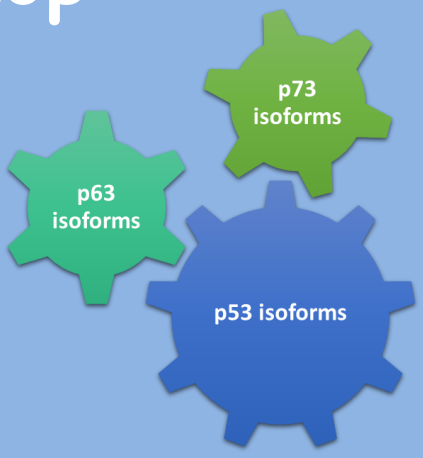
RUĐER BOŠKOVIĆ INSTITUTE, CROATIA

International p53/p63/p73 Isoforms Workshop



Book of abstracts

3-6 November 2019
Dubrovnik, Croatia

International p53/p63/p73 isoforms workshop

4th International p53 isoforms meeting

and

9th International p63/p73 workshop

Dubrovnik, Croatia

3rd -6th November 2019

CONTENTS

Dear Colleagues,

Welcome to the **International p53/p63/p73 isoforms workshop** in Dubrovnik!

The conference will for the first time combine two workshops – 4th International p53 isoforms meeting and 9th International p63/p73 workshop into one unique meeting and bring together researchers from the entire field as well as clinicians and pharmaceutical companies to discuss new developments and to generate fruitful collaborations.

Over the last decade, new findings in the field have dynamically reformed and broadened the roles of p53 and its family in cancer, ageing, development, immune response and metabolism. Understanding the p53 family isoforms' mechanisms offers exciting perspectives in the treatment of degenerative diseases such as cancer, neurodegeneration and other age-associated pathologies as well as infectious diseases.

We believe that the organisation of the International p53/p63/p73 isoform workshop is valuable opportunity for young scientists to present their work and discuss with p53 experts.

The historic town of Dubrovnik will host a p53 conference for the first time and give an opportunity for new networking between world class scientists. Picturesque Dubrovnik is situated on the very South of Croatia, surrounded by crystal clear Adriatic Sea and fortified with medieval walls.

The conference is organized by Ruđer Bošković Institute, Zagreb.

We are looking forward seeing you in Dubrovnik!

Sincerely,

on behalf of the Organising Committee

Neda Slade and Jean-Christophe Bourdon

COMMITEES

Scientific Committee:

Antony Braithwaite, University of Otago, New Zeeland

Jean-Christophe Bourdon, University of Dundee, UK

Yari Ciribilli, University of Trento, Italy

Volker Doetsch, Goethe Unviersity, Franckfurt, Germany

Bjorn Tore Gjertsen, University of Bergen, Norway

Curtis Harris, NCI NIH, USA

Gerry Melino, University of Leicester, UK

Thomas Meyer,Max Planck Institute, Germany

Alea Mills, CSHL, USA

Bertrand Mollereau, ENS Lyon, France

Pierre Roux, CRBM CNRSF, rance

Olivier Terrier, International Center for Infectiology Research, France

Organizing Committee

Neda Slade, Ruđer Bošković Institute, Croatia (Chair)

Ana Vidoš, Ruđer Bošković Institute, Croatia (Marketing & Event coordinator)

Jean-Christophe Bourdon, University of Dundee, UK

Martina Radić, Ruđer Bošković Institute, Croatia

Ana Dekanić, Ruđer Bošković Institute, Croatia

DUBROVNIK

Dubrovnik is an exceptional medieval stone walled city. With its UNESCO World Heritage listed sites, mild climate all year round and direct flights from over 50 large international airports, it is an ideal place to merge business and comfort.

Sitting proudly on the calm blue waters of the Adriatic, Dubrovnik is one of the world's most magnificent fortified cities and without doubt currently one of Europe's most fashionable destinations.

Steeped in history and virtually unchanged since the 13th Century the old quarter provides a fascinating distraction from the everyday's business. The city is a living museum and a live stage, and has an ideal connection between its historical past and the modern day. It is surrounded by medieval walls that are 1940 metres long and are preserved in their original form.

Dubrovnik is a wonderful pocket of the globe that still provide us with unique snapshots of the past.

VENUE

Of all the five-star hotels in Dubrovnik, the newly renovated Hotel Dubrovnik Palace has it all: a luxurious wellness and spa centre, high-tech conference rooms, superb gastronomy and spectacular sea views from every room.

Just a short distance from Dubrovnik Old Town, each of the recently fully renovated 308 contemporary rooms and suites all have private balconies and beautiful sea views. Great food and excellent service prevail. The Hotel Dubrovnik Palace was also the proud holder for

two consecutive years of two World Travel Awards: Croatia's Leading Hotel (2005/2006) and Croatia's Leading Spa Resort (2005-2007, 2011 /2012). Whether you're looking for rest and relaxation in understated luxury, sports and activities or a motivating environment for your business event, this five-star resort in Dubrovnik is worthy of all the awards that have been bestowed upon it. This hotel provides the largest conference center in Dubrovnik and state of the art congress and business facilities designed to meet every need.

https://www.adriaticluxuryhotels.com/en/hotel-dubrovnik-palace

ORGANIZERS AND SPONSORS

PROGRAMME

Sunday 3rd November 2019

Registration from 2 pm

15:00 – 15:10 Opening and introduction
Neda SLADE, *Ruđer Bošković Institute, Zagreb, Croatia*

Physiological roles, molecular and cellular mechanisms

Chairs: Bjørn Gjersten and Yari Ciribilli

15:10 – 15:50
Keynote Lecture sponsored by *Cancers*
Jean-Christophe BOURDON, *University of Dundee, UK*
Towards a p53 code?

15:50 – 16:15
Cecilia Blair LEVANDOWSKI, *University of Colorado Boulder ~~CUB~~,USA*
How does Δ40p53 alter WTp53 function?

16:15 – 16:40
Kelly AVERY-KIEJDA, *University of Newcastle, Australia*
The role of Δ40p53 in the response to DNA damaging therapies in breast cancer cells

16:40 – 17:05
Neda SLADE, *Ruđer Bošković Institute, Zagreb, Croatia*
Functional interplay between p53 and p53/p73 isoforms in human melanoma

Coffee break

17:25 – 17:40
Martin FISCHER, *Leibniz Institute on Aging – Fritz Lipmann Institute, Germany*
Dissecting the binding landscape and gene regulatory network of p63 and p53

17:40 – 17:55
Morgan A. SAMMONS, *State University of New York at Albany, USA*
Determinants of cis-regulatory activity for the p53 family of transcription factors

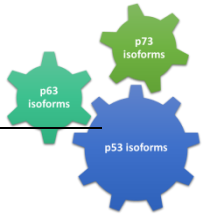
17:50 – 18:15
Alexander ZAIKA, *University of Miami Health System, USA*
Formation of protein adducts causes inactivation of p53 protein and its aggregation

18:15 – 18:40
Pierre ROUX, *CRBM CNRS, France*
Δ133p53ß isoform invasive activity: mechanism of action and regulation

18:40 – 18:55
Nicole HEINZL, *Medical University of Vienna, Austria*
The prognostic impact of p53 aggregates in high-grade serous ovarian cancer

18:55 – 19:20
Alea MILLS, *Cold Spring Harbor Laboratories, USA*
p53: dictator of epigenetic vulnerabilities in human glioma

19:20 – 21:30 Welcome dinner



Monday 4th November 2019

Ageing, stem cells and cell reprogramming. Multi-cellular organisation of tissue and organs

Chairs: Alea Mills, Jeffrey W. Pollard and Thomas F. Meyer

08:15 – 09:00
Keynote lecture
Curtis HARRIS, *NCI NIH, USA*
Aging and cancer: p53 isoforms

09:00 – 09:25
Margareta WILHELM, *Karolinska Institute, Stockholm, Sweden*
p53 controls genomic stability and temporal differentiation of human neural stem cells and affects neural organisation in human brain organoids

09:25 – 09:50
Juan J. TENA, *Andalusian Center for Development Biology CABD, Seville, Spain*
Roles of p63 during ectoderm specification

09:50 – 10:15
Ting ZHAO, *University of Zhejian, China*
p53 isoform delta113p53 promotes zebrafish heart regeneration via maintaining ROS homeostasis

10:15 – 10:40
Jo Huiqing ZHOU, *Radboud Institute of Molecular Life Sciences, Nijmegen, The Netherlands*
Transcription factor p63: guardian of the epithelial cell fate?

Coffee break

11:00 – 11:25
Maria C. MARIN, *Universidad de León, Spain*
p73 an architect of epithelial tissue

11:25 – 11:40
Yan SUN, *Cancer Research Centre of Lyon, UMR INSERM 1052 – CNRS 5286, Lyon, France*
Regulation of netrin-1 by p53 isoforms

11:40 – 11:55
Jin ZHANG, *University of California, Davis, USA*
Mutant p53 antagonizes p63/p73-mediated tumor suppression via Notch1

11:55 - 12:20
Caterina MISSERO, *University of Naples Federico II, Naples, Italy*
Overlapping transcriptional programs downstream of p63 and p73 promote cutaneous squamous cell carcinoma

12:20 – 12:45
Flash Poster presentations (2 min per poster)

12:50 – 13:30 Lunch

13:30 – 13:45 Posters

Maintenance and restoration of tissue integrity and function in response to infection, and oxidative stress. Role in the coordination of the immune response

Chairs: Jean-Christophe Bourdon and Curtis Harris

14:15 – 15:00

Keynote Lecture sponsored by *Cancers*

Jeffrey W. POLLARD, *University of Edinburgh, UK & Albert Einstein College of Medicine, USA*

Macrophage diversity plays an essential role in development, repair and disease

15:00 – 15:25

Max WELLENSTEIN, *Netherlands Cancer Institute, The Netherlands*

p53 status dictates pro-metastatic systemic inflammation in breast cancer

15:25 – 15:40

Marina KAZANTSEVA, *University of Otago, Dunedin, New Zealand*

Δ133p53β isoform regulates unique gene sets involved in immunosuppression, cell growth and cell migration

15:40 – 15:55

Hakim ECHCHANNAOUI, *Johannes Gutenberg University Mainz, Germany*

Targeting Δ133p53α as a novel T cell enhancer factor to improve cellular-based immunotherapy for cancer

15:55 – 16:10

Sunali MEHTA, *University of Otago, Dunedin, New Zealand*

Modulation of Dendritic Cell function by Δ133p53 isoforms

Coffee break

16:30 – 16:55

Thomas F. MEYER, *Max Planck Institute for Infection Biology, Germany*

Role of p53 in the interplay between host cell and pathogens

16:55 – 17:10

Yann BRETON *Centre de recherche du CHU de Québec-Université Laval, Canada*

An antiviral role for p53 against HIV-1 in macrophages and the implication of the p53 isoforms

17:10 – 17:25

Lucie CAPPUCCIO, *UMR754, Lyon, France and Institut Pasteur Shanghai, China*

Opposite effect of p53 on chikungunya virus replication in mammal and insect

17:25 – 17:40

Anaïs BLANCHET, *INSERM UMR_S1113, University of Strasbourg, France*

Identification of novel partners/regulators of p73 proteins in gastric cancer

17:40 – 18:05

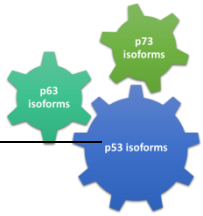
Christian GAIDDON, *INSERM, University of Strasbourg, France*

Role of the p53 family in muscle atrophy associated with ALS and cancer

18:05 – 18:30

Antony BRAITHWAITE, *University of Otago, New Zealand*

Elevated levels of Δ133p53β isoform are found in Rheumatoid Arthritis patients with hyperproliferative synovium and exaggerated immune cell infiltration



Tuesday 5th November 2019

Mutation and interplay

Chairs: Antony Braithwaite and Neda Slade

8:15 – 8:40
Elsa FLORES, *Moffitt Cancer Center, Tampa, Florida, USA*
Pan-cancer analysis reveals alternate mechanisms for AKT activation through TAp63 regulated oncogenic lncRNAs (TROLLs)

8:40 – 9:05
Volker DÖTSCH, *Goethe University, Frankfurt, Germany*
Mechanism of inhibition and activation of TAp63α in oocytes

9:05 – 9:20
Sebastien JORUIZ, *NCI NIH, USA*
Functional analysis of wild-type and mutant p53 isoforms

9:20 – 9:45
Tom VAN WEZEL, *Leiden University Medical Centre, The Netherlands*
Germline variant affecting p53β isoforms predisposes to various familial cancers

9:45 – 10:10
Daniele BERGAMASCHI, *Queen Mary London University, UK*
Impact of p63 upregulation on MAPK inhibitors resistance in melanoma

10:10 – 10:35
Luisa GUERRINI, *University of Milano, Italy*
Thalidomide teratogenicity uncovered- the central role of p63 and CRBN

Coffee break

11:00 – 11:25
Yari CIRIBILLI, *CIBIO-Centre for Integrative Biology, University of Trento, Italy*
Impact of p53 isoforms over-expression in lung cancer

11:25 – 11:50
James MANFREDI, *Icahn School of Medicine at Mount Sinai, USA.*
Tissue-specific tumor suppressor functions of p53 in vivo

11:50 – 12:15
Claude CARON DE FROMENTEL, *Cancer Research Center of Lyon, France*
DeltaNp73 expression impacts on stem-like cell properties of acute myeloid leukemia and hepatocellular carcinoma tumors

12:15 – 12:45
Flash Poster presentations (2 min per poster)

12:45 – 13:30 Lunch

13:30 – 14:15 Posters

Regulation, splicing and treatment

14:15 – 14:40
Michael KASTAN, *Duke Cancer Institute, Durham, USA*
DNA damage-induced alternative splicing of p53

14:40 – 14:55
Annette LASHAM, *University of Otago, New Zealand*

Quantitation and analysis of alternatively-spliced TP53 RNAs in a NZ breast cancer cohort, using a novel multiplex long amplicon digital PCR method

14:55 – 15:10

Jayanthi P. GUDIKOTE, *University of Texas M.D. Anderson Cancer Center, Houston, USA*
Targeting nonsense-mediated decay and mRNA splicing to activate p53 pathway in p53 mutant and non-mutant cancer cells

15:10 – 15:25

William TAYLOR, *Univ Rennes, CNRS, IGDR, UMR 6290, France*
The RNA Binding protein PTBP1 controls the alternative splicing of the C-terminal Exons of TP63 in HNSCC.

