast cancer, the molecular mechanism of p53LOH, physiological outcomes, and role in breast cancer development and progression were not comprehensively evaluated, especially in the context of DNA damaging treatments.

Using MMTV;ErbB2 model carrying both mutant p53 R172H/wild-type p53 alleles (heterozygous, H/+;ErbB2), we identified a novel oncogenic activity of mutant p53 (mutp53): the exacerbation of p53LOH in mammary tumors. We found that wild-type p53 (wtp53) allele is mostly transcriptionally competent and enables the maintenance of the genomic integrity in mutp53 heterozygous cells under normal conditions.
However, DNA damage promotes mutp53 stabilization in heterozygous cells that is associated with defective DNA repair and the aberrant cell cycle progression in the presence of inefficiently repaired DNA. In contrast to p53-/+;ErbB2 cells, irradiation of H/+;ErbB2 cells profoundly aggravates genomic instability that is coupled with deficient checkpoints, ultimately leading to p53LOH. As physiological outcomes of p53LOH, we observed the permanent stabilization of mutp53 protein, increased metastases and upregulation of the mTOR pathway only in the presence of mutp53 allele.

Hence, in mutp53 heterozygous cells, irradiation facilitates the selective pressure for p53LOH that enhances cancer cells fitness via upregulation of the mTOR pathway and provides the genetic plasticity for the acquisition of metastatic properties.

TOWARDS A PERSONALIZED AND APPROPRIATE MEDICAL MANAGEMENT OF TP53 VARIATION CARRIERS

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Over the last 5 years, our perception of the Li-Fraumeni syndrome has drastically changed: (1) germline TP53 pathogenic variations are often detected in cancer patients without familial history, highlighting that familial history is not mandatory to consider the presence of a germline TP53 pathogenic variations. Indeed, independently of the familial history, the mutation detection rate in children presenting with adrenocortical carcinomas or choroid plexus tumours has been estimated to 50% and, in females with breast carcinoma before 31 years, up to 6%. In these very suggestive clinical situations, molecular diagnosis laboratories should now ensure the detection of mosaic TP53 alterations; (2) germline TP53 pathogenic variations can be detected in families with only adult cancers, highlighting the variability of their penetrance.

Population and functional studies have revealed that the penetrance of germline TP53 variations has globally been overestimated and the variability of this penetrance underestimated. One factor underlying this variability is the type of alteration, dominant-negative missense variations are usually highly penetrant and associated with the severe forms of LFS characterized by childhood cancers.

In contrast, null mutations such as frameshift, nonsense mutations or genomic rearrangements and non dominant-negative missense variations, are predominantly identified in families with only adult cancers and have a lower penetrance. The founder p.Arg337His mutation, present in 0.3% of the population in southern Brazil, is a canonical low penetrant variation. Functional studies performed in lymphocytes have shown that this gradient is explained by a more drastic impact of dominant-negative missense variations on p53 DNA binding and transcriptional response to DNA damage.

The variability of the age of tumour onset observed in carriers within the same family supports the existence of genetic modifying factors, that should be identified by ongoing comparative exome studies. Germline TP53 mutations acting as permissive mutations to oncogenic stresses, the phenotypic expression is also probably dependent on environmental factors. Recent studies have confirmed that one key non-genetic modifying factor is, unfortunately, the chemotherapy or radiotherapy used for the treatment of the first tumour, explaining the remarkably high incidence of second primary tumours above 40%, in germline TP53 variation carriers.

In this context, it is difficult to have a dogmatic position, when considering the clinical management of germline TP53 variation carriers. The surveillance protocols proposed in 2016 and 2017 are based, from the first year of life, on abdominal ultrasound every 3-4 months, annual whole body MRI (WBMRI), annual brain MRI, and in females from 20 years on annual breast MRI. Numerous international studies published since have shown the clinical efficiency of WBMRI, with a 10% cancer detection rate. Nevertheless, considering the variability of TP53 variation penetrance, the benefits of such heavy protocols regardless to the psychological impacts should be carefully analyzed and discussed in each family. In order to determine the most appropriate starting age, it is probably reasonable to take into consideration the development of childhood tumours within the family, the type of germline TP53 variation and a previous cancer treatment based on radiotherapy or genotoxic radiotherapy.

This personalized medical management of germline TP53 variation carriers constitutes at the present time one of the most important challenges in clinical practice, especially in the context of the exponential increase of TP53 genotyping performed using enlarged cancer gene panels independently of the phenotype and leading to the detection of atypical variations requiring an expertise for a proper classification and quantification of their penetrance.

TARGETED THERAPY FOR THE AFRICAN-CENTRIC S47 VARIANT OF P53

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A non-synonymous SNP at codon 47 of TP53 exists in African descent populations (Pro47Ser, rs1800371, G/A). This SNP is second in frequency of coding region SNPs to the very common P72R SNP. The Pro47Ser variant, hereafter S47, has an allele frequency between 2-4% in African populations and a frequency of ~1.2% in African Americans; this variant has not been detected in Caucasian Americans. Interestingly, we found that mice expressing S47 in homozygous or heterozygous form are susceptible to hepatocellular carcinoma and other cancers. Mechanistically, we found that in both mouse embryonic fibroblasts (MEFs) from WT and S47 mice as well as human lymphoblastoid cells (LCLs) homozygous for WT p53 or the S47 variant are markedly impaired for programmed cell death in response to several genotoxic stresses. Our combined findings raised the possibility that cancer patients with S47 might benefit from a more personalized therapeutic regimen. To test this premise, we generated isogenic mouse and human tumor cell lines containing the WT and S47 forms of p53 and compared their response to chemotherapeutic drugs, with the goal of finding therapeutic compounds that are more efficacious in S47 tumors.

We found two compounds, cisplatin and inhibitors of BET proteins, that showed superior ability to induce cell death in S47 tumor cells, as well as superior efficacy on S47 tumors. The BET inhibitor OTX-015 and, to a greater extent, cisplatin caused dramatic decreases in the progression of S47 tumors in a xenograft model; interestingly, the ability of cisplatin to preferentially kill S47 tumor cells occurred in a transcription-independent manner, via the direct mitochondrial cell death pathway of p53. Moreover, we found that S47 tumor cells show altered metabolism and increased dependency on glycolysis, thus providing another potential therapeutic target for S47 individuals with cancer. Specifically, we found that S47 tumor cells are significantly more sensitive to the glycolytic poison 2-deoxy-D-glucose (2-DG). Taken together, our data provide a strong argument that targeted therapy can be successfully tailored to this TP53 genotype.couples in which one partner had LFS and identified mechanisms by which these couples cope with the intense physical and emotional demands of living with LFS. Similarly, we explored healthcare roles for managing LFS-related cancer risk assumed by parents, adolescents, and adult children in 23 additional families and characterized the family dynamics related to cancer risk.

The incorporation of epidemiologic and psychosocial research with clinical and molecular studies of LFS are essential in advancing our understanding of this complex syndrome and improving patient care.

SPECTRUM AND MUTATIONAL LANDSCAPE OF LFS TUMORS

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We created R172H (R175H in human) heterozygous p53 mutant mice in 8 different genetic backgrounds and collected tumors as they occur. We will describe the tumor spectrum including its dependence on age of onset, sex, and genetic background. The majority of the tumors were lymphomas and osteosarcomas with adenocarcinoma, spindle cell sarcoma and angiosarcoma being other prevalent tumor types. We sequenced the exome and mRNA of a collection of tumors including lymphomas, osteosarcomas and angiosarcomas. We discover that the majority of lymphomas were of B cell origin.

T cell lymphomas occur at earlier age of onset than B cell lymphomas. We will describe the tumor mutational landscape and pattern of recurrent oncogenic mutations in different tumor types and genetic backgrounds.

MUTANT P53 PROTECTS AGAINST DOXORUBICIN CARDIOMYOPATHY

Paul Hwang

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Doxorubicin (Dox) is a DNA damaging chemotherapeutic agent that is effective against a spectrum of cancer types, but a subset of patients can develop heart failure often many years after treatment.

Because of its prevalent use, even a small fraction of affected patients results in a large number suffering from this insidiously progressive cardiac complication. Cell death, including that induced by p53, has been proposed as a mechanism using mouse models. Most of these studies have used supra-clinical boluses of Dox to elicit acute cardiotoxicity, but a study using low dose injections of Dox suggested that inhibition of p53 actually exacerbates late-stage Dox cardiotoxicity.

To dissect the role of p53 in Dox cardiotoxicity, we used 3 distinct p53 genotype states in a divided low dose Dox exposure mouse model. We will present data showing that loss of mtDNA,but not acute p53-induced cell death associated with bolus Dox injections, contributes to cardiomyopathy pathogenesis induced by Dox with a more clinically relevant protocol.

We also report that a simple dietary supplement can prevent the development of Dox cardiomyopathy, and that mitochondrial markers associated with cardiomyopathy development can be observed in blood and skeletal muscle cells, which may have utility in the clinics.

ATYPICAL PHENOTYPES IN LI-FRAUMENI SYNDROME, TP53 GERMLINE PATHOGENIC VARIANTS AND CANCER RISK

Maria Isabel Achatz

Hospital Sirio Libanês - Brazil

Variable cancer spectrum and cancer penetrance in Li–Fraumeni syndrome (LFS) have been described in different populations. Reports of atypical presentations of the syndrome raised questions regarding the penetrance and prevalence of pathogenic germline TP53 variants. We reported higher than-expected population prevalence estimates composed of individuals unselected for cancer history of pathogenic and likely pathogenic germline TP53 variants in the gnomAD dataset (version r2.0.2, n = 138,632).
Variants were selected and classified based on our previously published algorithm and compared with alternative estimates based on three different classification databases: Clin-Var, HGMD, and the UMD_TP53 database. Conservatoive prevalence estimates of pathogenic and likely pathogenic TP53 variants were of one carrier in 3,555–5,476 individuals.

This study shows a higher-than-expected population prevalence of pathogenic and likely pathogenic germline TP53 variants. However, these estimates do not reflect the prevalence of the classical LFS phenotype, which is based upon family history of cancer.

THE ROLES OF INITIATING P53 MUTATIONS IN HUMAN CANCERS: THE ORDER OF MUTATIONS AND TUMOR CELL TYPE MATTERS

Arnold J. Levine, Nancy A. Jenkins, and Neal G. Copeland

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We propose that initiating truncal mutations play a special role in tumor formation by both enhancing the survival of the initiating cancer cell and by selecting for secondary mutations that contribute to tumor progression, and that these mutations often act in a tissue-preferred fashion.

We explain why inherited mutations in the p53 gene often have different tissue specificities compared to spontaneous mutations in the same gene. Initiating truncal mutations make excellent neo-antigens for immunotherapy, and understanding why one mutation selects for a second mutation in a particular tissue type could one-day aid in the design of gene-targeted combination therapies.

TRACING AND TARGETING TP53/PI3K MUTATIONS IN HNSCC PATIENTS

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HNSCC comprises 5.5% of all incidence cancers and is the sixth leading cancer worldwide. It is typically characterized by locoregional diffusion with 60% of patients affected by relapse. Unfortunately, advances in the surgical and medical treatments for HNSCC over the past two decades have not improved overall disease outcomes.
As a consequence, locoregional failure is the most common cause of death in HNSCC patients. TP53 is the most frequently mutated gene in head&neck squamous cell carcinomas (HNSCC) (http://www-p53.iarc.fr).

Recently, whole-exome sequencing analyses reported that TP53 mutations occur in HNSCC with a frequency of 72%. Almost 70% of HNSCC harbor genomic alterations in one of the major components of PI3K pathway, being PIK3CA, PTEN and PIK3R1 the most frequently mutated genes of the pathway. Data related to the tracing of TP53 and PI3K mutations in tumoral tissues and matched blood samples of HNSCC patients and the therapeutic targeting of the oncogenic network TP53/PI3K using the selective PI3K kinase inhibitor Alpelisib will be presented.

TARGETING MUTANT P53: A NEW APPROACH FOR THE TREATMENT OF PATIENTS WITH TRIPLE-NEGATIVE BREAST CANCER?

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The identification of a targeted therapy for patients with triple-negative breast cancer (TNBC) is one of the most urgent needs in breast cancer therapeutics. Since the p53 gene is mutated in approximately 80% of TNBC, it is an attractive therapeutic target for this subset of breast cancer patients. Using a large panel of breast cancer cell lines, we showed that treatment with 3 mutant p53 reactivating compounds, ie, APR-246, PK11007 or COTI-2 blocked cell growth and induced apoptosis.

For all 3 compounds, significantly lower IC50 values for growth inhibition were found in mutant p53 cell lines than in wild-type p53 cell lines, ie, p53 mutant cell lines were more responsive to growth inhibition than p53 wild-type cells. Additionally, a significant inverse correlation was found between IC50 values and p53 protein levels measured by ELISA ie, the higher the endogenous p53 protein level, the more sensitive the cell lines were to the mutant p53- reactivating compounds.

By staining with antibodies specific for correctly folded wild-type p53 (PAb1620) or unfolded mutant p53 (PAb240), we showed that the 3 compounds induced refolding of mutant p53 back to a wild-type conformation. Based on our findings, we conclude that targeting mutant p53 with APR-246, PK11007 or COTI-2 are potential approach for treating patients with p53-mutated triple-negative breast cancer. We are now planning to carry out such a trial with COTI-2.

SMALL-MOLECULE COMPOUNDS FULLY RESCUE A BATCH OF STRUCTURAL MUTANT P53 WITH SOLVED ATOM-LEVEL MECHANISM

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As titled, many small-molecule compounds are either hit in screening or rationally synthesized in our group. In assays quantitively comparing with wild-type p53, these compounds efficiently enhance a batch of structural mutant p53's thermal stability (5-6), protein folding (fully restored PAb1620 epitope), transcriptional activity (fully restored transcriptional activity in luciferase assay, fully restored express profile of p53 targets in RNAseq), tumor-suppressive functions (selective to structural mp53).

Rescue efficiency of these compounds for thousands of mutant p53s (covers >95% p53 mutations deposited in IARC, R18) are quantitively determined, providing the patient recruitment guide in the coming personalized clinical trials (based on individual mutant p53 rather than whether p53 is mutated). The talk with be ended with atom-level rescue mechanism (stapling four key residues that respond for modern-day human p53 thermal instability), progresses in industry, and progresses in clinical trial.

Reference
1.Tumor suppressor TP53 is the most frequently mutated gene in human cancers (Kandoth et al, Nature 2013)
2.TP53 is the most studied gene of all time, much more than the second TNF (Dolgin, Nature 2017).
3.TP53 keeps being the most cited gene every year since 2001, much more than the second APOE (Dolgin, Nature 2017). 4.So far, 415 clinical trials involving p53 have been registered on Clinicaltrials.gov. (https://clinicaltrials.gov) 5.So far, at least 45 groups have worked with mutant p53-based therapies (Sabapathy et al, Nat Rev Clin Oncol 2018). 6.So far, approx. 8 2 targeted anti-cancer drugs are approved with most of them inhibiting oncogenic proteins (Abramson, www.mycancergenome.org 2018), to our knowledge, none repairing tumor suppressors.
7. Together with Bcl-2, -catenin, c-myc, RAS, NF-kB, and others, p53 is hard to be targeted compared to routinely targeted kinase. It may partially attribute to three facts: 1) p53 is a transcription factor, 2) p53 apparently has 'no' druggable pocket on the protein surface except for a reported pocket unique to p53-Y220C, 3) p53 as a tumor suppressor need to be repaired but not inhibited. While Bcl-2 with an unconventional large pocket (a large BH3 pocket) is now drugged meanwhile KRAS-G12C with an unconventional allosteric pocket (GTP/GDP-binding pocket-neighboring allosteric pocket) is being drugged with substance progresses, we find p53 also harbors some unconventional pockets (largely mutant p53-specific, dynamic, odd-shape, generated by evolution, not overlapping with DNA-binding interface, manipulatable to rescue rather than inhibit p53 transcriptional activity), laying basis for our compounds to drug mutant p53.

MUTANT P53 AS A MASTER REGULATOR OF IMMUNE DYNAMICS DRIVING TUMORIGENESIS VIA EXOSOMES

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BACKGROUND: Exosomes are small spherical packages released by cells into the extracellular environment. It has become evident that exosomes convey information to neighboring or remote cells by delivering RNAs and proteins thus affecting signaling pathways in various physiological and pathological conditions including cancer. Mutations in TP53 are considered one of the most frequent genetic alterations in human cancer. Besides the abrogation of the wild-type (WT) p53-mediated tumor suppression, a distinct set of missense mutations was reported to endow mutant p53 proteins with novel activities termed gain-of-function (GOF). Even though mutations in TP53 are typically thought to arise in the tumor cells rather than in the stroma, the non-cell-autonomous effects of these mutants over the tumor microenvironment are poorly understood. In most solid cancers, a major component of the tumor stroma are macrophages referred to as tumor associated macrophages (TAMs) which mostly derive from peripheral blood monocytes recruited into the tumor mass. In recent years, TAMs have been extensively studied and proposed as a significant contributing factor to tumor progression. In the presented study, we investigated intercellular interactions mediated by ex somes in the context of cancers harboring mutant p53 and TAMs.