15:25 – 15:50

Xinbin CHEN *Comparative Oncology Laboratory, University of California, USA*
Targeting the p53-Rbm38 loop for tumor suppression

15:50 – 16:15

Sue HAUPT, *Peter MacCallum Cancer Centre, Melbourne, Australia*
In the loop with p53: isoforms and cancer

18:30 Sightseeing Dubrovnik

19:30 Gala dinner

Wednesday 6th November 2019

Biomarkers and targeting isoforms

Chairs: Claude Caron de Fromentel and Pierre Roux

08:15 – 09:00

The EMBO Keynote Lecture

Varda ROTTER, *Weizmann Institute of Science, Israel*

Development of a mutant p53-dependent novel cancer therapy

09:00 – 09:25

Bjørn GJERTSEN, *University of Bergen, Norway*

The landscape of p53 isoforms in normal blood cells and acute leukemia

09:25 – 09:40

GEMMA DOMÍNGUEZ, *CSIC-UAM, IdiPaz, Madrid, Spain*

Exosomal ΔNp73, TAp73 and Δ133p53 isoforms as early diagnosis markers in colorectal cancer

09:40 – 10:05

Klas WIMAN, *Karolinska Institute, Stockholm, Sweden*

Rescue of missense and nonsense mutant p53: from bench to bedside

10:05 – 10:30

Joanna ZAWACKA-PANKAU, *Stockholm, Sweden*

Drug repositioning to target p73 for improved cancer therapy

Coffee break

10:50 – 11:15

Ygal HAUPT, *Peter MacCallum Cancer Centre, Melbourne, Australia*

Targeting MDM proteins in wt and mutant p53 cancers

11:15 – 11:30

Giovanni MINERVINI, *University of Padova, Dept. Biomedical Sciences, Italy*

The E3 ubiquitin-protein ligase Mdm2 is a novel interactor of the von Hippel-Lindau tumor suppressor

11:30 – 11:45

Thomas G HOFMANN, *Institute of Toxicology, University of Mainz, Germany*

DAZAP2 acts as phosphorylation-dependent specifier of the p53 response controlling cancer cell chemosensitivity

11:45 – 12:10

Ivano AMELIO, *University of Cambridge, UK*

p53 family isoforms and mutations in response to microenvironmental stressors

12:10 – 12:35

Simon MCDADE, *Queen's University Belfast, UK*

Functional genomics identifies a novel p53 induced ligand-independent TRAIL-R2/FLIP complex as a novel therapeutic vulnerability

12:35 – 13:00

Jean-Christophe BOURDON, *University of Dundee, School of Medicine, UK*

Conclusion

Invited lectures

L1 Towards a p53 code?

JEAN-CHRISTOPHE BOURDON (1)

1) University of Dundee, Dundee Cancer Centre, School of Medicine, Dundee, DD1 9SY, United Kingdom

Over the last decade, genetic and experimental evidence indicate that the isoforms of the p53 gene family are involved in a broad range of pathologies: embryo malformation, premature ageing, (neuro)-degenerative diseases, inflammation/immunological defects, cancer and altered responses to ionising irradiation or infection, suggesting that the p53 pathway play a critical role in tissue maintenance and regeneration.

Deciphering the molecular mechanism of cell fate decision mediated by p53 pathway is poorly understood. Data indicate that the p53, p63 and p73 isoforms interact with each other to mediate the cell response. The generation of novel antibodies specific of the p53 isoforms enable to investigate the molecular mechanism and to explore the expression of the p53 isoform in normal and cancer tissue expressing WT and mutant TP53.

We investigated using p53 isoform specific siRNAs whether p53-mediated responses can be triggered in the absence of p53alpha and whether p53-mediated cell fate is determined by a dominant isoform or through the combination of co-expressed endogenous p53 protein isoforms.

The results are striking and lead us to reflect on the nature of p53 isoform and their biological roles.

L2 How does Δ40p53 alter WTp53 function?

CECILIA BLAIR LEVANDOWSKI (1), DYLAN TAATJES (1), ROBIN DOWELL (1), MARGARET GRUCA (1), TAYLOR JONES (1)

1) University of Colorado Boulder

Keywords: Δ40p53, PROseq, RNAseq, ChIPseq, CRISPR/Cas9

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 naturally occurring p53 isoform Δ40p53 is expressed in most cell types and is directly implicated in mammalian aging. For example, in mouse models, co-expression of WTp53 and Δ40p53 results in an accelerated aging phenotype with premature development of aging pathologies such as osteoporosis and Alzheimer's disease. The basic mechanisms driving these Δ40p53-dependent cellular and physiological changes remain poorly understood. Human Δ40p53 lacks the first N-terminal transactivation domain of WTp53, and Δ40p53 is preferentially translated during cell stress. The Δ40p53 isoform oligomerizes with WTp53 to form hetero-tetramers with altered function compared to WTp53 tetramers. Co-expression of Δ40p53 and WTp53 results in the formation of a mixed population Δ40p53:WTp53 tetramers, including "contaminating" WTp53 tetramers. This precludes a reliable functional comparison of Δ40p53:WTp53 tetramers vs. WTp53. To circumvent this issue, we developed and validated a strategy—based upon the native p53 tetramer structure—in which Δ40p53 is tethered to WTp53 (Δ40p53:WTp53) as a single transcript, resulting in a pure population of tetramers with a defined 2:2 ratio of Δ40p53 to WTp53. Using CRISPR/Cas9, human cell lines were generated in which Δ40p53:WTp53 (and controls) was inserted at the native TP53 locus. This enabled Δ40p53:WTp53 expression and induction in a physiologically relevant manner. Using these edited cell lines (Δ40p53:WTp53, WTp53, and WTp53:WTp53) in combination with precision run-on nuclear sequencing (PRO-seq), we have been able to determine how the Δ40p53 isoform alters WTp53 transcriptional activity, including changes in the non-coding transcriptome. These results have been complemented with ChIP-seq and other experiments to further define how Δ40p53:WTp53 alters the biological function of WTp53.

References: Lin, S.-C., et.al., Aging Cell(2013). 12(5), 863–872. This research was supported by the National Institute of Aging.

L3 The role of Δ40p53 in the response to DNA damaging therapies in breast cancer cells.

XIAJIE ZHANG (1), BRIANNA C MORTEN (2), HAMISH G CAMPBELL (3), ANTONY W BRAITHWAITE (4), KELLY A AVERY-KIEJDA (1)

1) Priority Research Centre for Cancer Research, Innovation and Translation, School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Australia.

2) Priority Research Centre for Cancer Research, Innovation and Translation, School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Australia

3) Children's Medical Research Institute, University of Sydney, NSW, Australia

4) Department of Pathology, School of Medicine, University of Otago, Dunedin, New Zealand.

Keywords: Δ40p53, breast cancer, DNA damage

Breast cancer is the most common malignancy in women. Nearly all deaths from breast cancer are a result of resistance to DNA-damaging therapies and the subsequent development of secondary cancers (metastases) and this remains a significant barrier to breast cancer survival. The tumour suppressor gene, p53, is a master regulator in the decision-making of cell fate outcome following DNA damage, by acting as a transcription factor to regulate the expression of a number of target genes involved in the processes of cell cycle arrest, DNA repair or apoptosis. How p53 decides a cell's fate depends on a myriad of signalling interactions and binding partners, including the differential expression of p53 isoforms. The p53 isoforms are known to play important roles in mediating the p53 response in a range of human cancers. The mutation frequency (~25%) of p53 in breast cancer is less than expected for a protein that plays a pivotal role in maintaining genomic integrity, suggesting that p53 is inactivated or that its function is modulated by mechanisms other than mutation. We have shown that the p53 isoform, Δ40p53, is abundantly expressed in breast cancer (1). Furthermore, a high Δ40p53:p53 ratio is associated with worse disease-free survival, indicating that disruption of p53 function by Δ40p53 may contribute to a more aggressive phenotype (2). However, the role of endogenous Δ40p53 in the response to DNA damage in breast cancer is unclear. The aim of this study was to investigate the role of Δ40p53 in the response to DNA damaging therapies in breast cancer cells. We have developed MCF-7 cells that overexpress Δ40p53 (LeGO-Δ40p53) or the empty vector (LeGO) as well as breast cancer

cells where Δ40p53 has been knocked down using shRNA. These cells were treated with different DNA damaging agents and downstream analysis was performed including real-time PCR, immunofluorescence and functional assays. Our in vitro studies have shown that Δ40p53-knockdown in breast cancer cells is associated with increased DNA damage and apoptosis; and decreased G2 arrest in response to DNA-damaging therapies, as well as increased expression of p53 and the p53-dependent apoptosis-related genes including BAX, Noxa and Puma. In contrast, a high Δ40p53 level (Δ40p53-overexpression) led to increased G2 arrest and decreased expression of these genes. These results suggest that Δ40p53 provides an intricate rheostat to the p53-mediated DNA damage response. The response of cells to DNA damage is particularly important for predicting chemosensitivity during treatment of breast cancer. Inhibition of Δ40p53 expression may serve as a potential target to enhance effectiveness of DNA-damaging therapies in breast cancer.

References: (1) Avery-Kiejda KA et al; Carcinogenesis 2014; 35(3), 586-596. doi:10.1093/carcin/bgt411 (2) Morten BC et al; Carcinogenesis 2016; 37(1): 81-6. doi: 10.1093/carcin/bgv164 This work was supported by grants from the Bloomfield Group Foundation and Pink Frangipani Ball through the Hunter Medical Research Institute (HMRI) (to KAK); the Australian Postgraduate Award and the MM Sawyer Scholarship through HMRI (to BCM). KAK is a Cancer Institute NSW Career Development Fellow.

L4 Functional interplay between p53 and p53/p73 isoforms in human melanoma

PETAR OZRETIĆ (1), MARTINA RADIĆ (1), NIKOLINA HANŽIĆ (1), BASTIEN PROUST (1), MAJA SABOL (1), YARI CIRIBILLI (2), IVAN MILAS (3), ZVONIMIR PULJIZ (3), NEDA SLADE (1)

1) Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

2) CIBIO, University of Trento, Italy

3) Sestre Milosrdnice University Hospital Center, Croatia

Unlike other tumors, TP53 is rarely mutated in melanoma; however, it fails to function as a tumor suppressor. We assume that its functions might be altered through interactions with several family members.

To elucidate the potential interplay among family members, we analysed the expression profiles of p53/p73 isoforms in a panel of melanoma cell lines, metastatic melanoma specimens and healthy corresponding tissue. Using qPCR, lower levels of Δ40p53 and ΔNp73 were observed in tumor samples compared to healthy tissue. Protein expression of Δ133p53α, Δ160p53α and ΔNp73α isoforms was elevated in tumor tissue, whereas ΔNp73β was downregulated. The results in melanoma cell lines, in general, support these findings. In addition, we correlated expression profiles with clinical features and outcome. Higher Δ133p53β and p53α mRNA expression had a negative impact on the overall survival. Shorter overall survival was also connected with lower p53β gene expression levels.

Furthermore, we examined the interactions between p53 and p53/p73 isoforms as well as their effect on p53 transactivation capacity in wild-type p53 melanoma cell lines. As expected, we have found interactions between p53α and small molecular weight p53 isoforms (p53β, p53γ, Δ40p53α, Δ40p53β and Δ133p53α) as well as with both ΔNp73α and ΔNp73β in melanoma cell lines. These results suggest that p53 and p73 isoforms may contribute to inactivation of p53 protein function in melanoma cells by forming the direct protein interactions. Consequently, reporter and apoptosis assay confirmed that p53 and p73 isoforms interfere with p53α activity thus regulating the expression of genes involved in cell cycle, apoptosis and senescence. In conclusion, all examined genes may have implications in melanoma development and functional inactivity of TP53. Targeting two independent pathways could be a promising strategy in melanoma treatment.

Reference: Ozretić P, Hanžić N, Proust B, Sabol M, Trnski D, Radić M, Musani V, Ciribilli Y, Milas I, Puljiz Z, Bosnar MH, Levanat S, Slade N. Expression profiles of p53/p73, NME and GLI families in metastatic melanoma tissue and cell lines. Sci Rep (2019) 9(1):12470.

L5 Formation of protein adducts causes inactivation of p53 protein and its aggregation

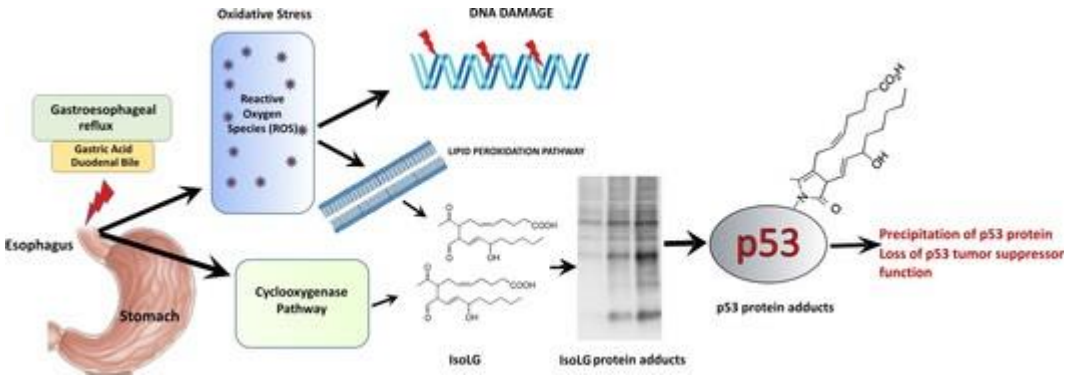
RAVINDRAN CASPA GOKULAN (1), KODISUNDARAM PAULRASU (1), ELENA ZAIKA (1), JOHN A. OATES (2), ALEXANDER ZAIKA (1)

1) University of Miami, Miami, FL, USA

2) Vanderbilt University, Nashville, TN, USA

Keywords: p53, p73, protein adducts

Oxidative stress is responsible for a plethora of pathological conditions, including cancer. One interesting example is esophageal adenocarcinoma (EAC) for which incidence is dramatically increased in many Western countries over the past several decades. This tumor is associated with esophageal tissue damage caused by gastroesophageal reflux disease (GERD). Due to GERD, cells located in the esophagus are periodically exposed to gastric acid and bile that induce reactive oxygen species and strong oxidative stress. Analyzing animal models of esophageal reflux injury and human tissues, we found that oxidative stress leads to the formation of isolevuglandins (isoLGs) protein adducts. Among affected proteins is wild type p53, which is covalently adducted by isoLG at lysine residues. The formation of p53 protein adducts significantly affects acetylation of p53 protein and its transcription activity, inhibiting expression of multiple p53 target genes. Due to strong reactivity and hydrophobicity of isoLGs, the adduction also causes the formation of p53 protein aggregates. Testing of chemical scavengers of isoLGs showed that these compounds can prevent adduction of p53 protein during oxidative stress resulting in restoration of p53-dependent transcription and inhibition of p53 protein aggregation. Our studies also show that other members of the p53 protein family differentially affected by isoLG and p73 protein is more protected against inhibitory activity of isoLGs compared to that of p53.



Scheme illustrating the formation of p53 protein adducts

L6 ∆133p53ß isoform invasive activity: mechanism of action and regulation

PIERRE ROUX (1)

1) Montpellier Cell Biology Research Center, Dynamics of cell invasion in cancer, Montpellier, France

Defects of the *TP53* tumor suppressor activity are a compulsory step to cancer formation. Inhibition of its tumor suppressor activity can be achieved through protein modifications either by mutations or by production of specific *TP53* variant proteins (isoforms).

It is currently thought that the oncogenic activities of mutant p53 proteins mainly result from a dominant-negative mechanism in which mutant p53 binds and inactivates wild type (WT) p53. However the mechanism of action by which *TP53* isoforms make cancerous cells is totally unknown. Our last results show that invasive activity of Δ133p53β is regulated through a reversible aggregation-dependent mechanism, which is independent of WT p53.

The p53 isoforms, in particular WT Δ133p53β, play a critical role in cancer progression and is a valuable biomarker for bad prognosis in human colorectal and breast cancers whatever the mutational status of the *TP53* gene. In cancer cells, the Δ133p53 isoforms promote cancer stem cell potential, invasion and metastasis via RhoA/ROCK signalling, IL6-dependent activation of JAK/STAT and protection from apoptosis. Although significant number of Δ133p53 isoforms functions in cancer progression are well established the mechanism of their action are totally unknown.

Recently we showed that WT Δ133p53β aggregates depend on associations with specific interacting partners, including ΔNp63 and the CCT complex, but not WT p53, and were observed in both cancer cell lines and in human tumour biopsies. Our study shows that WT Δ133p53β aggregation capacity inversely correlates with its activity. Δ133p53β oscillates between non-aggregated/active and aggregated/non-active states that critically influence cancer cell features such as migration and invasion. Our data allow us to conclude that the invasive activities associated with WT Δ133p53β isoform are not carried out by a dominant negative activity on WT p53. This constitutes an original mechanism of cancer progression that is completely different from the conventional mechanism of described for mutant p53.

L7 P53: Dictator of Epigenetic Vulnerabilities in Human Glioma

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1) Cold Spring Harbor Laboratory

Keywords: Glioma, P53, CHD5, chromatin regulators

Having identified P63 and shown it is a master regulator of development, cancer, cellular senescence, and aging, my team is also interested in P53 biology. We identified both tumor suppressors and oncogenes that modulate P53–mediated pathways. First, my laboratory identified CHD5 as a tumor suppressor mapping to human 1p36—a region of the genome frequently deleted in human cancer. We determined the mechanism whereby CHD5 regulates chromatin dynamics to maintain stem cell homeostasis, and defined how perturbation of these processes leads to cancer. We discovered that CHD5 is frequently inactivated in human glioma, and determined that CHD5's ability to bind unmodified histone H3 is essential for P53-mediated tumor suppression. Our recent work reveals that CHD5 regulates a ribosome biogenesis switch that dictates neural cell fate, and that CHD5 deficiency leads to excessive numbers of astrocytes at the expense of neurons. In parallel with our work identifying CHD5 as a tumor suppressor, our recent efforts to identify new oncogenic drivers of glioblastoma uncover a novel epigenetic vulnerability that serves as an Achilles' heel that when targeted shuts down tumor growth. We discovered that this target bypasses the requirement for inactivating P53, thereby epigenetically reprogramming the cell to a malignant oncogene-addicted state. We believe this target offers a novel therapeutic strategy for patients with glioblastoma in which P53 is intact, which make up the vast majority of cases.

L8 Aging and cancer: p53 isoforms

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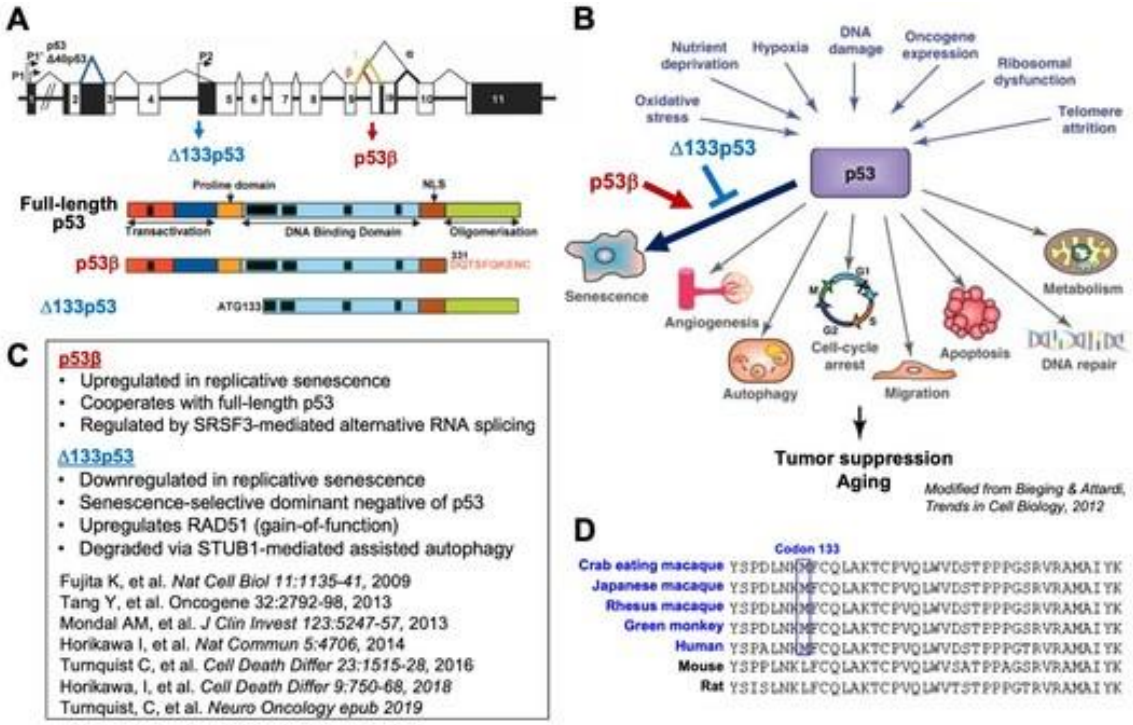
The p53 network is an intrinsic monitoring and responsive pathway of telomeric attrition and chronic stress involved in cellular aging and senescence (1). Cellular senescence, in cancer cells, can be a tumor suppressive mechanism that can be activated by p53. Cellular senescence of tumor stromal ceils 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" Δ133p53 and “co-transactivator" of wild-type p53, p53β, 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, Δ133p53 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).



References: References 1. Lozano G and Levine AJ (Eds): The p53 Protein: From Cell Regulation to Cancer. New York. Cold Spring Harbor Laboratory Press, 2016. 2. Joruiz SM et al., Cold Spring Harb Perspect Med. 6 (Aug 2016). 3. Campisi J, Annu Rev Physio! 75 (2013) 685-705. 4. Charni M et al., Cell Death and Differ 24 (2017) 8- 14.

L9 p53 controls genomic stability and temporal differentiation of human neural stem cells and affects neural organisation in human brain organoids

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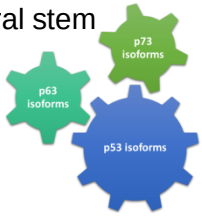
Keywords: p53, neural stem cells, brain organoids

The important role p53 play in repressing tumorigenesis is undeniably essential, however, its function during normal brain development is less understood. Interestingly, p53 is ubiquitously expressed throughout the whole mouse brain during early embryogenesis, and mouse models have shown that loss of p53 can in some instances lead to neural tube defects. However, little is known about the biological role of p53 in human brain development. In this study, we take advantage of human iPS cell-derived neural stem cells and brain organoids to study the role of p53 during human brain development. We knocked down (KD) p53 in human neuroepithelial-like stem (NES) cells derived from iPS cells. Upon p53 KD, NES cells show centrosome amplification and accumulation of aneuploid cells. Furthermore, we observed reduced proliferation rate, downregulation of genes important for oxidative phosphorylation, and a shift to glycolysis for their energy production. In addition, we observed a premature upregulation of neuronal differentiation genes in proliferating p53 KD NES. This was further potentiated during differentiation, p53 KD neurons display an increased pace of differentiating into immature neurons and exhibit a phenotype corresponding to more mature neurons. Using brain organoids, we modelled more specifically cortical neurogenesis. Here we found, in contrast to neural stem cells grown in monolayer, that p53 loss resulted in brain organoids with disorganized stem cell layer and reduced cortical progenitors and neurons. This demonstrates an important role for p53 in controlling genomic stability of neural stem cells and regulation of neuronal differentiation, as well as maintaining structural organization and proper neural stem cell function in human brain organoids.



L10 Roles of p63 during ectoderm specification

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1) Centro Andaluz de Biología del Desarrollo

Keywords: p63, ectoderm specification, gene regulation, pioneer factor

The transcription factor p63 is a master regulator of ectoderm development essential for epidermal specification. Although previous studies have highlighted the role of p63 triggering the epidermal differentiation program in several *in vitro* models, the mechanisms of target gene regulation by p63 in the complex context of a developing embryo remains poorly understood. Here, we used zebrafish embryos to analyze *in vivo* how p63 regulates the expression of its target genes during development. We generated *tp63* knock-out mutants that recapitulate human phenotypes and show down-regulated epidermal gene expression. Following p63-binding dynamics during development, we found two distinct functions clearly separated in space and time. During early development, p63 binds enhancers associated to neural genes, where it limits Sox3 binding and reduces the expression of these neural genes. Indeed, we show that p63 and Sox3 are co-expressed in the neural plate border. Later in development, p63 binds enhancers associated to epidermal genes and promotes their expression, acting as a pioneer factor, as it binds to non-accessible chromatin and is required for its opening. Therefore, our results suggest that p63 is an important regulator of cell fate decisions during ectoderm specification, promoting the epidermal fate and inhibiting the neural program.

L11 p53 isoform delta113p53 promotes zebrafish heart regeneration via maintaining ROS homeostasis

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Keywords: Δ113p53, Heart regeneration, Cardiomyocytes, Reactive oxygen species

Neonatal mice and adult zebrafish can fully regenerate their hearts through proliferation of pre-existing cardiomyocytes. Previous studies revealed that the p53 signal is activated during cardiac regeneration in neonatal mice, and hydrogen peroxide (H_2O_2) generated near the wound site acts as a novel signal to promote zebrafish heart regeneration. We recently demonstrated that the expression of the p53 isoform *Δ133p53* is highly induced upon the stresses of low-level reactive oxygen species (ROS) and coordinates with full-length p53 to promote cell survival via enhancing the expression of antioxidant genes. However, the function of the p53 signal in heart regeneration remains uncharacterized. Here, we find that the expression of *Δ113p53* is activated in cardiomyocytes at the resection site of zebrafish heart in a full-length p53- and ROS signal-dependent manner. Cell lineage tracing shows that Δ113p53 positive cardiomyocytes undergo cell proliferation and contribute to myocardium regeneration. More importantly, heart regeneration is impaired in *Δ113p53*$^{M/M}$ mutant zebrafish. The depletion of *Δ113p53* significantly decreases the proliferation frequency of cardiomyocytes, but has little effects on the activation and migration of *gata4* positive cells, as well as apoptotic activity. The live imaging of intact heart shows that the induction of H_2O_2 at the resection site is significantly higher in *Δ113p53*$^{M/M}$ mutants than in wild-type zebrafish, which may be resulted from the less induction of antioxidant genes in *Δ113p53*$^{M/M}$ mutants. Our finding demonstrates that the induction of *Δ113p53* in cardiomyocytes at the resection site functions to promote heart regeneration by increasing the expression of antioxidant genes to maintain redox homeostasis.

Fig.1.