RESULTS: Tumor cells harboring mutp53 were found to exert a non-cell-autonomous effect over macrophages. When exposed to tumor cells harboring mutp53, monocytes became polarized towards a distinguished subset of macrophages characterized by TAMs-related markers. The mutant p53 affected TAM were characterized as TNF-low/ IL-10high, over expressing CD-206 and CD163, with decreased phagocytic ability and increased invasion and matrix degradation potency. Investigating the exosomal transfer from mutp53 tumor cells to macrophages, revealed a mutp53-specific miRs signature led by miR-1246 promoting the TAM phenotype and creating an invasive front together with tumor cells. MiR-1246 was also found to be the top mutp53-associated miR in a cohort of 57 human colorectal resected tumors. In-situ hybridization using a specific miR-1246 probe, demonstrated a higher expression in mutp53 tumors, both in cancer cells and immune cells compared with WT p53 tumors.

CONCLUSIONS: Altogether, these findings are consistent with a microenvironmental role for specific "hot-spot" GOF p53 mutants tightening the interaction between the tumor cell and the immune compartment in colon cancer. We show that tumor cells interact with macrophages to promote immunosuppressed microenvironment using exosomes shed by the tumor cells. Such an interaction exacerbates tumorigenesis and leads to increased metastatic burden in mice and cancer patients. Deciphering the intricate regulation shared by the tumor cell and its surrounding macrophages may give rise to novel prognostic and diagnostic tools as well as to therapeutic approaches targeting of specific bacteria, TAM, tumor-promoting miRs and mutp53-specific subsets of exosomes.

PROBING THE DYNAMICS OF WILD TYPE AND MUTANT P53: NOVEL ROUTES TO RESTABILIZATION

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If we hypothesize that mutant p53 conformations can be restabilized to wild type conformation, then we can assume that mutant p53 must largely adopt conformations that are associated with different mutations; however there will also exist a small population of mutant p53 that adopts wild type conformation. This opens a new opportunity to probe the events that initiate the change of conformation from wild type to mutant, and therapeutically target mutant p53 with small molecules/peptides that can drive the population of mutant p53 molecules to adopt wild type conformation. Using simulations we find dynamical features, including a "druggable" pocket, that are common to a set of mutant p53 DNA binding domains and characterize the initial events that disrupt the wild type conformation.

These then can be subject to manipulation using small molecules and peptides to prevent the transition to mutant conformation and we take guidance from the known interactions of p53 with SV40 T-antigen and E6 proteins (Nucleic Acids Research, Volume 47, Issue 4, 28 February 2019, Pages 1637–1652).

MUTANT P53 INTERACTS WITH TANK-BINDING KINASE 1 (TBK1) TO INHIBIT ACTIVATION OF CANCER CELL-AUTONOMOUS INNATE IMMUNE RESPONSE

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Mutations in the p53 tumor suppressor occur very frequently in human cancer. Often, such mutations lead to the constitutive overproduction of mutant p53 proteins, which can exert a cancer-promoting gain of function (GOF). We have identified a mechanism by which mutant p53 controls both cell-autonomous and non-cell autonomous signaling to promote cancer cell proliferation and potentially suppress the immune system. Mutant p53 binds to multiple components of the cytoplasmic DNA sensing machinery, cGAS/STING/TBK1/IRF3, that controls the activation of the innate immune response.

We find that mutant p53, but not WT p53, binds to TANK binding protein kinase (TBK1) and inhibits both its basal and agonist-induced activity. The association of mutant p53 with TBK1 prevents the formation of a trimeric complex between TBK1-STING-IRF3, which is required for activation, nuclear translocation and transcriptional activity of IRF3. Mutant p53 knockdown restores TBK1 activity, resulting in the transcriptional induction of IRF3 target genes. Therefore, our study identifies a mechanism by which mutant p53 blocks the activation of the innate immune signaling pathway. Since this pathway plays a key role in how cells communicate with the immune system, its suppression likely contributes to immune evasion by cancer cells.
Thus, overriding mutant p53's inhibition of this pathway may ultimately lead to restored immune cell function and cancer cell eradication.

P53-REACTIVATING DRUG PRIMA-1MET REPURPOSING TO TREAT SEVERE SKIN INVOLVEMENT IN P63-RELATED ECTODERMAL DYSPLASIA SYNDROMES

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Ectodermal dysplasias are genetic diseases affecting the development and/or homeostasis of two or more ectodermal derivatives including hair, teeth, nails and certain glands. Among them, the syndrome AEC [Ankyloblepharon, Ectodermal dysplasia, cleft lip/palate, MIM106260] results from a heterozygous mutation in the SAM domain of the TP63 gene that belongs to the P53 gene family. The cutaneous involvement associates hypohidrosis, neonatal erythema and congenital erosions and in some patients aplasia cutis of the vertex, the back, the palms and the soles. In some patients, skin lesions never heal. Their treatment associates dressings and plastic surgery.

By the use of induced pluripotent stem cells (iPSC), we found that patient-derived-iPSC displayed altered epidermal commitment that can be partially rescued by PRIMA-1MET, a p53-reactivating small compound. PRIMA-1MET molecule is the subject of clinical trials on p53-dependent cancers. Primary keratinocytes isolated from two AEC patients with persistent aplasia cutis confirmed altered epidermal differentiation that can be efficiently rescued by PRIMA-1MET. The lack of curative treatment led us to repurpose the small compound for AEC patients. A formulated PRIMA-1MET ointment was topically applied in the two AEC patients of 9 and 10 years, respectively. Within the 10 months (patient 1) and 4 months (patient 2) of treatment, the involved areas reduced in size. Epidermis initially absent appears and almost covers the aplasia cutis. Its thickness increases progressively in the two patients. Oozing and pain (EVA 4 to 1) drastically decreased, Patient 2 gained in height and weight.

Therefore, topical treatment of chronic aplasia cutis that persists during the second decade in AEC patients with PRIMA-1MET is particularly encouraging. The potential mechanisms of action of the drug will be discussed.

GENOME WIDE CRISPR KNOCKOUT AND ACTIVATION SCREENS PROVIDE NOVEL INSIGHTS INTO THE MECHANISM OF ACTION OF APR-246 IN MUTANT P53 CANCER CELLS

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APR-246 induces cell death in cancer cells through mechanisms that converge on apoptosis. To date, published reports have highlighted two predominant mechanisms of action: (1) mut-p53 reactivation leading to p53 target gene expression and apoptosis, and (2) disruption of redox balance through inhibition of glutathione (GSH) and thioredoxin systems resulting in mitochondrial ROS accumulation and apoptosis. The second mechanism is enhanced by mut-p53 mediated deregulation of the transcription factor NRF2, a central regulator of redox balance1. To provide a more comprehensive understanding of APR-246 activity we undertook genome wide, pooled lentiviral CRISPR knockout and activation screens to identify genetic perturbations that alter sensitivity and resistance to APR-246 activity in mutant-p53 (R248Q) cancer cells.

To identify gene knockouts associated with sensitivity to APR-246, cells were transduced with a CRISPR library containing ~76,000 sgRNAs, targeting ~18,000 protein-coding genes. Transduced cells were exposed to a low (GI10) and a high dose (GI90) of APR-246 to identify sgRNAs that drop-out (sensitise) or are enriched (resistance) when challenged with APR-246 compared to vehicle controls for 8 days. Top hits were validated in single gene knockout assays. SLC7A11 and GCLM, both of which regulate de novo GSH synthesis were identified as key modulators of APR-246 sensitivity, consistent with our previous findings1. The screen also identified novel regulators of APR-246 sensitivity, including enzymes in the mitochondrial arm of one-carbon metabolism, some of which are also known to regulate mitochondrial oxidative phosphorylation. In contrast, the enrichment screen failed to identify gene knockouts that confer resistance, suggesting that individual gene knockout may not be sufficient to induce robust resistance to APR-246. In parallel, a CRISPRa library delivered ~56,000 sgRNAs, targeting the transcriptional activation of ~18,000 protein-coding genes. Concordant with the CRISPR knockout screen, resistance to APR-246 was accompanied by enrichment of GCLC activation, the catalytic subunit of the glutamate-cysteine ligase required for GSH synthesis. Intriguingly, activation of genes involved in the mitochondrial respiratory transport chain and mitochondrial ribosomal biogenesis also conferred resistance to APR-246. Together, these findings indicate a novel role of mitochondrial translation and maintenance of the electron transport chain in APR-246 resistance and potential mechanism of action.

1 Liu, D. S. et al. Inhibiting the system xC(-)/glutathione axis selectively targets cancers with mutant-p53 accumulation. Nature communications 8, 14844, doi:10.1038/ncomms14844 (2017).

P53 IN THE CLINIC – STILL A CHALLENGE

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TP53 has been studied in detail but still, there is no explicit consensus about the clinical value of TP53. There are as many clinical studies that show that p53 has an influence on the survival of cancer patients, as there are studies that show exactly the opposite.

By browsing data from literature and own published studies, the important difference of the "prognostic "and "predictive " marker type is demonstrated. By processing several clinical studies it is shown that TP53 is not a prognostic marker, but rather a purely predictive marker. We suspect that the neglect of the marker type can lead to misinterpreted results in the clinical p53 literature.

Finally, data from our prospective randomized colon cancer trial are processed demonstrating how the biomarker TP53 independently predicted effect of adjuvant 5-FU chemotherapy (p=0.0010) in a certain stage of lymph node positive colon cancer patients. Lymph-node positive colon cancers have been treated with 5-FU for decades and the varying efficacy of 5-FU treatment in these patients is a well-known but hitherto unexplainable clinical phenomenon.

The tumor stage specific interaction between TP53 status and survival (p=0.0374) we found in this cohort is likely to explain this clinical phenomenon seen in tens of thousands of colon cancer patients.

APR-246 FOR TREATMENT OF TP53 MUTATED TUMORS AND HEMATOLOGICAL MALIGNANCIES - CLINICAL STATUS AND MODE-OF-ACTION

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Mutation of the TP53 gene is associated with dire prognosis due to lack of treatment options over a wide variety of tumor types. The compound APR-246 (PRIMA-1Met) was developed to target and correct mutant p53, resulting from missense mutations in TP53. APR-246 is undergoing several phase II clinical trials and has recently entered clinical phase III in myelodysplastic syndrome.

Clinical data from early clinical studies will be presented, including data indicating activation of the p53 pathways in patients. APR-246 is converted to the Michael acceptor MQ covalently binding cysteines, including key cysteines on mutant p53. This has previously been shown to enhance mutant p53 core domain thermostability. In addition, MQ has been shown to inhibit thioredoxin reductase, thioredoxin and glutaredoxin, and to deplete cellular glutathione (GSH), all of which contributing to anti-tumor effects by increasing oxidative stress. These dual effects of APR-246 may synergize and provide an explanation for the strong synergy shown with a wide variety of therapeutic agents, ranging from DNA damaging agents to Bcl-2 antagonists. The chemistry and biology of MQ has been studied, leading to further insights into the mode-of-action of APR-246.

MISSENSE AND NONSENSE MUTANT TP53 AS TARGETS FOR NOVEL CANCER THERAPY

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The TP53 gene is frequently mutated in human tumors but the mutation frequency and type of mutations varies across different tumor types. Many TP53 mutations are missense mutations that cause single amino acid substitutions and disrupt p53's DNA binding and transcriptional transactivation activity.

We discovered the compounds PRIMA-1 and APR-246 (PRIMA-1Met) that restore wild type function to mutant p53. Both compounds are converted to the Michael acceptor MQ that binds covalently to p53 cysteines, including Cys277. MQ binding enhances p53 core domain thermostability. APR-246 shows strong synergy with chemotherapeutic drugs such as cisplatin, and with various other therapeutic agents. In addition, APR-246 targets redox homeostasis by inhibiting thioredoxin reductase, thioredoxin and glutaredoxin, and depleting cellular glutathione (GSH) via MQ. These effects can probably contribute to APR-246-induced tumor cell death. APR-246 is currently tested in a phase II clinical trial in high-grade serous ovarian cancer (HGSOC) and in a phase III trial in myelodysplastic sydrome (MDS). A smaller but still significant fraction of TP53 mutations are nonsense mutations resulting in expression of truncated and unstable p53. The most common nonsense TP53 mutation is R213X. Induction of translational readthrough by aminoglycoside antibiotics such as G418 has been shown to induce expression of full length p53 in tumor cells carrying nonsense mutant TP53.

Our experiments demonstrated that the combination of G418 with Mdm2 inhibitors, e.g. Nutlin, enhances levels of full length p53 and p53-induced cell death in R213X nonsense mutant TP53-carrying tumor cells. We have also screened chemical libraries and identified novel candidate compounds that are being validated for translational readthrough activity. We will aim at clinical trials with one or a few selected compounds. Thus, our results suggest that induction of translational readthrough is a feasible strategy for improved treatment of tumors with nonsense mutant TP53, and possibly other tumor suppressor genes with nonsense mutations.

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MUTATION-SPECIFIC LIFETIME CANCER RISK IN GERMLINE TP53 MUTATION CARRIERS

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BACKGROUND: Germline TP53 mutation predisposes to multiple cancers but the lifetime risk of cancer in carriers has been evaluated only in relatively small cases series. We have used data from IARC TP53 database (p53.iarc.fr), currently the most comprehensive dataset on germline TP53 mutation carrier, to analyze age-dependent and organ-specific excess cancer risk as compared to a reference non-carrier population. We have used a newly developed functional classification of p53 mutants based on transactivation clusters to estimate mutation-specific lifetime cancer risk in carriers (see Poster Lemonnier et al.).

METHOD: A total of 2,079 carriers of a pathogenic germline mutation in TP53 from 775 families were analyzed (excluding carriers of the atypical Brazilian founder mutation p.R337H). Iterative clustering of mutants was based on transcriptional data generated in experimental yeast assays by Kato et al. 2003 [1]. Data on Lifetime Risk of Developing or Dying of Cancer in the US population from the Surveillance, Epidemiology and End Results Program (SEER) [2] were extracted to constitute a reference population.

RESULTS: Overall, 2,550 tumors were reported in confirmed or obligate TP53 mutation carriers. Median ages (p25-p75) at cancer in carriers were 28 years (11-45 years) and 31 years (20-41 years) in males and in females, respectively (P = 0.085). After excluding female breast cancers, the median age became significantly lower in females than in males (25 versus 28 years, P = 0.023). After adjusting for gender, carriers had 78.7% risk over lifetime of developing cancer (95%CI: 76.9-80.4), representing a 2 fold excess risk compared to lifetime cancer risk in a reference population.

The risk of second cancer was 27.5% (95%CI: 24.4-30.6), with a significant difference between males and females (24.3% versus and 33.8%, P = 0.016). A significant lifetime excess risk (P<0.01) was observed for adrenal cortical carcinoma (258.1 fold), bone sarcomas (86.8 fold), soft-tissue sarcomas (38.9), brain cancers (21.9), stomach cancer (4.10) and breast cancers (3.65). However, other cancers did not show a significant lifetime excess risk. Excess risk was 40-100 in childhood and early adulthood (0-19 years), 3-5 in early adulthood (20-49 years) and 0.75 in late adulthood (above 50 years), suggesting that cancer risk is actually decreased in long-term surviving TP53 carriers as compared to an matched-matched normal population.

Significant differences in lifetime risk and in median ages of tumor onset were observed among carriers with mutations belonging to different functional clusters.

CONCLUSION: These provide risk estimates that may help to better predict cancer occurrence in carriers and evaluate the benefits of interventions aimed at prevention and early detection. They also underline the tissue and age-specific cancer risk associated with mutant TP53 carrier may be caused by functional differences in the contribution of p53 to the development and homeostasis of these tissues.

References

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THE INTERACTION OF P53 MUTANTS WITH THE HYPOXIC MICROENVIRONMENT

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The tumour suppressor protein p53 has an essential role in the response to cellular stress and somatic cells largely rely on p53 to overcome genotoxic stress and to maintain genomic integrity.
Inactivation of p53 can produce neomorphic forms of p53 (mutant p53) that exhibit oncogenic gains of function (GOF) activities. These mut-p53 forms can interact with stressors and modify the transcriptional and chromatin remodelling responses, leading to an abnormal cellular response to toxicity, such as hypoxia.
Here, we report that in non-small-cell-lung-cancer (NSCLC) p53 mutants exert a GOF effect on the major regulator of the hypoxia response, the hypoxia-inducible factor 1 (HIF-1). This GOF impinges on the regulation of a selective gene expression signature, involved in pro-tumourigenic non-cell-autonomous functions. Depletion of p53 mutants impairs the HIF-mediated upregulation of extracellular matrix (ECM) components, thus affecting tumourigenesis of NSCLC in vitro and in mouse models. Analysis of surgical resected human NSCLC indicates that this signalling correlates with poor prognosis. With this novel GOF effect of p53 mutants, we suggest a synergistic activity of p53 and HIF-1 with important implications for patient stratification to improve safety and effectiveness of therapies.