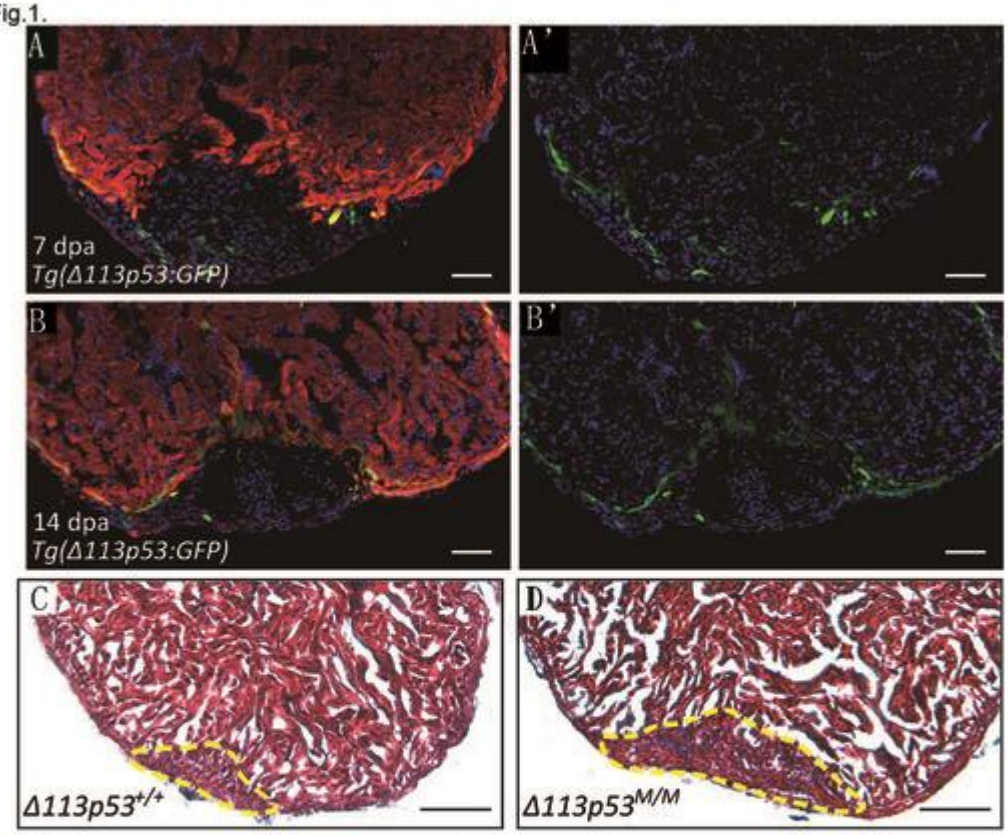
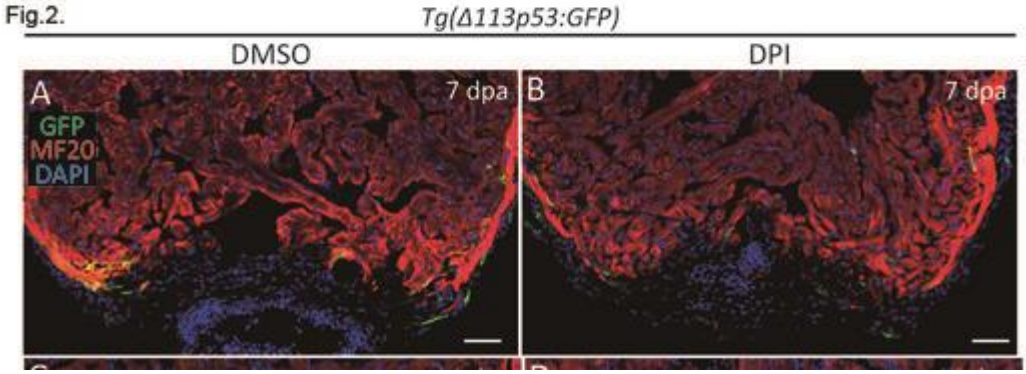


Fig.2.



Caption: Fig.1.Δ113p53 is induced to express in the cardiomyocytes at the resection site and contribute to myocardial regeneration. Fig.2.Activation of Δ113p53 depends on the elevation of H2O2 level and maintains ROS homeostasis.

L12 Transcription factor p63: guardian of the epithelial fate

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1) Radboud University

Mutations in transcription factor p63 are associated with developmental disorders that manifest defects in stratified epithelia including the epidermis. The underlying cellular and molecular mechanism is however not yet understood. We established an epidermal commitment model using human induced pluripotent stem cells (iPSCs) and characterized differentiation defects of iPSCs derived from ectrodactyly, ectodermal dysplasia, and cleft lip/palate (EEC) syndrome patients carrying p63 mutations. Transcriptome analyses revealed step-wise cell fate transitions during epidermal commitment: specification from multipotent simple epithelium to basal stratified epithelia, and ultimately to the mature epidermal fate. Differentiation defects of EEC iPSCs caused by p63 mutations occurred during the specification switch from the simple epithelium to the basal stratified epithelial fate. Single-cell transcriptome and pseudotime analyses of cell states identified mesodermal activation that was associated with the deviated commitment route of EEC iPSCs. Integrated analyses of differentially regulated genes and p63-dependent dynamic genomic enhancers during epidermal commitment suggest that p63 directly controls epidermal gene activation at the specification switch, and has an indirect effect on mesodermal gene repression. Importantly, inhibitors of mesodermal induction enhanced epidermal commitment of EEC iPSCs. Our findings demonstrate that p63 is required for specification of stratified epithelia, and that epidermal commitment defects caused by p63 mutations can be reversed by repressing mesodermal induction. This study provides insights into disease mechanisms underlying stratified epithelial defects caused by p63 mutations and suggests potential therapeutic strategies for the disease.

L13 p73 an Architect of Epithelial Tissue

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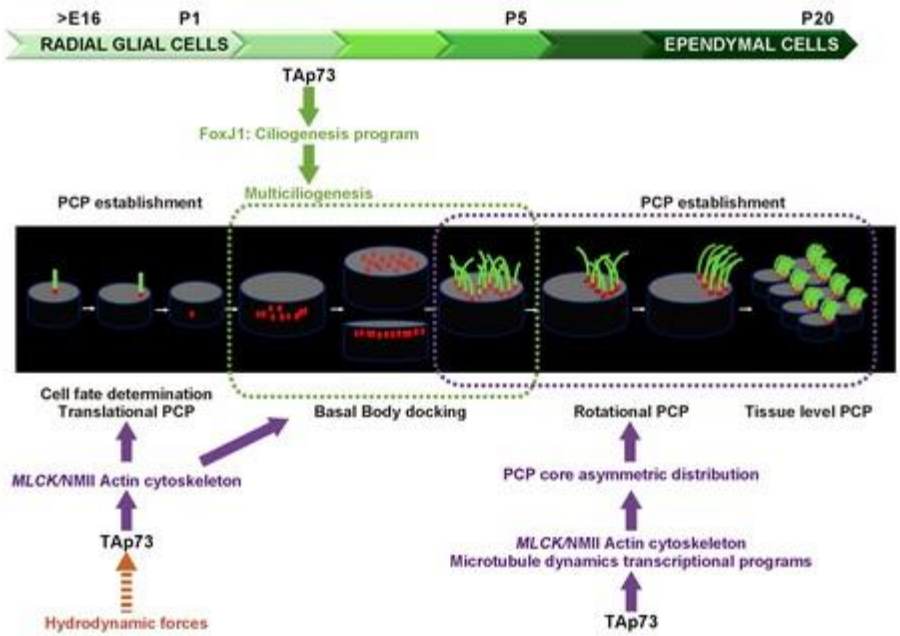
1) University of León

Intercellular junctional complexes and cellular polarity establish tissue structure and coordinated behaviors across epithelial sheets, setting up the basis of morphogenesis and tissual architecture. *Trp73* gene, like the other member of the p53 family of transcription factors, generate multiple isoforms named TA and DNp73, with different and, sometimes, antagonist functions. p73 is known to regulate many processes, like cell differentiation, cell metabolism or stem cell self-renewal. In fact, the *Trp73* null mice (p73KO) show multiple phenotypes like gastrointestinal and cranial hemorrhages, rhinitis or central nervous system defects. Our aim was to elucidate the common p73-regulated mechanism that underlies these defects and our working hypothesis is that p73 carry out an essential function in the establishment of the tissue architecture. To address this, we have analyzed the effect of p73-deficiency in different *in vitro* and *in vivo* models. In induced pluripotent stem cells, the lack of p73 lead to iPSC-clones with an impaired epithelial phenotype and altered stemness. A profound study of ependymal layer development and cytoarchitecture allowed us to identify a novel TAp73 function as an essential regulator of ciliogenesis and Planar cell Polarity (PCP). Moreover, p73 regulates PCP through the modulation of actin and microtubule cytoskeleton dynamics. Moreover, GO-analysis confirmed TAp73 role as a modulator of transcriptional programs regulating actin and microtubules dynamics, as well as cell-cell signaling pathways.



Caption: Trp73-deficiency affects ciliogenesis and the planar polarization of microtubule and actin networks resulting in lack of PCP and cilia disarrangement in Ependymal Cells.

References: Fuertes-Alvarez S. et al.,"p73 regulates ependymal planar cell polarity by modulating actin and microtubule cytoskeleton". Cell Death and Disease, 2018. Marta Martín-López et al., "p73 is required for appropriate BMP-induced mesenchymal-to-epithelial transition during somatic cell reprogramming. Cell Death and Disease 2017. Gonzalez-Cano et al.,"p73 is Required for Ependymal Cell Maturation and Neurogenic SVZ Cytoarchitecture" Developmental Neurobiology. 76(7):730-47. This research was supported by Grants SAF2015-71381-R from Spanish Ministerio de Economía y Competitividad cofinanced by FEDER funds (to MCM) and LE021P17 from Junta de Castilla y Leon, and from the Queen Elisabeth Medical Foundation to FT. MML and SFA were holders of predoctoral fellowships from the Junta de Castilla y León. LMA is supported by a predoctoral scholarship from the Asociación Española contra el Cáncer (AECC). JV is funded by a fellowship from the University of León. FT is a research Director of the FNRS. MW and ML are funded by the Deutsche Forschungsgemeinschaft (DFG) under grant number LI 2405/2.

L15 Overlapping transcriptional programs downstream of p63 and p73 promote cutaneous squamous cell carcinoma

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2) Radboud University Nijmegen

3) University of Lausanne

Keywords: squamous cell carcinoma, p63, p73, skin, EGFR

Aberrant expression of transcriptional regulators can affect oncogenic gene expression programs in cancer. Mutations in the tumor suppressor p53 are commonly found in UV damaged skin and are thought to protect damaged epidermal cells from senescence and or oncogene-induced apoptosis, favoring cancer formation. Here, we demonstrate that the p53 family members p63 and p73 are both frequently expressed at high levels in preneoplastic lesions and in cutaneous squamous cell carcinoma (cSCC). p63 and p73 form heterotetramers and jointly control a transcriptional program that promotes cell proliferation and tumorigenesis, at least in part by directly inducing several EGFR ligands. Treatment with EGFR ligands is sufficient to restore cell cycle progression in p63-depleted cells and to a lesser extent also in p73-depleted ones. The most highly expressed among these EGFR ligands is required to maintain SCC proliferative potential, anchorage independent growth, and to promote tumorigenesis. Thus, p63 and p73 collaborate as oncogenic drivers in cSCC by promoting cell proliferation through partially overlapping pathways.

L16 Macrophage diversity plays an essential role in development, repair and disease

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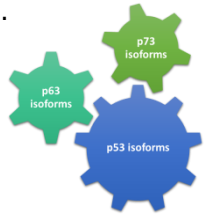
Macrophages are the most abundant cell type in the body, often accounting for 15% of the tissue. They have precise locations and territories in tissues and are often named according to their location. For example, Kupffer cells in the liver, microglia in the brain and Langerhans cells in the skin. They form a system that is akin to the nervous system extending through the entire body. It has been thought since the 1960's that all these cells in the adult arise from the bone marrow via a circulating monocytic progenitor population. However, recent studies have overthrown this concept and shown that many but not all populations arise from yolk sac derivatives and fetal liver progenitors and persist throughout life. Many of these macrophage populations are involved in development affecting processes such as branching morphogenesis and neural connectivity. They even control the patterning in Zebra fish! Furthermore these cells also help maintain homeostasis by detecting damage and repairing it as appropriate. This means that macrophages are also the first line in immune defenses detecting pathogen and responding directly or through activation of other immune cells particularly those of the acquired system. In fact, there is not a disease that macrophages do not play a part in usually beneficially but sometime exacerbating the disease such as in liver fibrosis.

Cancer is one of these diseases that is promoted by macrophages. We and others have shown that associated macrophages (TAMs) promote progression to malignancy. In this context TAMs stimulate angiogenesis, tumour cell motility and intravasation in the primary site while at distal sites another population of macrophage, metastasis associate macrophages (MAMs) promote extravasation, tumour cell survival and growth. TAMs are largely derived from circulating monocytes recruited by the tumour cells and then educated to differentiate into tumour promoting macrophages. Our studies have revealed mechanisms whereby these macrophages are recruited to the tumors and inhibition of these recruitment factors inhibits metastatic growth and also inhibits tumor progression. Thus macrophages become an important new target for cancer therapy in part through re-programming then to become anti-tumoral. Recent studies suggest that p53 may be involved in this reprogramming step.



L17 p53 status dictates pro-metastatic systemic inflammation in breast cancer

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Keywords: Metastasis, systemic inflammation, neutrophils, p53 loss, GEMMs

Metastasis remains the primary cause of death for breast cancer patients. The metastatic cascade is largely regulated by interactions between cancer cells and their microenvironment, both locally and systemically. Using a genetically engineered mouse model (GEMM) for breast cancer, we have previously shown that mammary tumors elicit a systemic inflammatory cascade, involving γδ T cells and neutrophils that promote metastasis. Breast cancer is a heterogeneous disease, and it is unknown whether and how the genetic make-up of primary tumors influences pro-metastatic inflammation. Therefore, we set out to dissect the cancer cell-intrinsic genetic events that activate systemic inflammation in breast cancer. Using a panel of 16 GEMMs for breast cancer with different tumor-initiating genetic modifications that recapitulate all subtypes of human breast cancer, we uncovered a role for cancer-cell-intrinsic p53 as a key regulator of pro-metastatic neutrophils. By combining GEMM tumor transcriptome profiling, in vitro mechanistic assays and in vivo intervention studies, we demonstrated that loss of p53 triggers systemic inflammation by WNT-dependent activation of macrophages. Pharmacological and genetic blockade of WNT secretion in p53-null cancer cells reverses macrophage activation and subsequent neutrophilic inflammation, resulting in reduced metastasis formation. In addition, comparison of p53-null tumors with those bearing p53 hotspot mutations reveal gain of immune-modulatory functions of specific p53 mutants. Together, these insights illustrate the importance of the genetic makeup of breast tumors in dictating pro-metastatic systemic inflammation, and set the stage for personalized immune intervention strategies for patients with cancer.

Reference: Wellenstein & De Visser, Immunity (2018) Coffelt et al., Nature (2015) Wellenstein et al., Nature (2019)

L18 Role of p53 in the interplay between host cell and pathogens

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1) Department of Molecular Biology Max Planck Institute for Infection Biology, Berlin, Germany

The epithelial surfaces are frequently affected by harsh encounters and it is thus not surprising that most cancers originate here. Several chronic bacterial infections have been implicated in human cancers, with Helicobacter pylori (Hp) representing the paradigm of a cancer-inducing bacterium. The pathology of Hp infections tends to follow a common program, starting with mild pathology that can progress towards active gastritis, gastric atrophy, intestinal metaplasia (GIM) and ultimately gastric adenocarcinoma in about 2% of cases. Intriguingly, GIM is usually cleared of Hp, suggesting this pathological state is not merely an intermediate stage towards cancer development, but can be viewed as a process of adaptive defense. This clearance of GIM from Hp has been attributed to differences in mucin synthesis (Muc2 instead of Muc5a/c and Muc6) and the altered glycosylation profile of the epithelium, where Hp is unable to survive. The changes are likely part of a mutational switch in gastric epithelium leading to GIM. Interestingly, the most frequent mutations in gastric cancer affect the central tumor suppressor protein p53, which has multiple functions and is subject to highly complex regulatory processes. Hp itself interferes with this protein's function via direct interaction with AKT/HDM2 and has been associated with accumulation of p53 mutations. Based on our recent observations we conclude that pathways inter-connected with p53 are involved in both Hp clearance from GIM and the GIM phenotype itself.

L19 Role of the p53 family in muscle atrophy associated with ALS and cancer

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1) Streinth Team, IRFAC, Inserm UMR_S1113, Strasbourg University, Strasbourg, France

Muscle atrophy is a co-morbidity cause of several human pathologies, such as cancers and degenerative diseases (ex. Amyotrophic Lateral Sclerosis; ALS). Mechanisms of muscle atrophy are complex and their understanding might help finding therapeutic solutions that are still lacking. Hence, to decipher the pathways involved in muscle atrophy we meta-analyzed transcriptomic experiments of muscles of ALS patients and ALS mouse models, uncovering a p53 deregulation as common denominator. We then characterized the induction of several p53 family members (p53, p63, p73) and a correlation between the levels of p53 family target genes and the severity of muscle atrophy in ALS patients and mice. In particular, we observed increased p63 protein levels in the fibers of atrophic muscles via denervation-dependent and -independent mechanisms. At a functional level, we demonstrated that TAp63 and p53 transactivate the promoter and increased the expression of *Trim63* (MuRF1), an effector of muscle atrophy.

To assess whether this p63/*Trim63* cascade is common to other pathological contexts impacting the muscles, we performed a transcriptomic experiment on an animal model of cancer-related muscle atrophy. Bioinformatics pathway analyses showed that deregulation of p63 activity was also observed. Using a TAp63 reporter mice we showed that TAp63 was induced in atrophic muscle and that TAp63 was involved in the regulation of *trim63*. In addition, we uncovered a functional interaction between p63 and the mechanotransduction HIPPO/YAP pathway in the regulation of *trim63* expression, which can be due to alteration of cell/cell interaction processes associated with muscular atrophy. Altogether, these results suggest a novel function for p63 as a contributor to muscular atrophic and plasticity processes *via* the regulation of multiple genes, including the muscle atrophy gene *Trim63*

L20 Elevated levels of Delta133p53beta isoform are found in Rheumatoid Arthritis patients with hyperproliferative synovium and exaggerated immune cell infiltration

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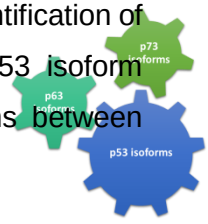
Keywords: Rheumatoid arthritis, inflammation, p53beta isoforms

Rheumatoid arthritis (RA) is a systemic autoimmune disease (AD) characterised by chronic inflammation resulting in destructive, debilitating arthritis affecting synovial joints initially, followed by multi-organ involvement. While its aetiology is unknown, environmental factors and/or biological factors (viral, hormonal) are presumed to trigger development of the disease.

Recent studies emphasizing the role of p53 in immune system modulation suggest that dysregulation in the delicate balancing act of co-expressed p53 isoforms leads to disease characterised by “wounds that never heal”, such as cancer and AD. Our mouse model of the Δ133p53 isoform **(**Δ122p53) is predisposed to developing lymphoma and has increased production of autoantibodies and pro-inflammatory cytokines reminiscent of an autoimmune phenotype, features of which are commonly seen in patients with RA.

To ascertain a role for Δ133p53 in the pathogenesis of human RA we analysed synovial joint tissue from a cohort of 32 RA patients and compared this with 20 osteoarthritis (OA) patients. Δ133p53 and other p53 isoform mRNA levels were determined by RTqPCR or digital droplet PCR (ddPCR). Cells expressing Δ133p53ß mRNA were also identified by RNAScope and by immunohistochemistry (IHC) using p53ß and Δ133p53αβγ specific antibodies. Immune cell infiltrate was identified by antibodies to CD20 (B cells), CD3 (T cells) and CD68 (macrophages). Three histopathological groups were evident in RA: (**1**) Lymphoid: distinct B cell follicle like structures (FLS); (**2)** Myeloid: predominantly macrophages, fewer immune cells displaying a diffuse pattern and no FLS; and (**3)** Fibroid: fibroblastic and pauci-immune. Semi-quantification of the immune cell infiltrate and the extent of proliferative synovium, along with p53 isoform expression data, allowed clustering algorithms to be used to look for associations between



isoform expression and these biological parameters. Results showed that RA Group 1 has more proliferative synovium, higher levels of Δ133p53ß mRNA in synoviocytes, endothelial cells, and in cells within and near FLS. Strong immunostaining for p53ß was found in a subset of cells that co-expressed CD90, a marker of 'activated' fibroblasts. These cells are found in the sub-lining of the synovium in RA and around FLS, and are thought to contribute to aggressive pathology and outcome in RA.

We propose that the function of Δ133p53 and/or p53 is in the recruitment of inflammatory cells, which if not controlled, contribute to aggressive pathology.

L21 Pan-cancer analysis reveals alternate mechanisms for AKT activation through TAp63 regulated oncogenic lncRNAs (TROLLs)

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1) Moffitt Cancer Center

Keywords: p53 family, lncRNA, AKT, PTEN, metastatic carcnima

Metastatic breast cancer is characterised by p53 mutations and activation of the PI3K/AKT pathway, two events responsible for the progression of this disease. Mutations in *TP53* lead to the inhibition of the tumour and metastasis suppressor *TAp63*, a p53 family member. By using the *TAp63* metastatic mammary adenocarcinoma mouse model and performing a mouse-human cross species analysis using models of human breast cancer progression, we identified two TAp63 regulated oncogenic lncRNAs or “TROLLs”. We unveiled via a pan-cancer analysis of human cancers and mouse models of breast cancer progression, that these two lncRNAs induce a PTEN-independent pathway of AKT activation to promote cancer progression by regulating the nuclear to cytoplasmic translocation of their effector, WDR26. Our data provide the preclinical rationale for the evaluation of these lncRNAs and WDR26 as novel therapeutic targets for the treatment of human tumours addicted to mutant p53 and the AKT pathway.

L22 Mechanism of inhibition and activation of TAp63α in oocytes

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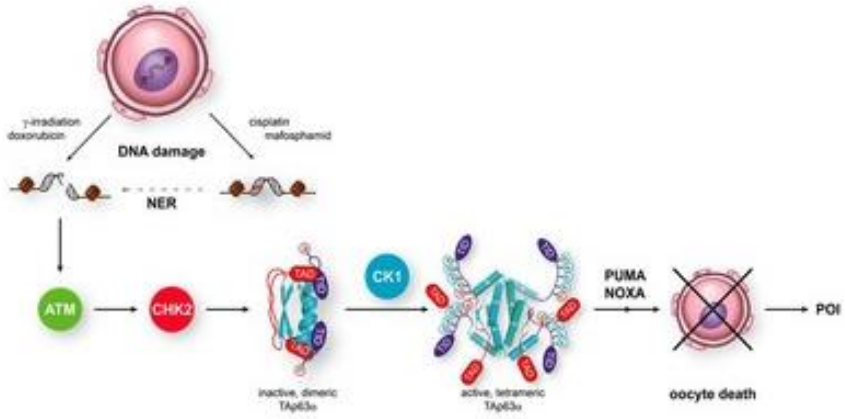
Keywords: TAp63a, oocyte, apoptosis, quality control, chemotherapy

We are investigating the mechanism by which the interaction between two domains that are unfolded in isolation gain structure and regulate the transcriptional activity of p63. This protein is expressed in high concentration in oocytes where it serves as a quality control factor. The C-terminal inhibitory domain interacts with the N-terminal transactivation domain as well as with the central oligomerization domain and keeps the protein in a dimeric, closed conformation by forming a β-sheet that blocks the tetramerization interface. The DNA binding affinity and the transactivation potential is strongly reduced in this inhibited conformation, explaining why the protein can accumulate to relatively high concentrations without initiating apoptosis in oocytes. This dimeric and closed conformation is formed co-translationally and forms a kinetically trapped meta-stable state. We have used a combination of many methods including NMR and SAXS to build a structural model that explains the tight regulation of the TAp63α and provide a structural mechanism of the phosphorylation driven activation process.



Mechanism of activation of TAp63a in oocytes.

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L23 Germline variant affecting p53β isoforms predisposes to various familial cancers

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Family history is a major risk factor for the development of many types of cancer, however genetic causes, underlying the familial aggregation of disease, are often not identified. Li-Fraumeni syndrome is a severe hereditary syndrome, which predisposes to a variety of early-onset cancers and is caused by pathogenic heterozygous mutations affecting the canonical isoform of the *TP53* gene, p53α. The clinical significance of genetic variants affecting the non-canonical p53 isoforms, p53β and p53γ, remains unknown. In search for novel cancer-predisposing variants, we identified a heterozygous germline variant affecting the p53β isoform, which was associated with an increased cancer risk.

We identified this variant in 27 individuals from four unrelated families, frequently diagnosed with various and multiple cancers per individual, especially colorectal, breast and thyroid cancer. This variant was absent or very rare in population databases and local cohorts. Somatic mutation analysis showed maintenance of this variant in 90% of the investigated neoplasia. Patient-derived B-lymphocyte cells showed increased mRNA expression of the *TP53*β isoform. Expression of the mutant isoform was detected at both the mRNA and protein level, using PCR-based approaches and a mutant-specific antibody. Based on the predicted secondary protein structure, the variant affected p53 oligomerization and thereby dysregulated

the expression of p53 target genes. This was supported by (i) co-immunoprecipitation, (ii) luciferase gene reporter assays, and (iii) altered expression of p53 target genes in patient-derived B-cells and transduced cell lines.

In conclusion, we are the first to show clinical, expression and functional data supporting the oncogenic effect of a variant affecting the p53β isoform. Phenotypic differences indicates a milder phenotype compared to classic Li-Fraumeni syndrome. Our work demonstrates the necessity of considering variants outside the canonical coding sequence, such as p53β, when investigating cancer predisposition in families and young individuals.

L24 Impact of p63 upregulation on MAPK inhibitors resistance in melanoma

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Keywords: BRAF, TP63, FBXW7, melanoma, MAPK inhbitors-resistance

MAPK pathway inhibition by combined use of BRAF and MEK inhibitors increases overall survival in advanced melanoma. Although these targeted drugs induce responses in most BRAF-mutant patients, a significant proportion acquire resistance to therapy and subsequently relapse. In recent years several mechanisms underlying acquired resistance have been identified. We have previously shown an important role for P63 as an inhibitor of p53-induced apoptosis in melanoma following genotoxic drug exposure. Here we investigate a possible role for p63 in acquired resistance to MAPK inhibition. We demonstrate significant correlation between p63 expression and BRAF/NRAS mutational status. We further show increased expression of p63 isoforms at mRNA and protein levels in melanoma cell lines chronically exposed to BRAF and MEK inhibition. We identify a mechanism by which the E3 ubiquitin ligase, FBXW7, controls p63 protein stability in MAPK inhibitor-resistant melanoma: resistant cell lines harbour reduced FBXW7 and enrichment of nuclear MDM2 and, consistent with this, FBXW7 inactivating mutations and MDM2 upregulation are observed in melanoma clinical samples. We provide evidence for a physiological interaction between MDM2 and FBXW7 which could explain these findings. Evidence for potential therapeutic relevance is provided by effects of the MDM2 inhibitor, Nutlin-3A; this restores FBXW7 expression and p63 degradation in a dose-dependent manner and renders MAPK inhibitor-resistant melanoma cells more

sensitive to apoptosis. Nutlin-3A may therefore increase the activity and overcome acquired resistance to MAPK inhibitors in melanoma therapy.

References: Funding support was provided by British Skin Foundation, and Barts and The London Charity. The authors declare no competing financial interests. We are grateful to Dr Gary Warnes, Flow Cytometry Core Facility Manager, for technical advice, to Rebecca Carroll and Laura Neal for the assistance with the tissue samples immunostaining and Dr Belen Martin for the technical support with fluorescence microscopy.

L25 Thalidomide teratogenicity uncovered- the central role of p63 and CRBN

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Keywords: p63, thalidomide, CRBN, teratogenicity

Severe developmental malformations were detected in human fetus in the 1950s when women used the anti-nausea and sedative drug Thalidomide in the first trimester of pregnancy[1]. The molecular mechanisms underlying Thalidomide teratogenicity were unknown after almost 6o years from its withdrawal from the market. Further studies revealed new potential therapeutic activities of thalidomide and its derivatives, leading to the approval of thalidomide for the treatment of leprosy and multiple myeloma. Cereblon (CRBN) is the primary target of thalidomide teratogenicity[2]. CRBN functions as a substrate receptor of the E3 ubiquitin ligase CRL4. Thalidomide and other ligands modulate substrate specificity of $CRL4^{CRBN}$, but the substrate responsible for thalidomide teratogenicity was unknown.