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MUTANT P53 ACCUMULATES UPON DELETION OF THE C-TERMINAL DOMAIN IN A NOVEL KNOCK-IN MOUSE

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p53 is best known as a transcription factor that elicits a vital tumor suppressive response in the presence of various oncogenic signals. The importance of the TP53 gene in protecting against tumorigenesis is emphasized by its mutation in 50% of human cancers. In these tumors, p53 harbors a high frequency of missense mutations that maintain expression of the full-length protein unlike most tumor suppressors that primarily succumb to nonsense mutations. The majority of p53 missense mutations perturb the DNA-binding domain and severely impair binding to canonical, tumor suppressive p53 target genes. Interestingly, numerous reports in cell culture highlight that mutant p53 is recruited to novel sites on DNA via protein-protein interactions and aberrantly activates expression of genes that promote tumorigenesis.
These studies not only highlight a possible mechanism mediating mutant p53 gain-of-function but also illuminate an attractive opportunity for therapeutic intervention. To further explore this hypothesis, we proposed that potential protein-protein interactions may require the C-terminal domain of p53. To this end, we utilized an existing $p53^{R172H}$ mouse model to develop a novel knock- in mouse using CRISPR/Cas9 that expresses $p53^{R172H}$ lacking the C-terminal 24 amino acids, $p53^{R172H\Delta24}$. Our preliminary observations show that $p53^{R172H\Delta24}$ accumulates to higher levels than $p53^{R172H}$ in several healthy organs including the liver, spleen, and thymus. This is in contrast to deletion of the C-terminal 24 amino acids from wild-type p53 which does not affect protein levels in either the thymus or liver.

This suggests that protein stability of wild-type and mutant p53 are regulated in distinct manners in these organs. In thymic tumors, both $p53^{R172H}$ and $p53^{R172H\Delta24}$ accumulate to similar levels and the onset of spontaneous tumors appears identical. Additionally, cyclohexamide experiments in mouse embryo fibroblasts show that $p53^{R172H\Delta24}$ is expressed at higher basal levels and has a longer half-life than the full-length mutant. Taken together, these observations suggest that the removal of the C-terminus stabilizes mutant p53, which is hypothesized to be a critical step in activating mutant p53 gain-of-function in tumors. Ongoing experiments will further detail the mechanisms mediating mutant p53 stability and characterize the transcriptomic signatures of $p53^{R172H}$ and $p53^{R172H\Delta24}$ in various organs.

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P53 ISOFORM FUNCTION IN GLIOBLASTOMA THERAPY

Jessica Beck

Glioblastoma is the most common primary brain tumor with a high rate of recurrence and 5-year survival of approximately 5%. Following diagnosis, patients may receive radiation treatment and temozolomide chemotherapy, both of which have been associated with induction of cellular senescence in cancer cells. Although the majority of primary glioblastomas have wildtype p53, tumors accumulate mutations following temozolomide treatment including mutations in p53.

The increase in p53 mutations in higher grade astrocytomas and in secondary glioblastomas suggests that p53 function may be important in temozolomide-induced senescence.

Cellular senescence is a critical barrier to tumor progression and is regulated by endogenous isoforms of p53 in primary human cells.

The p53 isoform, 133p53, is expressed in normal human tissues, prevents cellular senescence, and is upregulated in some cancer cells while p53 promotes senescence and is inhibited by high expression of SRSF3 in cancer cells.

Although studies have demonstrated the roles of these isoforms in senescence of primary human cells, their functions in tumor progression and malignancy are still being investigated.

Our current studies aim to investigate the roles of p53 isoforms and p53 mutations in the response of glioblastoma cells to temozolomide chemotherapy.

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THE P53 TARGET WIG-1 REGULATES CELLULAR REDOX BALANCE AND MITOCHONDRIAL RESPIRATION

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WIG-1 or ZMAT3 is a p53 target gene identified in our laboratory that encodes an RNA-binding protein which is induced upon cellular stress and regulates stability of multiple RNAs. The WIG-1 gene is located in a genomic region frequently amplified in cancer. We showed that high nuclear Wig-1 protein expression correlates with worse survival in cervical cancer. Using RNA-immunoprecipitation (RNA-IP), we identified novel Wig-1 mRNA targets involved in cellular redox regulation and mitochondrial respiration in human tumor cell lines (HCT116 and Saos-2) and in normal human diploid fibroblasts (HDFs).

Wig-1 binds and stabilizes Thioredoxin Reductase 1 (TrxR1) mRNA, thus contributing to the cellular antioxidant system. Wig-1 destabilizes SLC7A11 mRNA, a component of the cystine/glutamate antiporter system involved in maintenance of glutathione status as well as cell death by ferroptosis. Furthermore, by using a systems biology approach and high-throughput analysis of Wig-1 RNA-IP sequencing data, we found that Wig-1 potentially plays a critical role in mitochondrial respiratory functions. Thus, we are investigating the impact of Wig-1 knockdown on mitochondrial Oxygen Consumption Rate (OCR) using Seahorse technology. The overall aim of this study is to understand the role of Wig-1 within p53-mediated tumor suppression, notably through the regulation of energy metabolism and antioxidant defense.

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ATTENUATION OF P53-ALPHA ISOFORM TRANSACTIVATION BY INVERTED REPEAT SEQUENCES IN P53 TARGET SITES

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p53 is one of the most studied tumour suppressor proteins, playing important roles in regulating basic biological processes including cell cycle, apoptosis, senescence and metabolism. The human Tp53 gene contains alternative promoters and thanks to alternative splicing can produce several isoforms. p53 protein function is realized by binding to specific DNA response elements resulting in the transactivation of target genes.
Here we present results of p53alpha isoform obtained using a yeast isogenic system for in vivo transactivation studies in chromosomal context to specifically evaluate the influence of secondary DNA structures on transactivation.
We used a panel of S. cerevisiae haploid strains that are isogenic except for different p53 DNA binding sites positioned upstream of a luciferase reporter gene and chosen based on different propensities to form DNA structures. The targeting of the chosen p53 binding site was achieved by the Delitto Perfetto oligonucleotide targeting technique by the replacement of a double reporter ICORE cassette, facilitated by the induction of a single site-specific DNA double strand break.
The obtained yeast reporter strains differing in the p53 target site (with and without propensity to form cruciform structure) were transformed with a plasmid for the expression of p53alpha. Our results show that transactivation is correlated better with the relative propensity of a response element to form cruciform structure than to its predicted DNA binding affinity.

These results point to the fact that structural features of DNAs are an important determinant to its DNA-binding and transactivation function. In the follow-up experiments we would compare DNA-binding and transactivation potential of other p53 isoforms relevant in cancer development, expressed alone or co-expressed with p53alpha.

This work was supported by the Grant Agency of the Czech Republic (18-15548S).

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EFFLUX PUMP INHIBITORS POTENTIATE MUTANT P53 TARGETING COMPOUND APR-246 TUMOR KILLING BY INCREASING DRUG ACCUMULATION AND PERTURBING REDOX STATUS

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Mutation of the TP53 tumor suppressor gene occurs at high frequency in human tumors. In high-grade serous ovarian carcinoma TP53 mutations occur in up to 95% of the cases. Mutant p53-carrying tumors are in general more resistant to conventional anticancer drugs. We have previously identified the mutant p53-targeting compound APR-246 that is currently tested in a Phase II clinical trial in high grade serous ovarian cancer (platinum-sensitive) and in a Phase III trial in myelodysplastic syndrome (MDS). APR-246 induces refolding of mutant p53 to a wild type-like functional protein capable of inducing p53 downstream targets and eventually cell death. APR-246 is non-enzymatically converted to its active product methylene quinclidinone (MQ) which binds to cysteine residues in p53 and promotes refolding.

APR-246 also exhibits pro-oxidant activity as MQ binds and inactivates important antioxidants such as glutathione and thioredoxin reductase, which both are essential for the cellular defense against oxidative and electrophilic stress.

Multidrug resistance protein 1 (MRP1/ABCC1) plays an important role in efflux of unconjugated and glutathione-conjugated drugs, and thereby reduces drug accumulation and enhances tumor cell resistance. As MQ readily conjugates to glutathione, we hypothesized that MRP1 could be involved in the efflux of APR-246 and/or MQ from cells. Indeed, inhibition or knockdown of MRP1 leads to intracellular 14C-accumulation after 14C- APR-246 treatment in tumor cell lines of various origin (including several ovarian cancer cell lines). The label is situated in the part of APR-246 retained in the active drug MQ. Additionally, treatment with MRP1 inhibitors shifted intra- and extracellular thiol status. The compound accumulation and thiol status shift caused profound synergistic cell death upon combination treatment with APR-246 and MRP1 inhibition. Missense mutant TP53 status correlated with the degree of sensitivity to combination treatment. Normal fibroblasts were unaffected at similar concentrations. LC-MS analysis revealed that cellular APR-246 levels were unchanged after MRP1 inhibition while MQ conjugated to glutathione (GS-MQ) accumulated. Furthermore, we showed that GS-MQ conjugates are reversible under physiological conditions.

This suggests that GS-MQ could serve as a drug reservoir, increasing the availability of MQ for targeting of mutant p53.

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MUTANT P53 MODULATES THE UNFOLDED PROTEIN RESPONSE (UPR) AND IMPROVES CANCER CELLS' SURVIVAL UPON ENDOPLASMIC RETICULUM STRESS

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Missense alterations of the TP53 gene are frequent in human cancers, and generate mutant p53 proteins (mutp53) that can acquire oncogenic properties. Accumulating evidences indicate that mutant p53 proteins can modulate cell homeostatic processes, suggesting that mutp53 could increase resistance of tumour cells to intrinsic and extrinsic cancer-related stress conditions, thus providing a selective advantage during progression.

We provide evidence that mutant p53 can modify the Unfolded Protein Response (UPR) pathway and can increase survival upon endoplasmic reticulum (ER) stress, a condition to which cancer cells are exposed during tumour formation and progression as well as during therapy. Mechanistically, this effect involves activation of the pro-survival UPR effector ATF6, coupled with inhibition of pro-apoptotic UPR effectors JNK and CHOP. In a triple-negative breast cancer cell model with mutant p53, we find that ATF6 activation is necessary for viability and invasive phenotypes.

These results suggest that ATF6 inhibitors could be combined with mutp53-targeting drugs to specifically sensitize cancer cells to endogenous or chemotherapy-induced ER stress.

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RESVERATROL PREVENTS P53 AGGREGATION IN VITRO AND IN VIVO

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INTRODUCTION: One potential target for cancer therapeutics is the tumor suppressor p53, which is mutated in more than 50% of malignant tumors. Loss of function (LoF), dominant negative (DN) and gain of function (GoF) mutations in p53 are associated with amyloid aggregation. Resveratrol is a naturally occurring polyphenol that regulates many cellular targets involved in cancer signaling pathways, including those mediated by p53. OBJECTIVES: We tested the potential of resveratrol to prevent the aggregation of wild-type and mutant p53 in vitro, in human breast cancer cells (MCF-7, MDA-MB-231 and HCC-70 and) and in a nude mice xenograft model of breast cancer.

MATERIALS AND METHODS: p53 aggregation experiments were performed by using fluorescence spectroscopy techniques and immunofluorescence co-localization assays. Cell proliferation and cell migration were assessed using the trypan blue exclusion test of cell viability and the wound-healing assay, respectively.

DISCUSSION AND RESULTS: Based on our data, an interaction occurs between resveratrol and the wild-type p53 core domain (p53C). In addition, resveratrol and its derivatives pterostilbene and piceatannol inhibit mutant p53C aggregation in vitro. Furthermore, resveratrol reduces mutant p53 protein aggregation in MDA-MB-231 and HCC-70 cells but not in the wild-type p53 cell line MCF-7. To verify the effects of resveratrol on tumorigenicity, cell proliferation and cell migration assays were performed using MDA-MB-231 cells. Resveratrol significantly inhibit the proliferative and migratory capabilities of these cells. Finally, mutant p53 and p53 amyloid aggregates detected in the breast cancer tissues of nude mice, were reduced by resveratrol treatment.

CONCLUSIONS: Our study provides evidence that resveratrol directly modulates p53, enhancing our understanding of the mechanisms involved in p53 aggregation and its potential as a therapeutic strategy for cancer treatment.

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TARGETING P53 DEFICIENCY WITH ONCOLYTIC VIRUSES IN B-CELL MALIGNANCIES

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In Multiple Myeloma and Mantle Cell Lymphoma patients and in B-cell malignancies, TP53 deletion and TP53 mutations are frequently associated. Treatments for these diseases have improved in the past decade but patients with p53 deficiency (deletion and mutation) have a reduced response to all treatments. Although the role of p53 loss in tumor emergence was recently shown to be related to its loss of DNA repair coordination, resistance to therap is assumed to be related to the inability of the p53 defective protein to transactivate apoptotic genes such as BBC3 (Puma), PMAIP1 (Noxa) and BAX. However, since p53 coordinates cellular regulation in response to numerous stresses, including viral infection, its loss of function should induce tumor-specific vulnerabilities such as viral infection.
We thus hypothesized that p53 mutant cells would be highly permissive to oncolytic viruses. We assessed the sensitivity of myeloma and lymphoma cells to Measles Virus (RNA negative virus) and TVEC (double strand DNA), in relation to p53 using 35 cell lines and 40 primary samples. In cell lines, we showed that viral load (measured by N gene and DNA polymerase expression, respectively) and cell death were associated with TP53 status for both viruses: TP53abn cell lines were preferentially infected and killed by Measles Virus or TVEC when compared to the TP53wt cell lines ($p=0.046$ and $p=0.026$, respectively). Resistance to infection was not related to IFN production.

Interestingly, coculture of tumor cells with HS-5 stromal cells did not prevent any infection or death. Measles Virus infection occurred exclusively via CD46 (CD150 was mostly absent on myeloma and lymphoma cells), and we demonstrated using p53 activation/silencing that p53 repressed CD46 expression directly (ChIP assay) and indirectly (miRNA192).
Receptors mediating TVEC infection of myeloma/lymphoma cells and mechanisms supporting p53-related TVEC infection are under investigation. Myeloma cell death induced by MV or TVEC was not dependent on caspase activation but displayed features of autophagy. In patients' samples (mononuclear cells from bone marrow or peripheral blood), we showed that both viruses preferentially targeted myeloma/lymphoma cells and that p53 deficient tumor cells (assessed by FISH, DNA sequencing and lack of DR5 increase upon exposure to nutlin3a) were more infected/killed than p53 competent tumor cells.

In summary, myeloma and lymphoma cells were highly sensitive to Measles Virus or TVEC and resistance to infection was abrogated in p53 deficient cells, arguing for a clinical trial in refractory patients with p53 deficiency.

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MICRORNA-205-5P INHIBITS BRCA1 AND RAD17 EXPRESSION IMPAIRING DNA REPAIR AND PROMOTING GENOMIC INSTABILITY OF HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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RATIONALE: Defective DNA damage response (DDR) is frequently associated with tumorigenesis. Abrogation of DDR leads to genomic instability, one of the most common characteristics of human cancers. TP53 mutations with gain-of-function activity associate with tumors with high replicative stress, high genomic instability and a reduced patients survival. BRCA1 and RAD17 genes encode for two pivotal DNA repair proteins required for the proper cell cycle regulation and the maintenance of genomic stability.

METHODS: Initially, we evaluated whether miR-205-5p targeted BRCA1 and RAD17 expression. We subsequently assessed the effect of miR-205-5p on the DNA repair pathway by in vitro assays. In vivo analysis and association with clinical features of Head and Neck Squamous Cell Carcinoma (HNSCC) patients supported the experimental findings.

Results: In this study we found that BRCA1 and RAD17 are targets of miR-205-5p in head and neck cancers (HNSCC). Indeed, ectopic expression of miR-205-5p decreased BRCA1 and RAD17 expression in vitro and in vivo leading to inefficient repair of endogenous replicative DNA and increased chromosomal instability. Conversely, miR-205-5p downregulation reduced in vivo tumor growth leading to increased expression of BRCA1 and RAD17 genes. We documented that miR-205-5p expression was higher in tumoral and peritumoral HNSCC tissues than the non-tumoral tissues in patients exhibiting reduced local recurrence-free survival. Interestingly, miR-205-5p expression was significantly anti-correlated to that of its gene targets BRCA1 and RAD17.

CONCLUSIONS: Collectively these findings unveil a notable role of miR-205-5p in the control of DDR through its selective targeting of BRCA1 and RAD17 gene expression. The identification of predictive miRNAs expression in peritumoral tissues might have relevance for early detection of minimal residual disease and of pre-cancer molecular alterations implicated in malignant transformation. Furthermore, advancements in surveying microRNA molecular mechanisms can provide the tools to make these molecules attractive targets for targeted therapy.

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MUTANT-P53 ADDICTION IN TRIPLE NEGATIVE BREAST CANCER

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The TP53 tumor suppressor gene is inactivated in more than 50% of cancer patients. In breast cancer specifically, 84% of patients with triple negative/basal-like, 75% of HER2-amplified, and 100% of medullary subtype of breast cancer have mutated TP53- suggesting that p53 loss is a driver in this disease. We have developed a novel, and spontaneous, triple-negative, mutant p53-driven breast cancer mouse model.