The striking similarities between the phenotypic abnormalities of babies born from mother exposed to thalidomide during pregnancy and patients affected by syndromes associated with mutations in the p63 gene, prompted us to verify whether p63 could be the substrate responsible for Thalidomide teratogenicity. Here we show that TAp63□ and □Np63□ proteins are degraded by thalidomide by the CRBN E3 ubiquitin ligase complex. Thalidomide initiates its teratogenic effects by binding to CRBN and this enhances p63/CRBN interaction resulting in p63 degradation both *in vitro* and *in vivo*. Using a zebrafish model, we demonstrate that thalidomide exerts its teratogenic effects on pectoral fins and otic vesicle by binding to CRBN and inducing the degradation of zΔNp63α and zTAp63α, respectively[3]. The results shed new light on the molecular mechanisms of thalidomide teratogenicity and may contribute to the development of new thalidomide derivatives without teratogenic activity.

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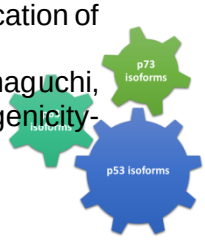

L26 Impact of p53 isoforms over-expression in human lung cancer cells

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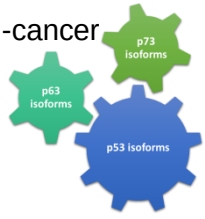
Keywords: p53 isoforms, chemotherapy, apoptosis, cell cycle, migration

Based on the data gathered over the past years by several laboratories using different human cell lines and animal models, we can now consider the array of p53 isoforms as a set of physiologically active and potent proteins. These data highlight that a p53-mediated cell activity is not driven by a single protein, the canonical FLp53 (or p53□), but is in fact the sum of the activities of the co-expressed p53 isoforms in a given tissue. The deregulation of p53 variants expression considerably affects cell homeostasis in its most crucial pathways as cell-cycle progression, programmed cell death, senescence, inflammation, stem-cell renewal, differentiation, and aging, and, in turn, leads to the onset of cancer and other diseases. Moreover, a key feature of the p53 protein is its ability to oligomerize forming homo- or hetero-tetramers, and each combination with single p53 isoform can have its unique impact.
In order to better unveil the role of p53 shorter isoforms in cancer biology and response to anti-cancer therapies, we generated a panel of stable clones from H1299 (p53 null) and A549 (wild-type p53) lung cancer-derived cells over-expressing Δ40p53α/β/γ, Δ133p53α/β/γ and Δ133p53α/β/γ isoforms or an empty vector as a control.

Using different assays such as viability (i.e., MTT), WB, qPCR, migration, proliferation and reporter assays we demonstrated that p53 shorter isoforms can negatively influence the functions of full-length p53□ protein. In particular, □40p53□ and □133p53□ can reduce the sensitivity of A549 cells to different chemotherapeutic drugs such as Doxorubicin, Cisplatin and Oxaliplatin, but not 5-Fluorouracil. Moreover, they can inhibit the ability of p53□ to increase the expression of p21 or BAK, and to induce apoptosis and cell cycle arrest.

Further, most of p53 shorter isoforms are able to enhance migration potential of A549 cells, while only □40p53□ and □133p53□ isoforms can favor proliferation.

Taken collectively, this study indicates that p53 shorter isoforms can influence and shape the processes regulated by p53, and a better knowledge of their specific functions or their expression levels in cancer cells can pave the way to increase the effectiveness of anti-cancer therapies.



L27 Tissue-specific tumor suppressor functions of p53 in vivo

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Keywords: p53, apoptosis, gene expression, tissue-specific, radiosensitivity

The p53 tumor suppressor mediates cellular responses to DNA damage including genotoxic stress triggered by Xray. Previous studies have implicated p53 as a key determinant of tissue-specific radiosensitivity in vivo. Indeed, whole body X-radiation of mice is shown to trigger an apoptotic response in the thymus and spleen, but not in the liver. The *Cdkn1a* gene encoding the cyclin-dependent kinase inhibitor p21 is upregulated in all tissues, whereas the apoptotic targets *Bbc3* (Puma), *Pmaip1* (Noxa), and *Bax* are selectively upregulated only in thymus and spleen, but not liver. Global gene expression analysis using RNA-seq demonstrates that there are both shared and tissue-specific genes that are upregulated in response to X-radiation in a p53-dependent manner. These include both protein-coding mRNA as well as long non-coding RNA. Identification as a p53 target gene was confirmed by ChIP-seq analyses. These studies confirm that the apoptotic targets are upregulated in thymus and spleen, but not liver. Intriguingly, robust gene occupancy by p53 can be detected in all tissues, including liver. This indicates that the failure to upregulate in liver occurs at a step subsequent to DNA binding. As the C-terminal 24 amino acids have been implicated in transcriptional co-factor binding, the radiation response in genetically engineered mice with a deletion in the endogenous p53 gene corresponding to this domain was examined. Surprisingly, deletion of the C-terminus confers p53-dependent upregulation of apoptotic genes in the liver. Thus, it is proposed that liver p53 interacts with a repressor protein via its C-terminus thereby preventing apoptotic gene activation in that tissue. One of the p53-dependent genes that is upregulated in a liver-specific manner, and not in thymus or spleen, is *Dnajb9*. The basis for the liver-specific gene regulation is at the level of gene occupancy as ChIP assays do not show binding by thymus or spleen p53. The Dnajb9 protein has previously been shown to exert anti-apoptotic effects. Taken together, the lack of radiation-induced apoptosis in the liver is likely due to two tissue-specific mechanisms: interference with upregulation of apoptotic targets mediated by the C-terminus of p53, and liver-specific occupancy by p53 on the *Dnajb9* gene, leading to suppression of an apoptotic response.

L28 DeltaNp73 expression impacts on stem-like cell properties of acute myeloid leukemia and hepatocellular carcinoma tumors

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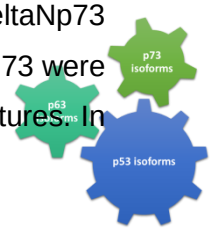
1) CRCL, INSERM U1052

2) HCL

Keywords: p53 family, DeltaNp73, Cancer Stem Cells, Acute Myeloid Leukemia, Hepatocellular Carcinoma

The p53 family plays an important role in carcinogenesis and cell stemness. *TP53*, *TP63* and *TP73* encode full-length (TA) and truncated (DeltaN, DeltaTA) isoforms. These latter have been found implicated in the maintenance of the stem cells pool of many tissues, as well as in the presence of cancer stem cells (CSCs) in tumors, such as breast carcinoma or acute promyelocytic leukemia. Acute myeloid leukemia (AML) and hepatocellular carcinoma (HCC) are two tumor types for which CSCs are thought to be responsible for radio-chemotherapeutic resistance and relapse. Thus, we investigated whether the truncated isoforms, in particular the DeltaTAp73s, could influence the presence of CSCs in these tumor types. In AML, we observed a correlation between the expression of BMPR1A receptor and DeltaNp73 in patients' samples at diagnosis. We then demonstrated that BMP4 activated the expression of DeltaNp73 through its binding to BMPR1A. DeltaNp73 in turn induced NANOG expression. This resulted in an increase of stem-like features in leukemic cells, as shown by ALDH activity and functional assays. In patients, high expression of either BMPR1A, DeltaNp73 or NANOG at diagnosis was associated with an increased rate of relapse (<3 years post-diagnosis). Interestingly, combining the three markers led to an increase in the clinical predictability for AML patient outcome at diagnosis, by identifying patients with a higher risk of relapse (33 and 86% risk of relapse in the low-expression- and the high expression group, respectively). In HCC, we analyzed the expression of p73 isoforms in 170 pairs of HCC tumors and corresponding peritumors. We also used liver cell lines to assess the consequences of DeltaNp73 expression on stem-cell properties. We showed that the expression of DeltaNp73 modulated those of some stemness factors. In addition, cells overexpressing DeltaNp73 were more prone to form large colonies and hepatospheres, two stem cell-associated features. In



HCC samples, the DeltaTAp73 isoforms were found to be overexpressed in more than 50% of HCC tumors. This overexpression was significantly associated with those of NANOG, SOX2 and OCT4. DeltaNp73, but not the other truncated isoforms, was also found to have a significant impact on patient outcome. Indeed, in multivariate analysis, DeltaNp73 was a prognostic factor for early recurrence (<2 years post surgery). In conclusion, in AML patients' samples, we identified DeltaNp73 as a new partner of a signaling cascade linking tumor environment alterations (increased concentration of BMP4), NANOG expression and stem-cell properties in a sub-population of leukemic cells, which could be responsible for resistance to treatment and relapse (Voëltzel *et al*., 2018, PMID: 30262802). We confirmed the association of DeltaNp73 expression and stem-cell features in HCC patients' samples. Altogether, our results show that DeltaNp73 is associated with stem-cell features, early relapse and therefore poor patients' outcome.

L29 DNA DAMAGE-INDUCED ALTERNATIVE SPLICING OF P53

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TP53 is the most commonly mutated gene in human cancers and levels of p53 protein increase following stress exposure, modulating cell cycle progression, cell death, and cellular senescence. A central dogma of the p53 field has been that the induction of p53 after DNA damage results from a transient increase in the half-life of p53 protein. In recent years, our laboratory demonstrated that this long-standing dogma is an incomplete picture of p53 regulation by uncovering a critical role for protein translational regulation in p53 induction after DNA damage (Takagi et al, Cell, 2005; Chen and Kastan, G&D, 2010; Chen et al, JBC, 2012). These investigations led to the discovery of a DNA damage-induced alternative splicing (AS) pathway that affects p53 and other gene products (Chen et al, Cancer Discovery, 2017). This new stress-induced alternative splicing pathway regulates induction of cellular senescence markers after DNA damage, thus expanding the repertoire of gene products that modulate cellular stress responses. This AS pathway includes damage-induced inhibition of the PIKK kinase, SMG1, and a site-specific, RPL26-dependent recruitment of the SRSF7 splicing factor to p53 pre-mRNA. The damage-induced AS of p53 RNA results in the generation of p53β RNA and protein, which, in turn, is specifically required for induction of cellular senescence markers after ionizing irradiation (IR). Over 10 different alternatively spliced forms of human p53 have been described, but definitive physiologic roles or mechanistic insights about these isoforms had been generally lacking until this linkage of p53β to IR-induced cellular senescence. Cellular models and reagents that enable differential regulation of full-length p53 and p53β suggest functional divergence between these two forms of p53 protein (apoptosis versus senescence, respectively). These two proteins differ in their carboxy-terminal domains, with replacement of the oligomerization domain of FLp53 protein with a unique 10 amino acid tail in p53β protein. Current and future studies focus on distinctions in the genomic DNA binding sites, protein interactome, induced transcriptome, and post-translational modifications of FLp53 versus p53β proteins.

L30 Targeting the p53-Rbm38 Loop for Tumor Suppression

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Keywords: Translational Regulation, Rbm38, eIF4E

p53 is activated upon exposure to a stress signal and induces an array of genes for growth suppression. Among these is Rbm38, a RNA-binding protein. Interestingly, Rbm38 interacts with eIF4E on the p53 mRNA and represses p53 mRNA translation by preventing eIF4E from binding to the p53 m7G cap. Thus, the mutual regulation between p53 and Rbm38 constitutes a feedback loop. Interestingly, Rbm38 mutations and amplification occur in multiple cancers and are correlated with poor prognosis and tumor progression. Additionally, Rbm38 phosphorylation at serine-195 disrupt its interaction with eIF4E, abrogate the ability of Rbm38 to inhibit the binding of eIF4E to the p53 m7G cap, and consequently convert Rbm38 from a repressor to an activator of p53 translation. Therefore, disrupting the Rbm38-eIF4E complex to enhance p53 expression may be explored as a novel therapeutic option for cancers that carry wild-type p53. To relieve Rbm38 repression of p53, peptides corresponding to the binding interface between Rbm38 and eIF4E, including an 8 amino acid peptide (Pep8) derived from Rbm38, were generated and showed to be effective in disrupting the Rbm38-eIF4E complex. Importantly, Pep8 alone, or together with a low dose of doxorubicin, was highly potent to induce p53 expression, leading to inhibition of colony/tumor sphere formation *in vitro* and suppression of xenograft tumors *in vivo* in Rbm38- and p53-dependent manners. Together, this novel approach may be explored as a therapeutic strategy for cancers that carry wild-type p53.

L31 In the loop with p53: isoforms and cancer

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Keywords: p53, p53 regulation, p53 interactors, cancer

Cancer prevention requires appropriate responses to genomic insults. A major orchestrator of these responses is the tumour suppressor p53. Intriguingly, p53 activities appear to be dictated by their cellular context. This implies that the molecular milieu in which p53 operates influences its potency. Additionally, the functional efficiency of p53 depends on its mutational state. We have defined vital pathways of regulation that are context dependent and critically fate determining for p53 tumour suppression capabilities.

L32 Development of a mutant p53-dependent novel cancer therapy

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In the last couple of years, we focused on the development of a p53 based therapy. This consist on the development of small peptides that can re-activate mutant p53 that has oncogenic activity into a wild type p53 that serves as the guardian of the genome and thus induce death of cancer cells.

In our studies regarding the activity of p53 small peptides that were shown to reactivate mutant p53, by conformational modulations into a functional wild type p53 that is capable to apoptosis. To that end we are improving and modulating the small peptides to induce higher activity by increasing their cell penetrance. Both in vitro and in vivo experiments using human cell lines, yielded significant augmentation of cell death in vitro and tumor rejection in vivo. In recent experiments we could clearly show that that these activities are specific for cancer cells rather that for in-vitro growing non-cancer cells.

As ovarian cancers express a high incidence of p53 mutations, we will consider these human carrying such tumors as primary targets for our novel mutant p53 based therapy.

L33 The landscape of p53 isoforms in normal blood cells and acute leukaemia

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Keywords: haematopoiesis, cancer, therapy, signal transduction

The blood and the bone marrow constitute a complex organ in terms of cell population control and regenerative capacity, were regulation of p53 play an important role. The additional dimension of isoforms of p53 provide the blood system with a regulatory system for a wide range of functions, from the dramatical cell death induction to finely tuned isoform modulations in differentiation and aging of cells. This is also evident in neoplastic development and malignancies deriving from hematopoietic and mesenchymal stem cells.