This is a K14-cre driven, p53wmR172H/flox; Rosa26LSL-CAS9 mouse model. I utilized a novel conditional allele that expresses wild type p53 due to insertion of a partial cDNA into the mutant p53R172H locus. In absence of Cre-recombinase, wildtype p53 is expressed; upon Cre recombination, the mutant p53R172H is expressed. The advantage of this novel allele is that mutant p53 expression is limited to the epithelial cells in the mammary gland leaving the surrounding stroma and immune cells wildtype for p53. Our aim is to understand if (1) mutant p53-driven breast cancers are dependent on mutant p53 for growth and maintenance in a somatic and spontaneously arising mammary tumors with an intact immune system that is wildtype for p53 and (2) how the tumor microenvironment changes post mutant p53 with drawal.

Our data show that p53-driven breast cancers are dependent on mutant p53 for growth and maintenance; in vivo withdrawal of mutant p53 curbs tumor growth and significantly extends survival. Interestingly, an increased T cell infiltration post mutant p53 withdrawal is observed. Given the increase of T cell infiltration in mammary tumors, we hypothesize that mutant p53 withdrawal may enhance antitumor effects of PD1 checkpoint blockade (currently testing). In summary, spontaneously arising mutant-p53 driven mammary tumors are addicted to mutant p53, and deletion of mutant p53 in vivo in cancer cells curbs tumor growth and enhances T cell infiltration.

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AUTOPHAGY-INDUCED MUTANT P53 DEGRADATION BY ZN(II)-CURC DEPENDS BY ENDOPLASMIC RETICULUM (ER) STRESS AND UNFOLDED PROTEIN RESPONSE (UPR) ACTIVATION

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Mutant p53 (mutp53) proteins, may acquire a misfolded and partially denatured conformation that results is accumulation in tumors of hyperstable mutp53 proteins that cannot undergo degradation. TP53 missense mutations not only abolish the tumor suppressive function, but may also acquire new tumorigenic driver activities, namely, gain-of-function (GOF). Cancer cells develop addiction to these mutp53 oncogenic functions in order to survive, therefore, targeting mutp53 can be therapeutically exploited to clear cancers carrying TP53 mutations.

In the last years, numerous strategies aimed at targeting mutp53 for its degradation or reactivation of wild-type p53 (inhibited by mutp53 as a dominant negative function), have been devised as novel anticancer therapies. However, other than just targeting mutp53 proteins, it is now becoming clear that an important anticancer strategy is to identify vulnerabilities in cancers imposed by molecular/cellular pathways that can regulate mutp53 and, vice versa, that can be regulated by mutp53 itself. Our previous studies demonstrated that mutp53 can undergo degradation through autophagy following treatment with Zn(II)-curcumin compound or natural compounds such as capsaicin and apigenin.
Management of autophagy is strictly connected to endopasmic reticulum (ER) stress that triggers the unfolded protein response (UPR). UPR may induce autophagy as a protective response to alleviate ER stress, and the reduction of autophagy may exacerbate the ER stress and modify UPR, changing the balance between its pro-survival and pro-apoptotic functions. In this study, we aim at evaluating the role of ER stress/UPR in autophagy-induced mutp53 degradation. We found that treatment of cancer cells carrying R175H or R273H p53 mutations (SKBR3, breast cancer cells and U373, glioblastoma, respectively) with Zn(II)-curc induced ER stress as evidenced by BiP activation and the UPR/IRE1 arm. Inhibition of ER stress by 4-PBA reduced both autophagy and mutp53 degradation.

Looking at cell viability, we found that inhibition of autophagy by cloroquine (CQ) slightly reduced the Zn(II)-curc-induced cell death, likely due to hyperstability of mutp53 and consequent impairment of wtp53 reactivation. On the other hand, Zn(II)-curc sensitized mutp53-carrying cells to drug cytotoxic effect, increasing cell death compared to the single treatments. These findings suggest that efficient treatment of cancers carrying mutp53 proteins may benefit of ER tress/UPR/autophagy activation.

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POST-TRANSLATIONAL MODIFICATIONS OF THE TUMOR SUPPRESSOR P53 DURING APL THERAPY

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Activation of the tumor suppressor p53 is a key step in the process of eradicating Acute Promyelocytic Leukemia (APL), one of the rare leukemic disorders curable by targeted therapy.
This disease is driven by the PML/RARA fusion, a powerful repressor of myeloid differentiation and a disorganizer of PML nuclear bodies (PML NB).

These structures recruit a wide variety of partners and were proposed to act as a platform for various post-translational modifications (ubiquitination, acetylation, phosphorylation, etc.). One of the key signaling pathways activated by PML and NBs is p53. Pml-/- cells are defective for p53-driven senescence. In several APL mouse models, we have demonstrated that retinoic acid and/or arsenic induce PML/RARA degradation, reformation of PML NB and activation of a subset of p53 target genes implicated in senescence. Studies in Pml-/- or p53-/- APLs have shown that both PML and p53 are essential for therapy-induced APL clearance.
In order to decipher the molecular basis of PML control over p53 activity and identify PML-dependent p53 post-translational modifications, we have reintroduced by retroviral transduction a tagged version of human p53 in p53-/- APLs.

Preliminary experiments indicate that therapeutic response, with retinoic acid and/or arsenic, is restored by adding wild-type p53. We will purify tagged p53 proteins and express p53 mutants in an effort to characterize the post-translational modifications which are critical for the activation of specific target genes yielding APL clearance in vivo.
These studies should provide important insight into the PML p53 cross-talks and also open important pharmacological perspectives for other types of cancers even when the PML gene is not rearranged.

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CLINICAL CHALLENGES INTERPRETING GERMLINE VARIANTS OF UNCERTAIN SIGNIFICANCE IN THE TP53 DNA-BINDING DOMAIN

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BACKGROUND: Screening for germline TP53 mutations predisposing to Li-Fraumeni syndrome (LFS) is complicated by the identification of genetic variants of uncertain significance (VUS). The most common mechanism of inactivating the p53 pathway is by single nucleotide alterations that generate missense mutations in exons encoding the sequence specific DNA-binding domain (DBD) (amino acids 96-292). Determining which genetic variants are pathogenic and which are neutral is a major challenge in clinical genetics [1].
Thus, pre-symptomatic genetic testing using a VUS to guide clinical care is not typically recommended. During investigation of possible genetic contributors to a complex pattern of cancer presentation in an Irish family, we identified a VUS in both the TP53 and FH genes. In addition we present data on an additional two pedigrees in which TP53 variants of uncertain significance were also characterised in the DBD, c.542G>A (p.Arg181His and c.572C>G (p. Pro191Arg).

METHODS: Data are presented on an Irish kindred (Figure 1) which satisfies the Chompret critera [2] for LFS. Non-synonymous single nucleotide substitution VUS in both TP53 (c.800G>A, p.R267Q) and FH (c.841A>C, p.T281P) were identified through diagnostic genetic testing. A family was referred with a likely pathogenic variant in TP53 c.542G>A (p.Arg181His) which was subsequently reclassified as a VUS (Figure 2) we also report c.572C>G (p.Pro191Arg) in affected siblings with childhood malignancies (Figure 3).

RESULTS: Co-seggregation genetic test results will be presented on each variant in addition to a summary of functional and in silico data

CONCLUSION: Although several lines of evidence can be evaluated to assess the clinical implications of these variants, usually none of these approaches can be used in isolation to obtain clinically useful interpretations and, for many TP53 variants in particular comprehensive data are lacking due to the rarity of LFS. The emerging phenotypic hetergeneity of TP53 variants is suggested by the lower pentrance suggested in each pedigree. Previous studies of LFS have included familes ascertained because of a striking cancer history, raising the question whether current LFS cancer risk estimates may be overestimation overall penetrance and underestimating the phenotypic spectrum. Efforts to characterise the TP53 VUS are ongoing.

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COMPOUND HETEROZYGOTE MUTATIONS IN THE TP53 AND FH GENES ASSOCIATED WITH MULTIPLE CANCER PRESENTATION

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BACKGROUND: One outcome of genetic testing is the identification of a genetic varian of uncertain significance (VUS). Determining which genetic variants are pathogenic and which are neutral is a major challe nge in clinical genetics [1]. Thus, pre-symptomatic genetic testing using a VUS to guide clinical care is not recommended. During investigation of possible genetic contributors to a complex pattern of cancer presentation in an Irish family, we identified a VUS in both the TP53 and FH genes. We present evidence to support our hypotheses that the compound heterozygotes discovered in the TP53 and FH genesunderlie the development of multiple neoplasms within this family.

METHODS: Data are presented on an Irish kindred (Figure 1) which satisfies the Chompret critera [2] for Li-Fraumeni syndrome (LFS). Non-synonymous single nucleotide substitution VUSs in both TP53 (c.800G>A, p.R267Q) and FH (c.841A>C, p.T281P) were identified through diagnostic genetic testing.

RESULTS: FH T281P co-segregates with the clinical features of Hereditary Leiomyomatois and renal cell cancer (HLRCC) in the proband and her maternal first cousin. Located in a highly conserved region of the gene, it is predicted as pathogenic by in silico analysis. TP53 R267Q located in the DNA binding domain co-segregates with three family members affected with core LFS associated cancers.
Tumour studies did not demonstrate evidence for loss of heterozygosity (LOH) for R267Q on two cases of brain tumours and one case of pre-menopausal breast cancer. Functional yeast transactivation assay results indicates that the TP53 variant alters p53 protein function [3]. TP53 R267Q also has a significantly reduced ability to induce p21WAF1/CIP1, which is an important downstream target of p53 in inducing G1/S arrest [4]. The bio-informatic tool REVEL (rare exome variant ensemle learner) score for R267Q is 0.902 indicating a high likelihood of pathogenicity [5].

CONCLUSION: Compound TP53 and FH heterozygotes in this family appear to have the most severe phenotypic expression indicating the increased pathogenicity of these variants. However, given the proband's sister shares both variants and is unaffected at age 57, the data suggests that TP53 R267Q may be either a low penetrant variant or a risk modifier of deleterious changes in the FH mitochondrial pathway. Efforts to characterise the TP53 VUS are ongoing.

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VITAMIN K DERIVED COMPOUNDS WITH PROMISING EFFECTS AGAINST MUTANT P53 AGGREGATION

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INTRODUCTION: The p53 protein is a tumor suppressor. In more than 50% of cases of cancer, this protein has at least one missense mutation. When this occurs, p53 has a less stable conformation and a greater tendency to form intracellular amyloid aggregates, contributing to the accumulation of p53 in tumors and their effects of gaining of function. The therapy available for the treatment of cancer, although vast, is still quite limited, and it is still necessary to study new therapeutic targets, such as the aggregated p53 protein, as well as new molecules. PRIMA-1 is a Michael acceptor molecule that is in phase II clinical study, which has already been tested in several types of cancer. It is known that PRIMA-1 is able to bind covalently to the cysteines present in the central domain of the mutant p53 protein, stabilizing it in a wild type like conformation and clear p53 aggregates.

OBJECTIVES: Investigate the mechanism of action of new drug candidates who are also potential Michael acceptors, as well as PRIMA-1.

RESULTS AND DISCUSSION: 21 new compounds were screened using the MTT reduction assay. 10 and 100 µM concentrations were used in breast and ovary tumor cell lines expressing mutant p53 (MDA-MB-231 and OVCAR-3) or wild type p53 (MCF-7 and A2780). After screening, these compounds were found to be ten times more potent than PRIMA-1. Three of them were selected for reducing the cell viability of mutant lines (RCP-03, RCP-17 and RCP18), but the RCP-17 was effective only for the mutant breast line. Then, the MDA-MB-231 cell was treated with 10 µM of the selected compounds and their extract were immunoprecipitated with antibody anti amyloid oligomers (A11), demonstrating that after treatment with RCP-17 and -18 we have a reduction of the p53 levels in the amyloid fraction. By western blotting, we found that when MDA-MB-231 were treated for 18 h with 10 µM with RCP-17 the level of p53 increases and when treated with RCP-18, decreases. Functional assays were also performed with compounds RCP-17 and -18. Both compounds shown to induce apoptosis by the Annexin / PI assay, but the compound RCP-17 shown more late apoptosis. Moreover, the RCP-18 compound was able to induce spheroid formation in the MDA-MB-231 cell line, which is characterized as line that does not form spheroids in the protocol used by our group, indicating a loss of function of mutant p53 in this line.

CONCLUSIONS: Compounds RCP-17 and -18 demonstrated potential in the elimination of mutant p53 aggregates.

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PI3K INHIBITORS CURTAIL MYC-DEPENDENT MUTANT P53 GAIN-OF-FUNCTION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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BACKGROUND: Mutation of TP53 gene is a hallmark of head and neck squamous cell carcinoma (HNSCC) not yet exploited therapeutically. TP53 mutation frequently leads to the synthesis of mutant p53 proteins with gain-of-function activity, associated with radioresistance and high incidence of local recurrences in HNSCC.

METHODS: mutant p53-associated functions were investigated through RNA-seq and Chromatin Immunoprecipitation analyses in HNSCC cells Cal27, carrying endogenous mutant p53H193L, and FaDu, carrying endogenous p53R248L. Prognostic power of mutant p53-associated transcriptome and mutant p53-MYC-associated signature were evaluated in HNSCC patients using the TCGA cohort. Drugs impinging on mutant p53-MYC-dependent transcriptome were identified interrogating Connectivity Map (https://clue.io) derived from the Library of Integrated Network-based Cellular Signatures (LINCS) database (http://lincs.hms.harvard.edu/).

RESULTS: Here, we identified a signature of transcripts directly controlled by gain-of-function mutant p53 protein and prognostic in HNSCC, which is highly enriched of MYC targets. Specifically, both in patient-derived xenografts (PDX) and cell lines of HNSCC treated with the PI3K-selective inhibitor Alpesilib (BYL719) the down-regulation of mutant p53/MYC-dependent signature correlates with response to this compound. At the molecular level, mutant p53 and YAP favor the binding of MYC to its target promoters and enhance MYC protein stability. Treatment with Alpelisib disrupts the interaction of MYC and mutant p53 proteins with MYC target promoters. Of note, depletion of MYC, mutant p53 or YAP potentiates the effectiveness of Alpelisib treatment.

CONCLUSION: Collectively, the blocking of this transcriptional network is an important determinant for the response to Alpelisib in HNSCC.

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VISUALIZATION OF THE UPTAKE AND SUBCELLULAR LOCALIZATION OF STAPLED PEPTIDES TARGETING MDM2/MDMX

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Stapled peptides hold remarkable promise as a novel class of therapeutic compounds, with enhanced serum stability, cellular uptake and proteolytic resistance when compared to non- stapled peptides. Because stapled peptides present a stabilised interface, their use is especially attractive when disruption of the binding between two proteins supports the therapeutic effect.

The E3 ubiquitin ligase MDM2, and its closely related homologue MDMX, interact with and inhibit the activity of the transcription factor p53. In normal cells, this constitutes a major feedback loop for specific and timely responses to various cellular stresses. In cancers expressing wild-type p53, the frequent overexpression of MDM2/MDMX leads to inactivation of p53-dependent tumour suppression. Inhibition of MDM2/MDMX has long been considered an attractive therapeutic strategy for these cancers. Stapled peptides targeting MDM2/MDMX and eliciting p53-dependent cell death in vitro and in vivo show potential as single chemotherapeutic agents or in combination therapies. Further studies of their mechanism of action and effects in various TP53 context, and internalisation using for example fluorescently tagged stapled peptides, are required to ensure the generalisation of their use. Imaging flow cytometry enables quantitative studies combining investigation of the expression levels and subcellular localization of fluorescently tagged stapled peptides, as well as expression levels of their target proteins, MDM2/MDMX and p53.

Here, we used fluorescent microscopy and imaging based flow cytometry to study the uptake of fluorescently labelled stapled peptides in vitro and in vivo. In vivo, we detected intracellular stapled peptides as early as two hours after injection of stapled peptides and in cells expressing wild type p53, p53 expression was significantly increased at six hours. We are currently investigating peptide uptake in cells expressing wild type and mutant p53 and the functional downstream consequences.

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A MUTATIONAL CHIP TO DETECT TP53, CDKN2A AND FAT1 MUTATIONS IN CCF-DNA OF HNSCC PATIENTS: A PILOT STUDY

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Head and neck squamous cell carcinoma (HNSCC) is characterized by a high incidence of relapse, which is the common cause of death in HNSCC patients. The identification of biomarkers supporting the management of HNSCC disease is still an unmet need in clinical oncology.

To this end we designed a mutational chip for Next-Generation-Sequencing (NGS) analysis to detect mutations in tumor tissues and matched plasma of HNSCC patients. The analysis of the HNSCC TCGA cohort revealed that TP53 (72%), CDKN2A (22%) and FAT1 (24%) are the most frequently mutated genes in HNSCCs. Notably TP53 mutations associate with poor outcome. A cohort of 250 fresh frozen tissues from HNSCC patients has been collected. Specifically, for each patient three biopsies representing non-tumoral (resection margin), peritumor (histologically tumor-free tissue at ≥ 1cm from the tumor) and tumor tissues were collected.