Varying cell types of the blood comprise widely different levels of p53 protein. Further, the isoform expression profile is also different. Age and sex seem to affect the general state of p53 in white blood cells, likely reflecting altered control mechanisms of p53. The question that raises is when and how leukocyte p53 is responding to physiological and pathological exposure.

Neoplastic myeloid disorders are frequently involving p53. Myelodysplasia, characterized by clonality and cytopenia, has frequently mutated TP53. Myeloproliferative diseases, like chronic myeloid leukaemia, has recurrent mutations that involve regulation of p53. The more aggressive blood cancers, like acute myeloid leukaemia has mutations in TP53 in 10% of the cases.

Analysis of p53 protein and p53 induced genes in patients with acute myeloid leukaemia indicate that the majority of early genes activated are involved in p53 regulation. This underscores the necessity of functional p53 for optimal response to chemotherapy. Further, the p53 pathophysiology in leukaemia provide a blueprint of how novel therapy and diagnostics may be used to optimize the therapy results and lengthen survival in relapsed and refractory disease. Together, p53 and its isoforms appear as a bar code for disease stratification. Using normal blood cells and leukaemia as models, we question if p53 is a nexus for therapy and diagnostics, integrating the multitude of mutations and epigenetic alternations of cancer.

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L34 Rescue of missense and nonsense mutant p53: from bench to bedside

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Keywords: TP53, missense mutation, APR-246, nonsense mutation, translational readthrough

The frequent mutation of the TP53 tumor suppressor gene in human tumors has stimulated efforts to reactivate mutant p53 as a therapeutic strategy. We have previously discovered the small molecules PRIMA-1 and APR-246 (PRIMA-1Met) that restore wild type function to missense mutant p53. Both compounds are converted to MQ, a Michael acceptor that binds covalently to p53 cysteines including Cys277. APR-246 synergizes with chemotherapeutic drugs such as cisplatin and various other therapeutic agents. Moreover, APR-246 targets redox homeostasis by inhibiting thioredoxin reductase, thioredoxin and glutaredoxin, and depleting cellular glutathione (GSH) via MQ, which presumably contributes to APR-246-induced tumor cell death. APR-246 is tested in several clinical trials, including 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. Aminoglycoside antibiotics can induce translational readthrough of R213X nonsense mutant TP53 in tumor cells and expression of full length p53. Our screening of chemical libraries identified novel candidate compounds that are now being validated for translational readthrough activity. Induction of translational readthrough is a promising strategy for improved treatment of tumors with nonsense mutant TP53, and possibly other tumor suppressor genes with nonsense mutations, e.g. RB1, APC and PTEN.

L35 Drug repositioning to target p73 for improved cancer therapy

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1) Karolinska Institute

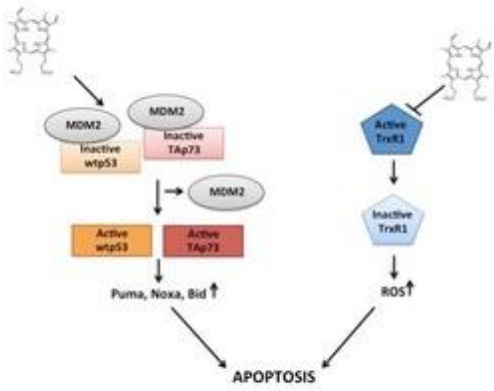
Keywords: drug repositioning, p73, thioredoxin reductase

Drug repositioning is an approach that strives at reusing existing drugs for new indications. It brings hope for the improved treatment of cancer patients by addressing the financial and treatment-related toxicity of current cancer care. There are multiple advantages of repurposing when compared with classical drug discovery route. Paramountly, there is a large amount of existing information to amass from including drug dosage and toxicity profiles.

The *T*p73 is expressed in two distinct isoforms: TA isoforms that function as tumor suppressors and ΔN isoforms recognized as oncogenes. ΔN isoforms are often overexpressed in cancers, bind to TA proteins and p53, and inhibit their function, thus acting as dominant-negative towards p53, p73, and p63. I strive to reinstate TA isoforms in cancer cells. In particular, my major interest is on tumors of poor clinical outcomes namely lung and pancreatic cancers which harbor *T*p53 mutations.

In drug-repurposing approach, it has been shown that small-molecule protoporphyrin IX (PpIX), a metabolite of aminolevulinic acid (ALA), a pro-drug applied to treat actinic keratosis, activates TAp73 and induces apoptosis in cancer cells in cells deficient for p53. Next, it was demonstrated that PpIX inhibits TAp73/MDM2 and TAp73/MDMX interactions. PpIX stabilizes TAp73 on protein levels in cells and *in vivo* (1). An analog of PpIX, verteporfin (benzoporphyrin derivative), a compound used to treat age-related macular degeneration activates p73 and inhibits thioredoxin reductase (TrxR), an oncogene and a key player in the defense of cancers against oxidative damage (2). In addition, to p73, PpIX potently activates wild-type p53 via inhibiting p53/MDM2 and p53/MDM4(X) interactions (3).

The findings have significant clinical relevance in that they may accelerate the repurposing of verteporfin and ALA/PpIX in oncology for improved therapy of cancers which bear *T*p53 gene mutations.



Protoporphyrin IX activates Tp73 and inhibits thioredoxin reductase in cancer cells.

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L36 Targeting MDM proteins in wild type and mutant p53 cancers

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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*. We targeted MDM4 using stapled peptide, inhibition of splicing, and *MDM4* transcription. We have also 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.

L37 p53 family isoforms and mutations in response to microenvironmental stressors

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The tumour suppressor protein p53, cooperated by its family members p63 and p73, has an essential role in the response to toxic injury. Somatic cells largely rely on p53 to overcome genotoxic stress and to maintain genomic integrity. Inactivation of p53 is indeed considered the “point of no return” for genomic instability.

In addition to the *canonical* p53 control of cell cycle arrest/apoptosis, recent evidence indicates that upon cellular stress p53 coordinates highly diverse processes, such as cellular metabolism, redox homeostasis, and inter-cellular communication and interaction with the external micro-environment. Hence, p53 is a critical factor in maintaining cellular homeostasis following a wide range of (micro)-environmental insults.

I will discuss the contribution of p53 family to the cellular response to microenvironmental stressors such oxidative stress and hypoxia, interactions with the cellular epigenome and the potential implications for acquisition of aggressive phenotype and genomic instability.

L38 Functional genomics identifies a novel p53 induced ligand-independent TRAIL-R2/FLIP complex as a novel therapeutic vulnerability

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2)

3) NUIG

Keywords: TP53, Apoptosis, TRAILR2, Caspase-8, HDAC

How p53 differentially activates cell cycle arrest versus cell death remains poorly understood. Here, we demonstrate that upregulation of canonical pro-apoptotic p53 target genes in colon cancer cells imposes a critical dependence on the long splice form of the caspase-8 regulator FLIP (FLIP(L)), which we identify as a direct p53 transcriptional target. Inhibiting FLIP(L) expression with siRNA or Class-I HDAC inhibitors promotes apoptosis in response to p53 activation by the MDM2 inhibitor Nutlin-3A, which otherwise predominantly induces cell-cycle arrest. When FLIP(L) upregulation is inhibited, apoptosis is induced in response to p53 activation via a novel ligand-independent TRAIL-R2/caspase-8 complex, which, by activating BID, induces mitochondrial-mediated apoptosis. Notably, FLIP(L) depletion inhibits p53-induced expression of the cell cycle regulator p21 and enhances p53-mediated upregulation of PUMA, with the latter activating mitochondrial-mediated apoptosis in FLIP(L)-depleted, Nutlin-3A-treated cells lacking TRAIL-R2/caspase-8. Thus, we report two previously undescribed, novel FLIP(L)-dependent mechanisms that determine cell fate following p53 activation.

Oral presentations

OP1 Dissecting the binding landscape and gene regulatory network of p63 and p53

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Keywords: p63, p53, gene regulatory network, binding landscape, meta-analysis

In contrast to the tumor suppressor p53 with its well-studied set of target genes, the gene regulatory network (GRN) of its phylogenetically ancient sibling p63 has been less well investigated to data. While multiple genome-wide p63 gene expression data sets have been generated in recent years, the target genes identified from discrete studies varies significantly and a robust p63 GRN has yet to be defined. Using a recently developed meta-analysis approach, that overcomes the limitation of individual studies by ranking of potential p63 target genes based on the number of data sets supporting a p63-dependent regulation. We find that genes commonly up-regulated by ΔNp63 are significantly enriched for sets of genes involved in cell cycle, E2F,DREAM, MYC target genes as well as mTORC1 signalling genes. In contrast, genes down-regulated by ΔNp63 enrich gene sets associated with oxidative phosphorylation, interferon response and epithelial mesenchymal transition. Most importantly, we find that ΔNp63-dependent gene regulation exhibits significant positive correlation with gene expression in squamous cell carcinomas (SCC), which underscores the oncogenic role of ΔNp63 in SCC. While early reports suggested that ΔNp63 interferes with target gene up-regulation by p53, our comparison of p63- and p53-dependent gene regulation shows that that ΔNp63 does not commonly interfere with target gene up-regulation by p53 and regulates largely distinct gene sets. Utilized the wealth of recent p63 and p53 ChIP-seq studies , we further establish a more precise global distinction between p53 and p63 binding sites and their underlying response elements (REs) and demonstrate that that p63 regulates many, if not most, of its target genes through enhancers

OP2 Determinants of cis-regulatory activity for the p53 family of transcription factors

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Keywords: enhancers, chromatin, p53, p63, transcription

Transcription factors in the p53 family perform diverse cellular functions across metazoan organisms, from safeguarding germ cell genome fidelity and tumor suppression to specification and maintenance of epithelial cell identity. The founding family member, p53 is most well-known as a powerful mammalian tumor suppressor, but the precise mechanisms by which its transcription factor activity results in organismal-level protection from cancer have yet to be resolved. Recent evidence across biological taxa suggest that the p53 family has an innate ability to recognize DNA sequences within the context of chromatinized DNA and are key factors for establishing transcriptional and cis-regulatory competence. We examined the nature of potential pioneer factor activities of p53 and p63 using genetic, biochemical, and genomic approaches. Our data suggest that p63 activity is critical for enhancer identity in epithelial cell types and may be responsible for establishing a permissive binding environment at transcriptional regulatory regions for other factors. p53 is not required for the establishment or maintenance of chromatin structure at promoters or enhancers, contrary to predictions based on its observed biochemical activities. Activity and DNA binding by p53 is strongly influenced by cis-regulatory element activity, with hundreds of novel p53 gene targets and genomic binding events occurring in a cell type-dependent manner. Additionally, using a massively parallel reporter assay (MPRA) across multiple cell types, we examined how cell type and co-occurring motifs influences p53-dependent enhancer activity. Our results suggest a wide range of transcription factors cooperate with p53 to drive transcriptional activity, including evidence that p63 is required for p53-dependent enhancer activity in epithelial cell types. Overall, our data suggest that p53 requires a series of transcription factor partners, including p63, to establish competence at cis-regulatory elements for broad, cell type-dependent transcriptional and tumor suppressor activity.

OP3 The prognostic impact of p53 aggregates in high-grade serous ovarian cancer

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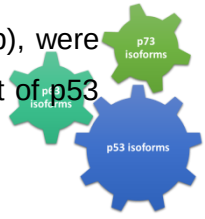
Keywords: ovarian cancer, aggregated p53, ReACp53

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 clinical relevance of p53 aggregates in HGSOC and the response to a novel peptide targeting aggregated p53. **Methods:** 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. A peptide restoring aggregated p53 (ReACp53) was tested on 9 ovarian cancer cell lines, where the baseline p53 aggregation level was known. The cell viability following treatment was used to determine response. **Results:** In 84.8% of patients carrying a missense mutation a 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). Response to ReACp53 was assessed in 5 missense mutated, 2 nonsense mutated and 2 cell lines carrying wild-type p53. There was a wide range of responses to ReACp53 (IC50: 3.3 to 16.7) depending on the amount of p53 aggregates. **Conclusions:** Our proof-of-concept study shows that p53 aggregation is an independent prognostic marker for survival and that the response to ReACp53 may depend on the p53 aggregation level.

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OP4 Regulation of netrin-1 by p53 isoforms

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Keywords: netrin-1, apoptosis, cancer, p53

Netrin-1 is a secreted protein initially discovered as an axon navigation cue during the development of nervous system. However, recently Netrin-1 has been involved in tumorigenesis regulation; indeed, the main Netrin-1 receptors, DCC and UNC5 family members, belong to the emerging family of dependence receptor. Such receptors share the particularity to be also active in the absence of their ligands, inducing a “negative signalling” that triggers apoptosis. Dependence receptors are candidate tumour suppressors. The overall hypothesis is that these receptors limit tumour development through apoptosis induction of tumour cells that would grow or migrate beyond the regions of ligand availability (Goldschneider and Mehlen, 2010). Consequently, tumour transformation is associated with the constitutive inhibition of the death signals induced by these receptors, which could be achieved, for example, by ligand up-regulation. Indeed, a Netrin-1 gain, correlated as a selective advantage for tumour progression, has recently been described in several human cancers (Paradisi and Mehlen, 2010). However, little is known about Netrin-1 expression regulation during tumour development.

Recently, we have shown that Netrin-1 and its main receptor UNC5B could be regulated by the transcription factor p53 (Paradisi et al., 2013). The TP53 gene can be expressed as 12 different isoforms (p53α, p53β, p53γ, Δ40p53α, Δ40p53β, Δ40p53γ, Δ133p53α, Δ133p53β, Δ133p53γ, Δ160p53α, D160p53β, and Δ160p53γ) through alternative initiation of translation, usage of alternative promoters, and/or alternative splicing. p53 isoforms are expressed differently for different cancer types and they also have different transcriptional activities and tumor-suppressor functions that can affect various other biological functions.