This cohort has been challenged with a customized mutational chip for NGS analysis of mutations that includes the entire CDS of the TP53, CDKN2A and FAT1 genes. We found that the TP53 (77,5%) and CDKN2A (22,5%) mutation frequency in tumors of our cohort was similar to that of TGCA. While the frequency of FAT1 (36%) mutations was higher in our dataset. Many of the identified FAT1 mutations have not been annotated yet. Preliminary data on matched plasma of HNSCC analysis at the surgery and post- surgery, showed that the sensitivity of our mutational chip for identifying mutations in circulating cell free DNA (ccf-DNA) from HNSCC patients is comparable to the sensitivity of dPCR technology.
This analysis is still ongoing and the related data will be presented.

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BIPOLAR CLUSTERING OF SUPERNUMERARY CENTROSOMES AND HIGH NEK2 EXPRESSION POSITIVELY CORRELATE WITH MUTANT P53 STATUS IN BREAST CANCER CELL LINES.

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Centrosome amplification (CA), the acquisition of three or more (supernumerary) centrosomes, results in defective, multipolar mitosis and aneuploidy, leading to a multitude of genetic alterations that leads to cancer initiation and promotion. CA is present in most human tumors, including breast cancer and colon cancer. The coexistence, in certain tumors and in tumor-derived cell lines, of multiple centrosomes together with high levels of aneuploidy suggests a positive correlation between CA and chromosome instability (CIN).
However, high-level CIN can also be deleterious. Interestingly, several cancer cell lines seem to overcome this problem by clustering their extra centrosomes at the two poles of the spindle during mitosis, thus ensuring bipolar chromosome segregation. The molecular mechanism responsible is still poorly understood. A number of studies have shown the role of p53 and of mitotic kinases, in regulating centrosome duplication, separation and maturation and subsequent mitotic spindle assembly during cell cycle progression. P53 is mutated in about 30% of breast cancer patients. Also, NIMA-related kinase 2 (Nek2), a mitotic kinase, is highly expressed in breast cancer. AIM: To determine the correlation between mutant p53 (mutp53), NEK2 and centrosome clustering in breast cancer. Methods: HER2-positive human and mouse mammary epithelium breast cancer cell lines (with wild-type (wt), mutant or heterozygous for p53) were used. NEK2 expression level in human breast cancer was analyzed from Metabric data available from http://www.cbioportal.org.
Quantitative RT-PCR and western blot were used to determine NEK2 expression and protein level in human and mouse breast cancer cell lines, respectively. Centrosome clustering was determined by staining for gamma-tubulin. Results: Analysis of Metabric cohort (2,433 patients) showed a significant low survival rate and increased NEK2 expression in patients with mutp53, as compared to those with wtp53. Additionally, by RT-PCR, NEK2 expression was significantly higher in HER2-positive human breast cancer cell lines carrying mutp53 as compared to those carrying wtp53. Mouse breast cancer cell lines heterozygous for mutp53 had higher Nek2 protein level compared to those with wtp53. Both human and mouse breast cancer cell lines carrying mutp53 had significantly higher percentage of centrosome clustering in mitotic cells, as compared to cells carrying wtp53. Suppression of mutp53 by shRNA in human breast cancer cell line MDA-MB231 resulted in reduced NEK2 expression after gamma-irradiation.

Conclusion: Our results indicate a positive correlation between mutp53, high NEK2 expression and centrosome clustering in breast cancer, suggesting that mutp53 may support cancer cell survival and tumor progression by promoting centrosome clustering, possibly through interaction with NEK2.

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TARGETING MKK3 AS NOVEL THERAPEUTIC PERSPECTIVE IN COLORECTAL CANCER.

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We previously defined the mitogen activated protein kinase (MAPK) kinase 3 (MKK3) as novel target of mutant p53 proteins providing novel insights for the understanding of mutant p53 gain- of-function activity. MKK3 is a specific activator of p38 MAP kinases (p38 MAPK), through which contributes to the regulation of several cellular functions.
We demonstrated that MKK3 genetic depletion induces autophagic cell death not only in mutp53 but also wild type (wt) and null-p53 tumor cell context, suggesting MKK3 as potential more generic target gene for the development of novel anticancer therapeutic strategies.

To validate MKK3 as therapeutic target, deeper analyses have been performed on a panel of authenticated CRC lines and primary colonocytes. Targeting of MKK3 univocally exerts antitumor effects in CRC cells but not primary cultures. While MKK3 depletion per se affects growth and survival by induction of sustained autophagy in some CRC lines, it potentiates 5-Fluorouracil (5- FU) antitumor efficacy in all of the tested CRC lines.
We demonstrate that MKK3, upon non-lethal 5-FU challenge, specifically activates p38 MAPK delta isoform, which sustains pro-survival signals by ERCC1 induction. Indeed, p38 MAPK delta silencing rescues growth impairment and boosts 5-FU effects in CRC cells in vitro and in vivo. Moreover, tissue micro-array (TMA) analysis in a cohort of 185 CRC patients showed significant correlation of high MKK3 levels with advanced tumor stages, and queries of publicly available datasets (TGCA) revealed higher MKK3 levels to correlate with shorter Overall Survival in CRC patients.

These results suggest MKK3 as a prognostic marker and as a novel and extremely attractive therapeutic target for the development of promising strategies for the management of CRC patients.

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P53 ISOFORMS AS MUTATION TARGETS IN CANCER : LESSONS FROM PUBLIC DATABASES

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BACKGROUND: TP53 is expressed as several isoforms through different mechanisms including internal initiation of translation, use of alternative promoters and alternative splicing. These isoforms differ from canonical p53 (TAp53) by their N- and/or C-terminal domains. Recent studies using whole-exome (WES) or whole-genome (WGS) rather than targeted sequencing have revealed that up to 25% of TP53 mutations in cancer occur in regions that differ between isoforms. In this study, we have analyzed these mutations in order to evaluate their predicted effect on isoform production.

METHODS: TP53 mutation data (WGS/WES) were selected from COSMIC (Catalogue of Somatic Mutations in Cancer, 7,188 mutations)[1] and ExAC (Exome Aggregation Consortium, 837 mutations)[2]. Mutations were assigned to specific transcripts and grouped into 4 types: (1) Isoform-defining (mutations preserving isoforms other than TAp53) (2) Isoform-specific (falling in regions specific for a particular isoform) (3) Isoform-repressing (impairing isoform production) and (4) Isoform-like (causing truncated products lacking N- or C-terminus, similar to p53 isoforms). In addition, we have used RNASeq data from an original lung cancer cohort to search for candidate transcriptomic signatures associated with mutations that differentially target p53 isoforms.

RESULTS: Types 1 and 4 represented, respectively, 8.2% and 7.9% of the COSMIC dataset, almost twice as much as p.R175H, the most frequent TP53 hotspot (4%). Type 2 and 3 were extremely rare (< 0.1%). Type 1 and 4 were virtually absent in ExAC, suggesting that they are cancer-specific. Most type 1 mutations were nonsense and indels/frameshift impairing TAp53 but preserving N-terminal isoforms, with Delta133p53 the most favored isoform. Type 4 mutations were mostly truncating mutations in the C-terminus, with p.R306* and p.R342* as most common hotspots. Type 2 included a handful of mutations occurring in alternative exons 9A or 9B, and type 3 included mutations potentially altering IRES upstream of AUG40. Preliminary data on transcriptomic patterns in relation with different mutation types will be presented.

CONCLUSION: The majority of TP53 mutations outside the DNA-binding domain may have a dual effect, with first interrupting the synthesis of TAp53alpha, and second preserving the synthesis of a "wild-type" N-or C-terminal isoform. We suggest that this mechanism may provide an alternative dominant-negative/gain-of-function effect.

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MUTANT P53: GAIN OF FICTION?

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In this abstract, I would like to share with the workshop participants some of the ideas I had planned to develop and discuss during my presentation at the meeting.
Gain-of-Function (GOF) properties have been consistently identified and assigned to many forms of mutant p53. This notion finds its roots in the original oncosexual ambiguity of the p53 protein, which was identified as both oncogene and a tumour suppressor. Over the past 20 years, multiple molecular properties have been discovered that support experimental GOF effects, including mutant-specific transcriptional effects, interactions with multiple host cell proteins, and effects on cell metabolism, miRNA expression, epigenetic landscapes, cell-cell and cell-matrix interactions and immune regulation.
Despite this wealth of experimental evidence there is still no consensus for defining which mutant has GOF effects. There is no clear structural or functional feature that distinguishes a GOF mutant from a simple Loss-of-Function (LOF). It is widely assumed that mutants considered as most severe (e.g p.R175H or p.R248W) have GOF effects, but there is no definite clinical evidence to substantiate this. In LFS, for example, these mutants are associated with a lifetime penetrance that is almost identical to nonsense or frameshift mutations (predicted to preclude p53 protein synthesis). The only borderline significant difference is that severe point mutants are associated with a higher rate of childhood cancer, although there is no evidence so far that this effect is caused by GOF properties. Likewise, comparison studies on transcriptomics patterns in tumours with somatic mutations have consistently failed to identify a signature "GOF transcriptome".
I would like to suggest that GOF may not be an intrinsic property of particular mutants. Rather, it is a "double act": it requires specific mutant characteristics as well a cell context enabling the mutant to home on an appropriate cellular target. Mutants that are most likely to achieve this are not necessarily the ones considered as a priori most severe. Rather, they may be rare mutants, with only partial LOF properties, but which may target important mechanisms in a cell-specific manner. In my view, the best example of such a GOF mutant is p.R249S associated with Hepatitis B hepatocellular carcinoma (HB-HCC) and aflatoxin exposure.
Experimentally, this mutant is a weak LOF. So far it has never been observed in a LFS family. It is rare in cancers other than HB-HCC, suggesting that beyond the specific mutagenic context, it does not exert a strong tumorigenic effect. In transformed hepatocytes, it appears to bind and interfere with the HBV oncoprotein HBx, and there is evidence that their association an potent oncogenic effect in hepatocytes. Thus, this mutant has the features of a almost "pure" GOF: no strong effect on its own, conditional to the presence of a viral protein, tissue-and cell-restricted effects.
This paradigm should inspire us to revisit the biology of many "non-hotspot" mutants, in particular those with skewed tissue distributions, in search for yet unidentified but clinically meaningful GOF mechanisms.
This abstract will not be presented as a poster.

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TREATMENT WITH BEMCENTINIB MODULATES P53 PROTEIN ISOFORMS, FLT3 AND AXL EXPRESSION IN ACUTE MYELOID LEUKEMIA (AML) CELLS

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BACKGROUND: Acute myeloid leukemia (AML) is an aggressive blood cancer with poor survival characterized by the accumulation of malignant immature myeloid progenitor cells in the bone marrow and peripheral blood. In de novo AML, the most frequent mutated genes are NPM1 (30%) and Fms-like tyrosine kinase 3 (FLT3) receptor (25%), while mutations in TP53 (5-10%) are highly associated with a complex karyotype, demonstrated mutually exclusive with NPM1 and FLT3 mutations. FLT3 mutations are associated with a bad prognosis, while NPM1 mutations are associated with a slightly better prognosis. We have demonstrated that p53 full-length protein expression correlated with presence of mutated FLT3 where as NPM1 mutation correlated with p53 and isoform expression. Based on this we wanted to investigate the effect of clinically relevant concentrations (maximum 700nM) of the drug bemcentinib – a kinase inhibitor of the receptor tyrosine kinase Axl, currently in clinical trials for AML – on p53 full-length and isoform expression in AML cells.

METHODS: The AML cell lines Molm-13 (p53wt), MV4-11 (p53wt) and HL-60 (p53 deleted) were untreated and treated for 24h with bemcentinib (100, 250 and 500nM) and cells were lysed before investigated by immunoblotting by p53 isoform specific antibodies (p53, p53, p53, 133p53 and 40p53). In additionwe studied the expression of several other proteins such as MDM2, Axl, FLT3 and autophagy (LC3B and p62).

RESULTS: Interestingly, the full-length p53andp53/ expression increased after treatment with 100nM bemcentinib before profoundly decreased following treatment with 250nM and 500nM. Preliminary results for the expression of 133p53/ showed the opposite tendency with decreased expression after treatment with 100nM bemcentinib followed by an increase with increasing concentrations, while the expression of 133p53remained unchanged. Preliminary results showed a reduction in expression of 40p53 (and) after treatment with 500nM bemcentinib. We also found a decrease in the expression of MDM2 after bemcentinib treatment. On the other hand, the expression level of the fully-glycosylated receptors Axl and FLT3 proteins are strongly increased with increasing dose of bemcentinib, whereas markers of autophagy (LC3B and p62) indicate a decreased level of autophagy after treatment.

CONCLUSION: Our results indicate that bemcentinib treatment affects the expression of p53 full-length and isoforms in a concentration dependent manner, decreases autophagy and increases the stability of fully-glycosylated receptor tyrosine kinases such as Axl and FLT3.
Studies are ongoing to further map the expression levels of the p53 isoforms and to characterize the molecular pathways involved.

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TP53 MUTATIONS IN SMALL CELL LUNG CANCER

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Small Cell Lung Cancers have one of the highest mutational burdens of all tumour types. With over 80% of cases demonstrating a p53 mutation of one type or another, it is clear that a change in the behaviour of p53 is an important step in the formation of SCLCs. It is often stated that p53 function is almost universally lost in SCLC, but a growing body of evidence suggests that some p53 mutations may exhibit a gain of function (GoF), which could impact SCLC progression or treatment.
These GoF mutations produce p53 proteins with additional capabilities such as increased stability and alternative binding to other promotors. The outcome of these mutations on cells could be as drastic as an increase in migration, resistance to chemotherapy or an increase in growth rate.

In this project, we aim to functionally characterise the main p53 mutations found in SCLC, with the hypothesis that they will categorise into functional groups or families. Using part of our generated library, we have already noticed differences between mutants. We are in the process of setting up a number of functional automated assays to assess the remaining wild-type activity of the mutants, as well as any GoF features and dominant negative activity, which they may have accrued.

When analysed we will determine if different groups of mutants correlate to clinical as well as molecular characteristics of disease with the potential to develop into a biomarker.

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BIOCHEMICAL PROPERTIES OF THE P53 MOLECULE NECESSARY FOR THE REGULATION OF DNA REPLICATION FORK PROGRESSION IN CONCERT WITH POL

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DNA damage tolerance facilitates the progression of replication forks that have encountered obstacles on the template strand. It involves either translesion DNA synthesis (TLS) initiated by proliferating cell nuclear antigen (PCNA) monoubiquitination or less well-characterized fork reversal and template switch mechanisms. Recently, we discovered that p53 together with the TLS-POL regulates the replication dynamics to allow a novel DNA damage tolerance pathway and thereby might have protective roles during replication. Notably, this novel p53 activity was lost in the exonuclease-deficient but transcriptionally active p53(H115N) mutant. In the following, we dissected the structural properties of the p53 molecule necessary for the regulation of DNA replication fork progression. "Idling" processes between p53 and POL trigger a deceleration of nascent DNA elongation, which can be detected by DNA fiber spreading assays.

Moreover, both proteins stabilize each other at replication barriers which can be detected by in situ proximity ligation assays (PLA). We show that the novel tolerance pathway can be discriminated from p53`s transcriptional-transactivation functions by three separation of function mutants such as p53(H115N). The concerted action of p53 and POL at the replication fork requires the integrity of p53`s N-terminus as well as its oligomerization domain (OD). We will also present data identifying three independent residues of p53 involved in such a replication function of p53. Furthermore, we discuss modulatory functions of p53`s C-terminus for the p53-POL dependent replication phenotype. Altogether our results demonstrate that the p53-POL complex at replication forks depend on the formation of the p53 tetramer and on the integrity of p53`s N-terminus.

Our findings further elucidate the molecular properties of p53 necessary in the p53-POL dependent DNA damage response to endogenous or exogenous replication stress.

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TREATMENT OF MUTANT P53 BREAST CANCERS THROUGH COMBINED MDM4 INHIBITION AND P53 REACTIVATION

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Breast cancer (BC) is the most frequently occurring cancer in women in western countries and is a major cause of cancer-related deaths. The greatest survival risk for BC patients is spreading disease that does not respond to treatment. Dismantling the mechanism of dispersal, through targeting its essential components is a rational therapeutic approach.
Mutation of the major tumour suppressor p53, may not only drain its powerful cancer suppressive capacity, but diabolically may also confer cancer-promoting properties: where its ability to drive metastasis is the greatest survival threat. Reactivation of mutant p53 conformation to impose normal function has been achieved chemically and the lead compound APR-246 is currently in clinical trials. Potentiating this process of reactivation appears to be critical for therapeutic value.

We discovered that MDM4 targeting holds untapped capacity to target breast cancers with mutant p53 and can be combined with APR-246 for enhanced response. Normally, MDM4 acts as a critical regulator of the tumour suppressor wild type (wt) p53, whose controlled behavior is vital for its correct cellular function. However, high MDM4 protein levels exhibit oncogenic capacity in a range of cancers, including BC. The ability of MDM4 to inhibit wt p53 functions is a rational explanation for its elevated levels in BC, and also explains the inhibitory power of its ablation.
However, our identification of high levels of mutant p53 also in BCs with mutant p53 was unexpected and the explanation of its occurrence is less obvious.
Critically however, in mutant p53 BC, we identified that high levels of MDM4 promoted cancer. We hypothesized that the cellular impact of chemical conversion of mutant p53 to wt would be empowered by reducing MDM4. Specifically, our approach is to reactivate wt p53 from the mutant form using APR-246, and coincidently remove MDM4 restraint, in order to instate tumour suppression.

Our unpublished results demonstrate the power of this combined treatment in both in vitro and in vivo mutant p53 BC models.

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THE PROGNOSTIC IMPACT OF P53 AGGREGATES IN HIGH-GRADE SEROUS OVARIAN CANCER

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BACKGROUND: Prions are defined as infectious proteins with the ability to self-propagate, induce template misfolding and being transmissible. In the past, they were observed in neurodegenerative diseases, e.g. BSE. However, recent findings have shown that the tumour suppressor protein p53 also carries prion-like properties. Upwards of 96% of high-grade serous ovarian cancers (HGSOC) contain TP53 mutations, which may lead to aggregated protein. Herein, we describe the novel p53-Seprion-ELISA and its use for evaluating the clinical relevance of p53 aggregates in HGSOC.

METHODS: The p53-Seprion-ELISA is based on a ligand that specifically binds aggregated proteins. To prove that the assay specifically detects p53 aggregates, two ovarian cancer cell lines were fractionated and analysed using the p53-Seprion-ELISA as well as blue native PAGE, followed by Western Blot. Further, fresh-frozen tumour tissues of 81 HGSOC patients from the EU-funded OVCAD study (at least 5-year follow up), were analysed. Then, the patients were classified into 3 groups according to their amount of p53 aggregates. We performed Kruskal-Wallis and Wilcoxon tests to evaluate the relationship between p53 aggregates and Ki67 index as well as p53-autoantibody (p53-AAb) levels, log-rank tests to compare progression-free (PFS) and overall survival (OS), and Cox regression to determine hazard ratios for both survival outcomes.

RESULTS: The comparison of results of BN-PAGE and the p53-Seprion-ELISA proved the ELISA's high specificity in capturing aggregated p53 only, and that the signal is not proportional to the total amount of p53 protein present in the respective sample. Further, in 84.8% of patients carrying a missense mutationa significant induction of p53 aggregation could be detected. The aggregation propensity varied considerably within those samples carrying mutations leading to the same amino acid change, e.g. R175H. A multivariable Cox regression analysis was performed considering other prognostic factors, which are significantly associated with OS in HGSOC (age, FIGO stage and presence of residual tumour).

The analysis showed a superiority of the group with extensive p53 aggregation in OS and PFS as compared to patients with negative to moderate p53 aggregation levels (P values 0.025 and 0.011). A similar association with PFS was observed (P values 0.030 and 0.008). The group with extensive p53 aggregation had a significantly higher Ki67 index as compared to patients with no/moderate aggregated p53 (P value 0.035) and the level of p53-AAb varied significantly between the 3 groups (P value 0.024).

CONCLUSIONS: For the first time, we were able to demonstrate that the p53-Seprion-ELISA allows specific and sensitive detection of p53 aggregates and is suitable for large-scale screening. Moreover, our proof-of-concept study shows that p53 aggregation is an independent prognostic marker for survival.

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IDENTIFICATION OF NOVEL COMPOUNDS THAT INDUCE TRANSLATIONAL READTHROUGH OF NONSENSE MUTANT TP53

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The tumor suppressor gene TP53 codes for a transcription factor (p53) whose function is to maintain cellular integrity by regulating key processes including DNA repair, cell cycle progression, metabolism, apoptosis and senescence. TP53 is inactivated by mutations in 50% of all human tumors.
The majority of TP53 mutations are missense mutations. However, 10% of TP53 mutations are nonsense mutations that lead to expression of a truncated and non-functional p53 protein.

An estimated 900 000 cancer patients diagnosed worldwide 2018 have tumors with nonsense TP53 mutation. The most common TP53 nonsense mutation is R213X, which is also the sixth most common TP53 mutation overall. Other common TP53 nonsense mutations are R196X and R342X. There is no available targeted treatment for nonsense mutant TP53-carrying tumors. Therefore, we have performed a high-throughput screening of chemical libraries and performed data mining with the aim of identifying novel compounds that can induce translational readthrough and expression of full-length p53 protein.

Our screenings generated more than 80 candidate readthrough inducing compounds (CRICs). We are currently confirming the readthrough-inducing capacity of these compounds by assessing expression of full-length p53 protein in tumor cells carrying R213X nonsense mutant TP53. We will also assess the biological effect of induction of full-length p53 protein in tumors cells.

Our goal is to take one or more of the identified compounds to clinical trials in cancer patients with nonsense mutant TP53.

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MISFOLDED MUTANT P53-DEPENDENT ONCOGENIC FUNCTION OF HSP40/DNAJA1

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HSP40 is a molecular chaperone involved in protein refolding and stabilization/degradation of misfoldedproteins. Although its binding activity to mutant p53 (mutp53) is reported, biological contributions of HSP40 to cancer progression and the functional association with mutp53 are largely unknown. Our recent work has revealed that DNAJA1, a HSP40 family member, favorably binds to and stabilizes conformational or misfolded mutp53. DNAJA1 knockdown induces proteasomal degradation of p53 mutants with misfolded conformation with little effects on the levels of wild-type p53 (wtp53) and DNA contact mutants.

Based on these observations, we hypothesized that DNAJA1 promotes cancer progression in a manner dependent on misfolded mutp53. To test this hypothesis, we examined the effects of DNAJA1 knockdown/knockout on malignant properties of cancer cells, by mainly focusing on head and neck squamous cell carcinoma (HNSCC) cells, due to high frequency of p53 mutations in tumors. Our immunohistochemistry for DNAJA1 using HNSCC tissue microarrays showed increased DNAJA1 levels in tumors as compared with normal tissues. Depletion of DNAJA1 resulted in reduced proliferation, migration, and cell-matrix adhesion of HNSCC (CAL33, HN31) cells with decreased expression of conformational/misfolded mutp53 (R175H, C176F). These DNAJA1-mediated phenotypes were not observed in FaDu cells expressing R248L mutp53.

Phenotypes induced by DNAJA1 depletion in cells expressing conformational/misfolded mutp53 were substantially rescued by overexpression of DNAJA1 or mutp53. In an in vivo orthotopic (floor of the mouth) model, knockdown of DNAJA1 in HN31 cells (C176F) reduced primary tumor growth as well as metastases to the lungs or lymph nodes. Similarly, knockdown of DNAJA1 significantly reduced the number of lung metastasis following tail vein injections of HN31 cells. RNA sequencing analyses using HN31 cells with or without knockdown of DNAJA1 or mutp53 showed that 77.2% of downregulated and 65.9% of upregulated genes by mutp53 knockdown were overlapped with those altered by DNAJA1 knockdown.

Within 1,714 genes involved in cancer-related pathways, there was a positive correlation in over 600 differentially expressed genes (DEGs) between DNAJA1 knockdown and mutp53 knockdown, of which 5 DEGs showed more or less than 2.5 times log2fold changes (5.56 folds). Importantly, TCGA data analyses of HNSCC cases carrying p53 missense mutations revealed that groups with moderate and high DNAJA1 expression showed significantly worse prognosis than the group with low DNAJA1 expression, only when cases with four DNA contact p53 mutants were excluded. These results indicate that DNAJA1 promoted HNSCC progression largely dependent on mutp53 with misfolded conformation, suggesting the possibility of DNAJA1 as a novel therapeutic target for cancers carrying conformational/misfolded mutp53.

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FUNCTIONAL CLASSIFICATION OF P53 MUTANTS BASED ON ITERATIVE CLUSTERING OF EXPERIMENTAL YEAST P53 TRANSACTIVATION DATA

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BACKGROUND: TP53 variants impact a broad spectrum of cellular functions and pathways, with potential mutant-specific functional and clinical consequences.1,2 Yeast-based transcriptional assays have provided high-resolution mapping of missense mutations based on transactivation data towards 8 different p53 DNA response elements, with relation to p53 structure and function.3 We performed iterations of unsupervised clustering to enhance the strength of discrimination among transactivation data, retrieved clusters of variants and analyzed their representation and penetrance in Li-Fraumeni Syndrome (LFS) population and gnomAD datasets.

METHODS. Transactivation data3 on 2,655 mutants were matched with IARC TP53 Database4. Three iterations of hierarchical clustering were computed in R on the transactivation scores. Subclusters with >50 mutations were considered for next iteration. Heatmaps and dendrograms were computed and extracted. The cluster characteristics were visualized on pareto chart for categorical variables. The variation of mutants localization between clusters was visualized with gaussian kernel density plots and empirical cumulative distribution function (ecdf). The statistical significance was tested with 2 (categorical variables), t-test (numerical variables), and Anderson-Darling (ranked distribution, ecdf). We compared representation and penetrance of our clusters in LFS and gnomAD.

RESULTS: One cluster, named Triple-One ("1_1_1"), included non-functional variants (>99%), and was mostly associated to the proximal section of the DNA binding domain (DBD) and to loops L2 and L3 of p53 protein. Triple-One was overrepresented in LFS (~70%), with a higher penetrance than nonsense/frameshift variants and other variant clusters (50% at 30 years).
Another cluster, "1_1_3", included partially functional variants (69%), and targeted mostly the distal part of the DBD, as well as loops and beta-strands of the DNA-binding scaffold. It was underrepresented in LFS (~10%), but penetrance was surprisingly higher than Triple-One in LFS children up to 7 years probably due to high-risk of adrenal cortical carcinoma. Triple-One and "1_1_3" are equally underrepresented in gnomAD (<5%). The cluster named "1_3" is composed of partially functional variants (63%) located in DBD (51%), and regulation domain (19%). The "1_3" cluster had the lowest penetrance in LFS, which was almost stationary up to age 30 (10-15%) and then reached 60% at age 50. Clusters "2_1" and "2_2" included variants located outside of DBD and were mostly composed of transcriptionally functional variants (96% and 93% respectively). Clusters "1_3", "2_1" and "2_2" accounted for 30, 15 and ~50% of gnomAD.

CONCLUSION: Our iterative clustering method on transactivation data retrieved clusters with specific localization of p53. They differed in terms of severity and penetrance in LFS population and were differently represented in LFS and gnomAD. Further work is ongoing to exploit immunogenicity of the clusters, and extend their exploration to further datasets.

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ΔNP53 AS A REGULATOR OF P53 BIOLOGY

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The aging population is rapidly expanding, and with it, the prevalence of chronic diseases such as diabetes, cancer, and Alzheimer's disease. As our understanding of the biology of aging advances, the complexity of the aging process becomes more apparent. The ΔNp53 isoform, which is expressed in most cell types, is directly implicated in mammalian aging. This project focuses on elucidating the basic mechanisms whereby the naturally occurring ΔNp53 isoform alters wild-type p53 (WTp53) function. In mice, co-expression of WTp53 and ΔNp53 results in an accelerated aging phenotype with premature development of aging pathologies such as osteoporosis and Alzheimer's disease.
The basic mechanisms driving these ΔNp53-dependent cellular and physiological changes remain poorly understood.
Human ΔNp53 lacks the first N-terminal transactivation domain of WTp53 (residues 1-39), and is preferentially translated during cell stress. ΔNp53 oligomerizes with WTp53 to form hetero-tetramers with altered function compared to WTp53 tetramers. Co-expression of ΔNp53 and WTp53 results in the formation of a mixed population ΔNp53:WTp53 tetramers, including "contaminating" WTp53 tetramers. This precludes a reliable functional comparison of ΔNp53:WTp53 tetramers vs. WTp53. Tocircumvent this issue, we developed a strategy—based upon the native p53 tetramer structure—in which ΔNp53 is tethered to WTp53 (ΔNp53:WTp53), resulting in a pure population of tetramers with a 2:2 ratio of ΔNp53 to WTp53. Using CRISPR/Cas9, we generated cell lines where we replaced native, WTp53 with WTp53, WTp53:WTp53, or ΔNp53:WTp53.
Using ChIP-seq and precision run-on nuclear sequencing (PRO-seq) in these cell lines, we have begun to define how ΔNp53 alters WTp53 function in a physiologically relevant context.

Additionally, we observed ΔNp53:WTp53-specific phenotypic changes under conditions of repeated p53 activation. These results have more clearly defined the basic mechanisms by which ΔNp53 alters WTp53 function in human cells.

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MUTANT P53 DRIVES CLONAL HEMATOPOIESIS AND HEMATOLOGICAL MALIGNANCIES

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Clonal hematopoiesis of indeterminate potential (CHIP) increases with age and occurs when a single mutant hematopoietic stem cell (HSC) contributes to a significant clonal proportion of mature blood lineages. TP53 ranks in the top five among genes that were mutated in CHIP. CHIP is associated with increased risks of hematological neoplasms, including myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). While TP53 mutations have been identified in CHIP and hematological malignancies, the molecular mechanisms by which mutant p53 drives the pathogenesis of CHIP and hematological malignancies are largely unknown.

We have been investigating the role of p53 in normal and malignant hematopoiesis. We discovered that wild-type p53 plays a critical role in maintaining hematopoietic stem cell (HSC) quiescence (Liu et al., Cell Stem Cell, 2009) and identified Necdin as a p53 target gene in HSCs (Asai et al., Blood, 2012). We discovered that mutant p53 confers a competitive advantage to HSCs following transplantation and promotes HSC expansion after genotoxic stress (Chen et al., Leukemia, 2018). Mechanistically, mutant p53 interacts with EZH2 and enhances its association with the chromatin, thereby increasing the levels of H3K27m3 in genes regulating HSC self-renewal and differentiation. Further, genetic and pharmacological inhibition of EZH2 decrease the repopulating potential of p53 mutant HSCs.

Given that most homozygous p53-/- and p53R248W/R248W mice develop spontaneous tumors, including lymphoma and sarcoma, and die within 3 to 6 months after birth, we tested whether heterozygous mutant p53 mice would develop myeloid malignancies during aging. We maintained p53+/+ and p53R248W/+ mice for more than a year and monitored their survival and tumor development. p53R248W/+ mice show extended life span compared to p53-/- and p53R248W/R248W mice in that most of these mice died within 16-18 months after birth. Approximately 60 % of middle-aged p53R248W/+ mice develop MDS. Other middle-aged p53R248W/+ mice develop lymphoma and sarcoma. Further, we discovered that mutant p53 and FLT3 mutation (FLT3-ITD) cooperate in leukemia development in mice. Mutant p53 expands the hematopoietic stem and progenitor cell pool in a FLT3-ITD background and enhances the self-renewal potential of FLT3-ITD+ leukemia-initiating cells (LICs) (Nabinger et al., Leukemia, 2019).

In summary, we demonstrate that mutant p53 plays a critical role in the pathogenesis of CHIP and hematological malignancies. Our work will likely establish epigenetic regulator EZH2 as a novel therapeutic target for preventing CHIP progression and treating hematological malignancies with TP53 mutations.

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IDENTIFYING AND CHARACTERIZING NOVEL REGULATORS OF P53 GAIN-OF-FUNCTION MUTANTS

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Tumour protein p53 is the most commonly inactivated tumour suppressor in all cancers. Nearly half of all alterations in p53 are missense mutations clustered in six "hotspots", resulting in the expression of full-length point-mutated p53 proteins that not only lose its wild-type (WT) activities, but also gain novel oncogenic functions (p53 GOF). Compared to those lacking p53 expression, tumours expressing p53 GOF are more invasive, metastatic, and proliferative, and show increased genome instability and chemoresistance. Moreover, GOF p53 may function in a dominant negative manner, suppressing the functions of WT p53 in tumours that retain one WT allele. Similar to WT p53, GOF p53 protein in phenotypically normal tissues is kept at a low level; however, GOF p53s become highly accumulated in tumours, thereby actively promoting cancer.

This indicates a tight regulatory network that is perturbed in tumours. Indeed, lowering the expression of GOF p53 reduces growth and metastasis, and triggers tumour regression, suggesting that tumours have become addicted to GOF p53. Therefore, understanding the regulatory and interaction network of p53 GOF could enable us to design effective strategies for treating many cancers. To identify these physiological regulators of GOF p53, we employed an unbiased functional genomics approach coupled with a novel in vivo proteomics screen.
Specifically, we first generated a fluorescence-reporter-based p53 protein stability sensor and performed genome-wide loss-of-function CRISPR screens to identify genes that regulate the GOF p53 stability and their signaling network. In an orthogonal approach, we also set out to map the in vivo interactome of GOF p53 using proximity-dependent biotin identification (BioID) complimented with affinity-purification mass spectrometry (AP-MS). We shall functionally characterize overlapping hits, and explore their underlying molecular mechanisms.

Taken together, by elucidating the specific regulatory network of GOF p53 through our screenings in physiological conditions, we aim to find new ways to therapeutically target GOF p53 mutant tumours, which may benefit a substantial subset of patients.

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LKB1 IS A CRITICAL REGULATOR OF NEURAL CREST CELLS THROUGH LIMITATION OF OXIDATIVE DNA DAMAGES AND P53 SIGNALING.

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The neural crest is composed of highly multipotent cells called Neural Crest Cells (NCC). These cells delaminate from the neural tube and migrate toward target organs where they give rise to a broad range of derivatives such as osteocytes and chondrocytes of the craniofacial skeleton, pigment cells, neurons and glial cells of the enteric nervous system and sensory neurons and Schwann cells of the peripheral nerves. Defects in NCC formation lead to a wide group of human disorders called neurocristopathies. Controlling oxidative stress and in turn the activation of the tumor suppressor and transcription factor p53 has recently been shown to be essential for correct formation of NCC. We recently uncovered that the master kinase LKB1 is essential for NCC formation.
We therefore explored if p53 signaling contributes to NCC phenotypes associated with Lkb1 loss.

By using a NCC line that can be cultivated as progenitors or committed into glial cells in vitro, we demonstrated that NCC defects upon Lkb1 loss were due in part to oxidative stress and DNA damage accumulation leading to p53 hyperactivation. Although Lkb1 knockdown impairs glial differentiation, loss of p53 combined with Lkb1 knockdown rescued glial commitment in vitro. To explore p53 contribution in vivo, we used genetically engineered mice we generated to inactivate Lkb1 before NCC migration toward their target organs (E8.5 ; Ht-PA::Cre Lkb1F/F mice). These mice died at birth due to craniofacial malformations resulting from disturbed cranial NCC delamination, polarization and survival. Ablation of one allele of p53 in addition to the conditional ablation of Lkb1 in NCC prevented the craniofacial malformations allowing a better survival of the animals.

However, the animals died few days after birth due to a complex phenotype associating defective digestive motricity, hindlimb paralysis and coat depigmentation. Altogether, these data highlight LKB1 as a critical regulator of NCC through limitation of oxidative DNA damages and p53 signaling.

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MUTANT P53 IN PANCREATIC CANCER: BIOLOGICAL AND THERAPEUTIC IMPLICATIONS.

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Nearly 70% of all ductal adenocarcinoma (PDAC) tumor harbor a mutation in the TP53 gene rendering it second only to KRAS mutation among the genetic alterations in this type of cancer.
Ductal adenocarcinoma of the pancreas (PDAC) is an almost uniformly lethal disease, largely because it eludes diagnosis until very advanced stages. PDAC is also usually resistant to all forms of cytotoxic chemotherapies and ionizing radiation. As a result, the 5-year survival rate of pancreatic cancer is less than 10%, and most of the patients die within the first 2 years.

Although p53 has been studied extensively in a number of cancers, investigations that have explored its roles in pancreatic cancer biology and treatment are still relatively limited. For many years, mutant p53 proteins have been considered to be undruggable. More recently, a number of compounds that restore tumor suppressive functionality to at least some mutants have been described, and were shown to exhibit anticancer activity in preclinical models expressing mutant p53. It has yet to be shown however, whether any mutant p53 reactivating compound has efficacy for the treatment of human pancreatic cancer patients. Taking advantage of mutant p53-reactivating peptides (pCAP250) recently developed at the Weizmann we are assesing the therapeutic utility of targeting mutant p53 in PDAC, either alone or in combination with standard-of-care treatment or additional targeted therapies.

pCAP250 can effectively induce cell death under tissue culture conditions in a spectrum pancreatic cancer cell lines. In order to elucidate the mechanism of action of the pCAP in our experimental system, we performed RNA-Seq analysis. PANC-1 cells were treated with either pCAP250 or scrambled peptide. Further analyses revealed a number of interesting genes and pathways that are modulated by pCAP and may account for the observed induction of cell death. Genes related with "Unfolded Protein Response" are upregulated at an early timepoint (CHOP and ATF3). This suggests that pCAP may induce UPR stress apoptosis.

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MUTANT P53 DEPENDENT METABOLIC CHANGES IN BREAST CANCER CELL LINES

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TP53 is the most mutated gene in cancer, with about 50% of cancers harboring a single missense mutation, generally located in the DNA binding domain. Previous studies demonstrated that mutations in p53 transcription factor lead to perturbations of cellular metabolism. Because metabolic status impacts on epigenetic modifications and gene expression program, we hypothesize that mutant p53 could dysregulate epigenetic landscape as a consequence of metabolic changes.
Using breast cancer cell lines, we propose to uncover mutant p53-dependent metabolic perturbations as well as subsequent epigenetic defects. As a first approach, we selected breast cancer cell lines expressing mutant p53 and generated derivatives in which endogenous mutant p53 is inactivated by a specific shRNA. This strategy enables to compare the same cancer cell lines expressing mutant p53 or not expressing mutant p53.

Here, we present an untargeted metabolomic investigation using 1H Nuclear Magnetic Resonance (NMR) spectroscopy. NMR metabolic profiles allow to unambiguously identify 52 metabolites and characterize some metabolic differences that are dependent of p53.
We present our first results together with prospects for investigation of metabolic-dependent perturbations of epigenetic landscapes.

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UNDERSTANDING THE IMPACT OF P53 MUTATIONS BY INTERROGATING ITS PROTEIN INTERACTING NETWORK

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Missense mutations, the most common type of mutation in TP53, often result in abundant expression of mutant p53 which contributes to cancer pathogenesis. p53 mutants not only lack tumor suppressive activities but, can convert p53 into a cancer promoting protein. Transcriptionally incompetent mutant p53 drives cell proliferation, migration, invasion, drug resistance, survival and genomic instability, by exploiting its protein binding partners. Intrinsic differences exist in p53 mutants and two main classes of p53 mutations have been described.

'Contact' mutations (mutations of codons 248 or 273) and 'Structural or conformational' mutation (for instance of codon 175) that alter protein-DNA interaction domain or stabilization of tertiary structure, respectively. Structural mutants are believed to severely disrupt the p53 conformation, whereas contact mutations maintain overall spatial structure of p53 but may still have differences in intrinsically disordered protein (IDP) regions. Here, we hypothesize that the protein interacting network of different p53 mutants may differ from each other and from wild type. Moreover, cellular proteins that retain capacity to bind to mutant p53, might be functionally impacted differentially by interaction with mutant or wildtype p53. Thus, mutant p53 interactome analysis may reveal the mechanism by which mutant p53 orchestrates changes in gene expression largely different from its wild type form. I propose to comprehensively analyze the protein interaction network of most common p53 mutants (R273H and R175H) with the goal of devising new therapeutic strategies for mutant p53 harboring tumors.

Specifically, I have performed mutant p53 immunoprecipitation and use mass-spectrometric method to systematically profile the mutant p53 interactome in human PDAC cell line (PANC1). Few results of the screening are validated by different protein-protein interaction assays. Functional consequences of novel mutant p53 interactions to be investigated in both in-vitro and in-vivo models. Finally, drug screens with chemical libraries will be conducted to search for inhibitors of binding between mutant p53 and therapeutically relevant binding partners.

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RCP DEPENDENT RECYCLING OF P-GLYCOPROTEIN CAUSES CHEMORESISTANCE IN MUTANT P53 CELLS

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p53 is the most frequently mutated gene in human cancers. Mutations lead to loss of p53 protein expression or the expression of a mutant p53 protein. Mutant proteins haven't only lost wild type function, but also acquire novel functions in promoting metastasis and chemoresistance. Previously, we uncovered a role for RCP (Rab Coupling Protein) in driving mutant p53 dependent invasion and metastasis. RCP is a membrane bound protein involved in the endosomal recycling process. It is overexpressed in a variety of cancers and we characterised in mutant p53 cells that it transports integrins and growth factor receptors from intracellular vesicles back to the plasma membrane to facilitate cell migration and invasion. In a screen to detect novel RCP interacting proteins, we discovered P-glycoprotein and various other drug transporters. Given that mutant p53 also conveys chemoresistance in cancer cells, we hypothesised that mutant p53 through RCP dependent recycling of P-glycoprotein could promote chemoresistance.

The interaction between RCP and P-glycoprotein was verified in several cell lines both endogenously and exogenously. We also determined that mutant p53 driven chemoresistance was dependent on RCP in vivo and in vitro. This increased sensitivity coincided with a decreased delivery of P-glycoprotein to the plasma membrane upon cisplatin or etoposide treatment, a retention of P-glycoprotein substrate and an increase in the expression of cleaved caspase 3. RCP is known to be delivered to the plasma membrane upon Using proximity ligation assays we noticed that mutant p53 expression profoundly enhanced the co-localisation of P-glycoprotein and RCP, which was decreased upon cisplatin treatment.

In conclusion our data are indicative for a role of mutant p53 in promoting the interaction between RCP and P-glycoprotein to facilitate P-glycoprotein membrane expression and drug efflux upon drug treatment. Given that several drug transporters were found to interact to a similar domain in RCP, future work will be directed to identify molecules or peptides that prevent RCP from interacting with those transporters to promote drug sensitivity.

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THE HUMAN P53 BINDING AND EXPRESSION RESOURCE (BAER) SHOWS P53 MUTANT DEFECTS IN BINDING AND ASSOCIATED EXPRESSION

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The stress-inducible master regulator p53 modulates many important biological cellular processes in its role as a transcription factor that extend well beyond the traditional role of "guardian of the genome" against human cancer. It is, therefore, important to know as much as we can about p53 function, where it binds in the genome, transcriptional targets and impact on expression. We recently developed* the human p53 binding and expression resource (BAER) of human cis-regulatory information derived from ChIP-seq and gene expression profiling, which maps the genome-wide locations of wildtype p53 binding sites and associated changes in expression across a matrix of experimental conditions.

Currently, the p53 BAER contains 58 data sets from genome-wide human wildtype p53 ChIP-seq studies of which 16 have associated gene expression data. In addition, we generated a toolkit (p53 cistrome R Shiny) that allows one to mine, filter and view p53 cistrome data.
The p53 BAER is a user-friendly resource that can be easily personalized with other available public or private "omic" information to allow better understanding of p53 functions. Here, we extend this analysis pipeline to four publicly available human mutant p53 genome-wide chromatin binding studies that include the hotspot mutants R248W, R273H and R280K, which affect the interactions between the p53 protein and the DNA. Comparisons between the mutants and wildtype p53 revealed by the p53 BAER analysis will be presented.

The human p53 BAER hub and the p53 cistrome R shiny is available at:
https://www.niehs.nih.gov/research/resources/databases/p53/index.cfm

*Reference: Nguyen TT, et al., Revealing a human p53 universe.
Nucleic Acids Res., 2018; 46(16):8153-8167.

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ASSESSING INHIBITION OF DNA REPAIR IN TP53 DEFICIENT MYELOMA CELLS

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Human myeloma cell lines (HMCLs) are widely used for the assessment of (new) drug efficacy in vitro or in vivo in mouse models. HMCLs have been mainly derived from patients at ultimate relapse and frequently from extramedullary disease, (peripheral blood, pleural effusion or ascites). Of HMCLs derived from patients at diagnosis most were from primary plasma cell leukemia. We performed a whole exon sequencing (WES) in 33 independent HMCLs, which are collectively representative of myeloma oncogenomic heterogeneity.
After global SNP enrichment analysis on 609,585 bi-allelic SNPs (SNPRelate package), three groups of HMCLs were identified: a group gathering HMCLs of Pacific/Japanese origin and a cluster encompassing all other HMCLs except one, which was individualized as African ethnicity. Because of the lack of normal DNA from patients from whom the HMCLs were derived, we could not easily discriminate the constitutive SNPs from the tumor-associated mutations. To remove ethnic-related SNPs, HMCLs were filtered with Global Allele Frequencies, plus East Asian frequencies for the Pacific/Japanese cluster and African frequencies for MM1S. We then excluded variants shared by more than 3 HMCLs in the panel: indeed, the most mutated genes in HMCLs and myeloma patients, i.e., RAS and TP53 never displayed more than 3 identical variants across the HMCL collection.

The five genes the most frequently altered in HMCLs (mutation, indel, frameshift..) were PRKD2, KRAS, NRAS, CDKN2C and TP53 (with alterations occurring in 21% to 67%). We next compared mutation rates in HMCLs with those in patients at diagnosis or relapse. By contrast to KRAS and NRAS mutation rate, which were similar in HMCLs and MM patients (or slightly increased), TP53 mutation rate (67%) was dramatically increased in HMCLs when compared to primary myeloma cells at diagnosis (5.5%) or relapse (19%). TP53 hits (mutation and deletion) were mutually exclusive to deletion/mutation in ATM/ATR ($p = 0.03$), as observed in B cell malignancies. Global analysis of altered pathways showed that MAPK, p53 and DNA repair were the most frequently altered pathways (80% of HMCLs).
We next decided to determine whether deficiencies in DNA repair pathways could constitute therapeuticvulnerabilities in p53 deficient cells. We assessed efficacy of drugs targeting several DNA repair pathways according to the presence of hits within p53 and DNA repair pathways. We used PARP inhibitor or DNA-PK inhibitor to inhibit NHEJ in HMCLs with mutations in HR, ATR inhibitor to inhibit HR in HMCLs with mutations in NHEJ, and USP1 inhibitor to inhibit Fanconi pathway. We combined these inhibitors with a DNA G-quadruplex stabilizer (CX5461) that induces DNA break during replication. We show that CX5461 combination with DNA-PK or ATR inhibitor was highly efficient in p53 deficient HMCLs.

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P53 CONTROLS A GLOBAL EPIGENETIC PROGRAM TO SUPPRESS TUMOR DEVELOPMENT

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Although the induction of growth arrest and apoptosis genes is essential for p53 tumor suppression, emerging evidence suggests the existence of yet unidentified p53 effectors. In this study, we investigated the role of wild type and mutant p53 in the control of epigenome and we showed that p53 is a critical regulator of the epigenetic state of the cells. Acute p53 deletion resulted in profound changes in DNA methylome resembling those in breast cancers and Li-Fraumeni patients. Analysis of the global histone landscape revealed higher levels of histone activating marks upon p53 deletion.

Using several cancer cell and mouse models, we found that p53 inhibits a wide range of histone modifiers and chromatin remodelers, including a number of cancer drivers. Moreover, mutant p53 gain of functions include upregulation of multiple epigenetic factors. Inhibition of p53 epigenetic targets contributed to tumor suppression in vitro and in vivo. Finally, analysis of transcriptomic and proteomic patient data revealed that elevated expression of epigenetic factors is associated with p53 mutations and poor survival. Our findings uncover p53-mediated regulation of epigenome as a new mode of its tumor suppression activity, which have implications for anti-cancer therapy.

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PRIMA-1 INHIBITS AMYLOID AGGREGATION OF MUTANT P53 IN CANCER CELLS

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p53 mutants can form amyloid-like structures that accumulate in cells. p53 reactivation with induction of massive apoptosis-1 (PRIMA-1) and its primary active metabolite, 2-methylene-3-quinuclidinone (MQ), can restore unfolded p53 mutants to a native conformation that induces apoptosis and activates several p53 target genes. However, whether PRIMA-1 can clear p53 aggregates is unclear. In this study, we investigated whether PRIMA-1 can restore aggregated mutant p53 to a native form. We observed that the p53 mutant protein is more sensitive to both PRIMA-1 and MQ aggregation inhibition than wild-type p53. Results with anti-amyloid oligomer antibody assays revealed that PRIMA-1 reverses mutant p53 aggregate accumulation in cancer cells.

Size-exclusion chromatography of the lysates from mutant p53–containing breast cancer and ovarian cell lines confirmed that PRIMA-1 substantially decreases p53 aggregates. We also show that MDA-MB-231 cell lysates can "seed" aggregation of the central core domain of recombinant wild-type p53, corroborating the prion-like behavior of mutant p53. We also noted that this aggregation effect was inhibited by MQ and PRIMA-1. This study provides the first demonstration that PRIMA-1 can rescue amyloid-state p53 mutants, a strategy that could be further explored as a cancer treatment.

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A FUNCTIONAL GENOMICS APPROACH TO DETERMINING MUTANT P53 GAIN-OF-FUNCTION PHENOTYPES AND MECHANISMS IN TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that lacks traditional clinical targets; as a result, cytotoxic chemotherapy is the current standard of care. Development of targeted therapies for TNBC is challenging due to molecular heterogeneity and a lack of therapeutically targetable, high-frequency "driver" alterations.

The most unifying feature across TNBC cases is that ~80% harbor a mutation in the tumor suppressor gene TP53. Mutations in p53 are commonly missense and have been proposed to result in gain-of-function (GOF) activity leading to novel oncogenic phenotypes, although the mechanistic underpinnings of this GOF activity are not understood. To study p53 GOF mutant proteins, our lab developed two isogenic cell line models (non-transformed mammary epithelial and TNBC cell lines) using CRISPR/Cas-mediated genome editing. The models include clonal cell lines expressing two common "hotspot mutant" p53 proteins (R175H and R273H), wild-type (WT) protein, or no p53 protein (Null).
This panel of cell lines allows for the study of various forms of p53, all expressed and regulated by the endogenous gene promoter and without the confounding effects caused by ectopic and unregulated overexpression.
Additionally, these models afford a unique opportunity to both dissect novel and evaluate proposed GOF mechanisms and phenotypes that stem from loss of functional (LOF) p53 and/or concomitant gain of mutant p53 protein expression. I will deploy biochemical techniques and analysis of an array of genomics data sets generated from our cell line models to evaluate the relationship between mutant p53 and p73 interactions and CIN. Through these aims I will test the hypothesis that discovery and dissection of mutant p53 LOF and/or GOF mechanisms will lead to the identification of novel pre-clinical targets for TNBC. I anticipate that the dissection of novel mechanisms as well as the evaluation of the reproducibility of proposed mechanisms for mutant p53 GOF phenotypes will improve the current understanding of the role mutant p53 in tumorigenesis.

The results generated from our studies have the potential for clinical translation, not only in TNBC (for which the need for a targeted therapy is critical), but also in other types of human cancer that have high-frequency p53 mutation.

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WILDTYPE P53 VERSUS MUTANT P53: FIGHTING TO CONTROL THE STRESS-INDUCED CHAPERONE SYSTEM

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A known prerequisite for gain-of-function (GOF) is stabilization of the mutant p53 protein via the HSF1-HSP90 axis, which itself is linked to aberrant protein conformation. A second necessary prerequisite enabling mutant p53 stabilization and thus GOF in vivo is loss of the remaining wild-type p53 allele termed loss-of-heterozygosity (LOH) in heterozygous tumors carrying a GOF mut53 allele. Whether and how these 2 conditions are linked is currently unknown.
Here we show that mutp53 stabilization is linked to LOH via heat-shock factor 1 (HSF1). We identified that wildtype p53 (wtp53) potently represses the activity of HSF1, the master transcription factor of the inducible heat-shock response (HSR), a core oncogenic stress pathway that renders tumor cells resistant to proteotoxic stress. HSR plays a key role in stabilizing oncogenic proteins including mutant p53 (mutp53), receptor tyrosine kinases like HER2 and cell cycle regulating kinases such as CDKs. We show that wtp53 suppresses the critical activating phosphorylation of HSF1 at Ser326, which is the functional hallmark of the tumor-promoting HSR. Consequently, wtp53 activation inhibits HSF1 target gene expression including HSP90 and other heat shock proteins. We identified an indirect repression mechanism of HSF1 by wtp53. wtp53 activates p21 leading to CDK4/6 inhibition and suppression of E2F target gene expression. We identified E2F target gene MLK3 to regulate HSF1. MLK3 normally signals to the MAPK pathway including MEK1, and MEK1 is well-known to phosphorylate and activate HSF1 on Ser326. Thus, p53-mediated inhibition of the cell cycle and down-regulation of the E2F target MLK3 suppresses HSF1 activity.

Our findings could explain why in an autochthonous mouse model of colorectal cancer (AOM/DSS), heterozygous mutp53 R248Q tumors (mutp53/+) fail to stabilize mutp53. In contrast, wtp53-deficient mutp53/- tumors strongly and homogeneously stabilize mutp53 in all cells within the tumor. Taken together, this indicates a potent inhibition of the HSF1-HSP90-mutp53 stabilization axis by the remaining wtp53 allele in vivo. Importantly, once the wtp53 allele is lost, an oncogenic 'flood gate' is opened since not only the HSF1 repression by wtp53 is lost but unopposed mutp53 now stimulates HSF1 activity as one of its GOF functions resulting in a massive positive feedforward loop to drive cancer as previously shown.
Our studies demonstrate an opposing regulation of HSF1 by p53 in tumors cells. While wtp53 represses HSF1 activity, mutp53 stimulates HSF1 functions. This antagonistic behavior is reminiscent of the opposing regulation of the MVA pathway by wtp53 vs mutp53.

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THE EFFECT OF TUMOR ARCHITECTURE ON P53 DYNAMICS AND ACTIVITY

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Measurements of p53 protein in single breast cancer cells showed that as a response to DNA damaging drugs, there is a substantial cell-to-cell variation in p53 abundance, activity and localization over time (p53 dynamics), leading to diverse cell fate outcomes. The goal of this research is to study the dynamics of wild type (WT) and mutant p53 in a three-dimensional (3D) model of breast cancer spheroids, more closely resembling an in vivo tumor and its microenvironment.

To model the tumor microenvironment in vitro, we utilize breast cancer cancer cells co-cultured with normal and cancer-associated fibroblasts (CAFs), and monitor p53 dynamics and cell fate over time after DNA damage using quantitative live single-cell microscopy.
We expect to gain new insights into wild type and mutant p53 dynamics and activity with the goal to reveal whether variations in these dynamics may also contribute to evolution of tumor heterogeneity and therapy failure.

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THE TRANSCRIPTIONAL ACTIVITY RESCUE SPECTRUM FOR MUTANT P53-RESCUING COMPOUNDS AS-001, L-902, GA, AND SG

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BACKGROUND: Tumor suppressor TP53 is the most frequently mutated gene in human cancer. We recently identified the compound AS-001 that can directly binds and efficiently restores mutant p53 with tumor-suppressive function with solved atom-level mechanism. Our studies further showed that AS-001 could only rescue a set of structurally instable mutant p53 (termed structural mutant p53), suggesting a necessity of mapping the AS-001 rescue spectrum for all cancer-associated p53 mutations. Such rescue spectrum can guide patient selection for the coming mutant p53-based personalized trial, wherein patients harboring mutations that can be efficiently rescued by AS-001 will be recruited.

RESULTS: We compiled all 21,789 cancer cases involving p53 point mutations from IARC (R19). A dual-luciferase assay was applied to evaluate the efficiencies of AS-001, L-902 (oral version AS-001), enhanced version AS-001 (GA) and the new-mechanism compound (SG) in rescuing the transcriptional activity (TA) for all compiled p53 point mutations. So far, we completed tests for approx. 400 frequent p53 point mutations, which covers approx. 10,000 cancer cases record in IARC (R19). We found:

1. Domain-dependent rescue: a majority of mutations on p53 DNA-binding domain (DBD) (approx. 60%) can be efficiently rescued with TA by compounds whereas only a few (approx. 10%) mutations outside DBD domain can be significantly rescued.
2. Structural mutation-specific rescue: compounds preferentially rescued TA of the structural mutant p53 over the non-structural mutant p53, exemplified by structural hotspot p53-R282W restored with > 85% TA and contacting hotspot p53-R273H barely rescued with TA.
3. Individual mutation-specific rescue: some structural mutant p53, exemplified by germline structural hotspot p53-M133T restored with > 90% TA and p53-M133R barely rescued.
4. The four tested compounds show similar rescue patterns, with compound SG showing more diversity.

FUTURE: The remained cancer-associated mutations will be cloned and tested, aiming to draw a comprehensive TA rescue spectrum. Rescue spectrums regarding cell-growth inhibition and xenograft tumor-growth inhibition will also be mapped. The mapped rescue spectrums will be used to guide patient selection in the coming mutant p53-based personalized trial in 2020.

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MUTANT P53 INDUCES TRANSCRIPTIONAL REPROGRAMMING AS A FOUNDATION FOR METASTASIS CAPACITY

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Esophageal cancer is one of the most common cancers where TP53 is mutated frequently, and this is a cardinal feature. Esophageal squamous cell carcinoma (ESCC) is the major subtype of esophageal cancer, and is one of the most lethal cancers worldwide, and with genomic properties that overlap with head/neck SCC and lung SCC. ESCC frequently evolves to lung metastasis, which leads to a dismal prognosis.

P53R175H (homologous to Trp53R172H in mice) is a common hot spot mutation. How metastasis is regulated by p53R175H in ESCC, and indeed cancer in general, remains to be elucidated. To investigate p53R175H mediated molecular mechanisms in ESCC and to extrapolate to other cancers, our lab has utilized germline Trp53R172H/- mice, and generated esophageal specific Trp53-/- mice and Trp53+/+ mice treated with the 4-NQO esophageal- specific carcinogen (a common exposure in humans) to model ESCC. In the primary Trp53R172H/- tumor cell lines established from these mouse models, we depleted Trp53R172H and observed a marked reduction in cell invasion and migration in vitro and lung metastasis burden in a tail-vein injection model in comparing these isogenic cells. Furthermore, to understand the molecular basis for mutant p53 driven lung metastasis, we performed bulk RNA-seq to compare gene expression profiles of metastatic and primary Trp53R172H/- and Trp53-/- cells.

We performed Gene Set Enrichment Analysis (GSEA) and identified YAP-BIRC5 axis as a potential mediator of Trp53R172H mediated metastasis. As a target gene of YAP, BIRC5 encodes an anti-apoptotic protein, namely Survivin. We are able to demonstrate that Survivin expression increases in the presence of Trp53R172H. We are now determining whether transcription of BIRC5 is regulated by the mutant p53-YAP complex. Taken together, this study will unravel new insights into how mutant p53 mediates metastasis. Profiling metastatic and primary Trp53R172H/- and Trp53-/- cells will also facilitate identification of novel therapeutic targets to improve patient survival in ESCC and other SCCs.

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WILD-TYPE P53 CANCER CELLS DISPLAY MUTANT P53 PHENOTYPES WHEN EXPOSED TO HEAVY METALS

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The tumour suppressor p53 is mutated in 50% of all cancers and up to 90% in small lung cancers for example. Unlike most other tumour suppressors, mutations in p53 often lead to gain-of-function, with deleterious consequences. Not only does p53 lose its tumour suppressor abilities, but it can act as a dominant negative inhibitor on wild-type p53 (WTp53) leading to reduced apoptosis.
Additionally, it can also gain oncogenic functions in increased proliferation, invasion, metastasis and chemo-/radio-resistance.
The WTp53 protein DNA-binding domain is stabilized in its native conformation by a Zinc ion. Using conformation-specific antibodies for p53, we show with Immunoprecipitation and in-cell western that metals such as copper and cadmium interfere with the conformation of p53. This leads to unfolding of its protein structure in a concentration- and time-dependent fashion. In turn, this unfolding leads to a mutant-p53-like phenotype of the WTp53 proteins exposed to metal. Two different invasion assays show that metal treatment increases invasion, and that this invasion is dependent on p53.

Mutant p53 proteins such as p53-R175H physically interact with specific other proteins, such as TAp63 Dicer and Argonaute-2, leading to an altered transcriptional programme.
We have shown that copper-exposed WTp53 shows the same behaviour as mutant p53 and co-immuno-precipitates p63, Dicer and Argonaute-2. Going forward, we want to better understand the cellular consequences of metal-induced unfolding of p53. How is transcription affected? How do metal transporters affect the outcome? In addition, we want to characterise metal-exposed WTp53 and various p53 mutants to compare their structure and function.
Since metal-unfolded WTp53 with a mutant phenotype is to this day inconspicuous when sequenced, we hope that a better characterization of unfolded WTp53 will inform how patientscan be treated in the future.

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"POSITIVE DOMINANCE" OF WILD-TYPE P53 OVER MUTANT P53 - AN UNDERESTIMATED PHENOMENON?

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Oligomerization of p53 allows for a direct interplay between wt and mutant p53 proteins if both are present in the same cells – where a mutant p53's dominant-negative effect known to inactivate wt p53, co-exists with an opposite mechanism – a "dominant-positive" suppression of the mutant p53's gain-of-function activity by wt p53. We determined the oligomerization efficiency of wt and mutant p53 in living cells using FRET-based assays and describe wt p53 to be more efficient than mutant p53 in entering p53 oligomers.

The biased p53 oligomerization helped to interpret earlier reports of a low efficiency of the wt p53 inactivation via the dominant-negative effect, while it also implied that the "dominant-positive" effect could be more pronounced. Indeed, we show that at similar wt:mutant p53 concentrations in cells – the mutant p53 gain-of- function stimulation of gene transcription and cell migration is more efficiently inhibited than the wt p53's tumor-suppressive transactivation and suppression of cell migration. These results suggest that the frequent mutant p53 accumulation in human tumor cells does not only directly strengthen its gain-of-function and dominant-negative activity, but also protects the oncogenic p53 mutants from the functional dominance of wt p53.

The question remains how often this mechanism affects the actual tumorigenesis and anti-cancer therapies in humans.

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A NEW P53R245W GERMLINE MODEL SHOWS MUTANT SPECIFIC DIFFERENCES IN STABILITY

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Emerging evidence suggests that missense p53 mutations identified in human cancer patients are associated with poor prognosis and drug resistance due to gain-of-function (GOF) activities.

Interestingly, these missense mutations may contribute to tumor progression and drug resistance by different mechanisms depending upon p53 mutation types and cell context. Numerous studies have implicated differences between p53 missense mutations with regard to cell proliferation, tumor development and metastasis. Since the p53R248W mutation is one of the most frequent missense mutations among human cancers, and previously our lab and others failed to generate a faithful p53R245W (corresponding to human p53R248W) model, we generated a conditional p53wm-R245W/+ knock-in mouse model, which we subsequently converted to germline p53R245W/+ mice by crossing to ZP3-Cre mice. p53R245W/R245W mice were generated at normal Mendelian ratio in p53R245W/+ crosses. The embryonic lethality of Mdm2 or Mdm4 loss was also rescued by p53R245W/R245W, demonstrating loss of function of p53R245W mutation.

To investigate specific GOF of the p53R245W allele in the germline, we monitored tumorigenesis of p53R245W/+ cohorts in comparison to p53R172H/+ mice, and found differences in tumor spectra and osteosarcoma metastasis between these two cohorts. In addition, p53R245W protein was stabilized by loss of Mdm2 or Mdm4, indicating Mdm2 and Mdm4 regulate p53R245W similarly to wild type p53. However, loss of Mdm2 in p53R245W/R245W mice did not accelerate the tumorigenesis, which is different from the observation in p53172H//R172H mice. These results indicate that different p53 hot spot mutations may have different mechanisms in tumorigenesis and metastasis.

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ALPHA-KETOGLUTARATE CONTRIBUTES TO P53-MEDIATED CELL FATE CHANGES DURING TUMOR SUPPRESSION

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Metabolites play well-established roles in supporting the growth and malignant progression of cancer cells, yet whether metabolites contribute to tumor suppression remains an open question. Here, we show that induction of the tumor suppressor p53 in the context of mouse pancreatic ductal adenocarcinoma (PDAC) rewires glucose and glutamine metabolism to support the accumulation of the metabolite alpha-ketoglutarate (aKG) at the expense of downstream metabolites. We find that this metabolic shift is dependent on p53 transcriptional activity, which drives dynamic rewiring of the tricarboxylic acid (TCA) cycle machinery. Restoration of p53 activity in vivo or in vitro downregulates PDAC-associated genes and upregulates genes associated with a pre-malignant, pancreatic intraepithelial neoplastic (PanIN)-like state. Moreover, supplementation with cell-permeable aKG or the enhancement of aKG levels through silencing of oxoglutarate (aKG) dehydrogenase (Ogdh) expression is sufficient to recapitulate the PDAC to PanIN switch even in the absence of wild-type p53 function. We find that in both mouse and human pancreatic cancer, loss of the aKG-dependent chromatin modification 5-hydroxymethylcytosine (5hmC) marks the progression from preneoplastic to malignant lesions.

Conversely, we observe that p53 restoration or Ogdh silencing in pancreatic tumors drives accumulation of 5hmC and promotes differentiation in vivo. Together, these results implicate aKG as a critical effector of p53-mediated differentiation and tumor suppression.

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ZINC METALLOCHAPERONES REACTIVATE MUTANT P53 USING AN ON/OFF SWITCH MECHANISM: A NEW PARADIGM IN CANCER THERAPEUTICS

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Zinc metallochaperones (ZMCs) are a new class of anti-cancer drugs that reactivate zinc deficient mutant p53 by raising and buffering intracellular zinc levels sufficiently to restore zinc binding. In vitro pharmacodynamics (PD) of ZMCs indicates that p53 mutant activity is ON by 4-6 hours and is OFF by 24. We performed in vitro mechanistic studies and identified that cellular zinc homeostasis functions as an OFF switch in ZMC PD indicating that a brief period of p53 mutant reactivation is sufficient for on-target efficacy.

We conducted pre-clinical pharmacokinetic (PK), PD and efficacy studies using the genetically engineered murine pancreatic cancer model (KPC) and murine breast cancer models with Brca1 deficiency. In vivo PK studies indicate that ZMCs have a short half life (<30 minutes), which is sufficient to significantly improve survival in mice expressing a zinc deficient allele (p53R172H) while having no effect in mice expressing a non-zinc deficient allele (p53R270H or p53-/-). We synthesized a novel formulation of the drug in complex with zinc and demonstrate this significantly improves survival over ZMC1. Lastly, we show that ZMC1 plus olaparib is a highly effective combination.

Pancreatic cancer therapy suffers from a lack of effective chemotherapy. TP53 is second only to KRAS as the most commonly mutated gene in pancreatic cancer with point mutations occurring in 75% of patients. On the other hand, virtually all BRCA1 deficient breast cancers (BDBC) harbor TP53 mutations.

These results validate a novel therapeutic approach for pancreatic cancer and BDBC through reactivation of mutant p53.

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