To better characterize p53 regulation of Netrin-1, we generated several stable cell lines inducible for the main p53 isoforms, and interestingly we found that Δ40p53α was able to regulate Netrin-1 and UNC5B expression at transcriptional and protein levels, in an manner comparable to p53α. This regulation seems to be independent to p53 status, as we were able to observe it in cancer cell lines wild-type or null for p53. Moreover, using luciferase assay and chromatin immunoprecipitation assay, we have shown that Δ40p53α directly binds and activates Netrin-1 promoter. Finally, we observed a positive correlation between Δ40p53α and Netrin-1 expression in ovarian tumor biopsies, confirming that Δ40p53α could be a biomarker of Netrin-1 highly expressing tumors.

OP5 Mutant p53 Antagonizes p63/p73-Mediated Tumor Suppression via Notch1

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Keywords: mutant p53, Gain of Function, p63/p73, Notch1

p53 is the most frequently mutated genes in human cancers and mutant p53 has a gain-of-function (GOF) that promotes tumor progression and therapeutic resistance. One of the major GOF activities of mutant p53 is to suppress two other p53 family proteins, p63 and p73. However, the molecular basis is not fully understood. Here, we examined whether mutant p53 antagonizes p63/p73-mediated tumor suppression *in vivo* by using mutant p53-R270H knockin and TAp63/p73-deficient mouse models. We found that knockin mutant p53-R270H shortened the life span of $p73^{+/-}$ mice and subjected $TAp63^{+/-}$ or $p73^{+/-}$ mice to T-lymphoblastic lymphomas (TLBLs). To unravel the underlying mechanism, we showed that mutant p53 formed a complex with Notch1 intracellular domain (NICD) and antagonized p63/p73-mediated repression of HES1 and ECM1. As a result, HES1 and ECM1 were overexpressed in $TAp63^{+/-}$;$p53^{R270H/-}$ and $p73^{+/-}$;$p53^{R270H/-}$ TLBLs, suggesting that normal function of HES1 and ECM1 in T cell activation is hyperactivated, leading to lymphomagenesis. *Together, our data reveal a previously unappreciated mechanism by which GOF mutant p53* hijacks p63/p73-regulated transcriptional program via the Notch1 pathway.

OP6 Δ133p53β isoform regulates unique gene sets involved in immunosuppression, cell growth and cell migration

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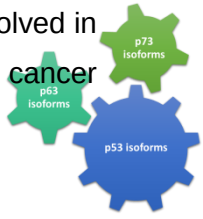
Keywords: p53 isoforms, clonal cell lines, gene set enrichment analysis, immunosuppression

The precise roles of the Δ133p53 isoforms in cancers are largely unknown. We have evidence from brain and prostate cancers that Δ133p53β is increased in highly aggressive cancers, with many poor prognostic features: the tumours have high PD-1+ T cells and CD163+/CSF1R+ M2 macrophages, stronger angiogenic and cell migration signatures and a poorer response to treatment. RNA sequencing of cancers showed that the Δ133p53β was associated with pathways involved in immune signalling, along with a transcriptional enrichment for PD-1 signalling, migration (the Rho GTPase signalling pathway) and angiogenesis. To investigate whether any genes in these pathways are directly regulated by Δ133p53 isoforms, we generated stable transfectants expressing individual isoforms on the H1299 p53-null cell background. Gene enrichment analysis of transcriptomic data on these cell lines identified 925 genes to be significantly altered in the Δ133p53β clones. Among the unique genes enriched in these cells were genes associated with pro-cancer inflammation including checkpoint markers Programmed cell death-ligand 1, *CD274*/PD-L1 and a novel biomarker for cancer progression, the Poliovirus Receptor (*PVR*/CD155), genes involved in tumour migration and metastasis including Melanoma Adhesion Molecule, *MCAM*/CD146 and *CDH2*/N-cadherin, and genes associated with enhanced tumour growth including the Insulin Receptor, *INSR* and Fibroblast Growth Factor 1, *FGF1*. The 558-gene signature unique to Δ133p53β clones shares 100 and 96 common gene signatures derived from brain and prostate cancers with high Δ133p53β levels, respectively including *CD274*, *MCAM*, *CDH2* and *FGF1* genes. Expression of PD-L1, CD155 and CD146 was further confirmed to be increased on the surface of Δ133p53β expressing cells. Overall, Δ133p53β isoform controls unique gene sets involved in creating an immune suppressive microenvironment conducive to promoting cancer progression.



OP7 Targeting delta133p53alpha as a novel T cell enhancer factor to improve cellular-based immunotherapy for cancer

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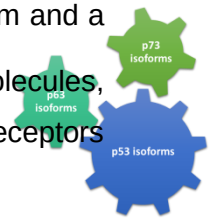
Keywords: delta133p53alpha, T lymphocytes, immunotherapy, cancer

Adoptive transfer of genetically modified T lymphocytes with tumor antigen (TA)-specific receptor has proven efficacy in cancer immunotherapy. However, in many patients the overall benefit is still limited due to various tumor escape mechanisms. Cell damage and metabolic/hypoxic stress in the tumor microenvironment (TME) can lead to a dysfunctional anti-tumor T cell response called T cell senescence. Few studies have demonstrated the critical role of p53 isoforms in the regulation of cellular senescence mainly in tumor cells. However, their role in tumor infiltrating lymphocytes (TILs) remains largely unexplored. Based on the pioneered work of *Harris' lab.*[1] who provided first evidences of the role of □133p53□ in regulating T cell proliferation, we analyzed the cellular phenotype as well as the effector function of the □133p53□-modified TA-specific human T cells.

T cells from healthy donors were engineered to co-express a TA-specific T cell receptor (TCR) and the □133p53□ isoform. Modified T cells were characterized for the expression of key activating/inhibitory molecules, homing markers and their proliferation capacity by flow cytometry. The effector functions i.e. cytokine secretion and antigen-specific killing capacity were assessed by Luminex immunoassay and long-term tumor colony-forming assay. Furthermore, we evaluated the therapeutic potential of □133p53□-modified TA-specific T cells in mouse xenograft model.

Analyses of human $CD8^+$ T cells simultaneously engineered with · 133p53 · -isoform and a TA-specific TCR revealed reduced cell surface expression of T-cell inhibitory molecules, senescence markers, increased expression of homing receptors and chemokine receptors



upon TA stimulation. Importantly, while control T cells exhibited replicative senescence after chronic antigen stimulation, □133p53□-expressing T cells remained highly proliferative, showed a stronger cytokine release and enhanced tumor-specific killing capacity *in vitro*. In line with these results, first *in vivo* xenograft study, demonstrated a superior anti-tumor response of □133p53□-overexpressing T cells.

These findings shed light on a new role of □133p53□ in human T lymphocyte function and could be exploited as a novel approach to circumvent tumor-mediated T cell dysfunction with high potential for cancer immunotherapy.

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OP8 Modulation of Dendritic Cell function by delta133p53 isoforms

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Keywords: Dendritic cells, D133p53, isoforms, NFkB

Our mouse model of the Δ133p53 isoforms (designated Δ122p53), as well as studies on several human cancers, have shown that one or more of the delta133p53 family regulate genes involved in immune regulation resulting in a pro-inflammatory environment. Using the pro-inflammatory phenotype of the Δ122p53 mice we asked whether Dendritic Cells (DC) from Δ122p53 mice could have prophylactic vaccine capacity. To explore this possibility we have phenotypically characterised Δ122p53 DCs. The data show they express surface activation markers, have an activated NFκB pathway 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 Δ122p53 DCs could provoke an anti-tumour response. To do this mice were injected with DCs from Δ122p53 or wild type (WT) p53 mice pulsed with OVA or left unpulsed, and subsequently challenged with B16-OVA cells. Results showed that Δ122p53 DCs could elicit a partial anti-tumour response without exposure to OVA. However, the anti-tumour response after exposure to OVA from Δ122p53 DCs or WT DCs was not significantly different. We have further demonstrated that antigen independent anti-tumour protection by Δ122p53 DCs require natural killer (NK) cells and CD8+ T-cells. Given this, we suggest that incorporating human Δ133p53 isoform in anti-tumour vaccines may improve their efficacy.

OP9 An antiviral role for p53 against HIV-1 in macrophages and the implication of the p53 isoforms

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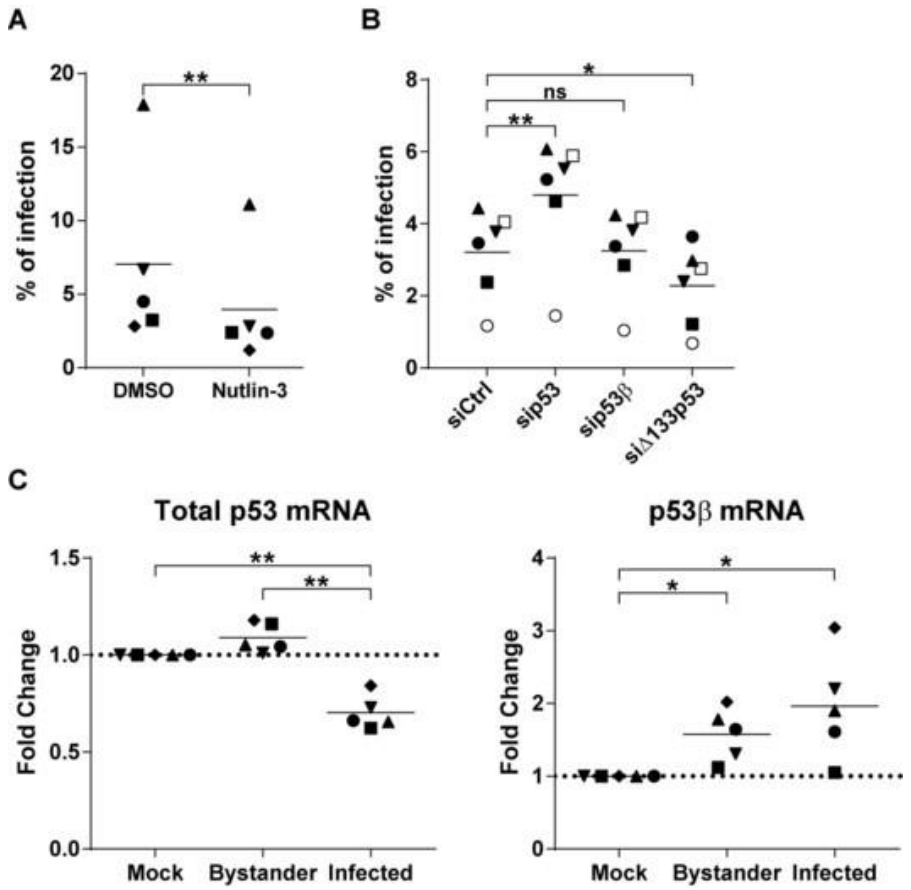
Keywords: HIV-1, Macrophages, MDM2, p53, SAMHD1

Macrophages play an important role in the establishment and propagation of HIV-1 infection. Upon exposure to HIV-1, only a small proportion of macrophages are productively infected. Transcriptomic analyses performed to compare infected and uninfected (bystander) populations revealed MDM2 as a positive regulator of HIV-1 infection in macrophages. To better understand the role of MDM2 in HIV infection, monocyte-derived macrophages (MDMs) were transfected with target-specific siRNAs and exposed to a fully competent HIV-1 virus expressing a small GPI-anchored reporter (HSA). In some experiments, MDMs were treated with Nutlin-3, a chemical inhibitor of the MDM2-p53 interaction, before being infected (Fig A). Infection was measured by flow cytometry and showed that MDM2 knockdown reduced the proportion of productively infected macrophages, associated with a decrease in HIV-1 reverse transcription and integration, two critical steps for the replication cycle. Similar results were observed with Nutlin-3 treatment. As expected, knockdown or inhibition of MDM2 resulted in a significant increase in the expression of p53-induced genes, including p21 (*CDKN1A)*, leading to a reduced level of phosphorylated/inactivated SAMHD1 at the time of infection. SAMHD1 is a strong restriction factor against HIV-1 in macrophages, interfering with the reverse transcription of the viral RNA.To further investigate the role of p53 in HIV infection, we transfected an siRNA targeting all isoforms (sip53) or specific to one variant in macrophages before exposure to HIV. In contrast to the broad p53 knockdown, specific knockdown of Δ133p53 reduced the number of productively infected cells (Fig B) and virus production, whereas knockdown of p53β had no effect. We also studied the expression pattern of p53 isoforms in infected macrophages (Fig C). Infected MDMs were sorted on the basis of HSA expression, then lysed for mRNAs and proteins analysis in both populations. Analysis of total p53 mRNA or the TA/Δ40p53 subclass only indicated a decrease in the infected population compared to the bystander cells. However, an increase in mRNA expression of the β and γ isoforms was seen in the bystander and infected populations compared to cells unexposed to

HIV. At the protein level, HIV-infected MDMs show a stabilization of full-length p53 and an increase in p53β compared to the bystanders. Altogether, our results indicate that the resistance to HIV-1 integration associated with MDM2 silencing requires the activation of p53. Experiments with Nutlin-3 suggest that the observed resistance to HIV-1 results more from the release/activation of p53 rather than the absence of MDM2 *per se*. The MDM2 expression level and the p53 activation state influence the amount of active SAMHD1 and are therefore important factors in the overall susceptibility of MDMs to HIV-1 infection. This study highlighted the important role of p53 and its isoforms in antiviral immunity.



(A-B) Percentage of HIV-infected MDMs following (A) Nutlin-3 treatment or (B) transfection of siRNA targeting all p53 isoforms or a specific subclass. (C) Expression of total p53 mRNA or p53β subclass in bystander or infected cells.

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OP10 Opposite effect of p53 on chikungunya virus replication in mammal and insect

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Keywords: arbovirus, chikungunya, insect, muscle cell

In the past few years, we have witnessed a resurgence or onset of emerging infectious diseases causing important global health threats. Most of these diseases are caused by arboviruses (ARthropod BOrne viruses): these viruses share the particularity to be transmitted to vertebrate hosts by hematophagous arthropod vectors. Among the different arboviruses, the re-emerging chikungunya virus (CHIKV, *Togaviridae* family) moved into previously unaffected areas, mainly following the extension of its vector *Aedes albopictus* ("Tiger mosquito") and is associated with millions of affected people. CHIKV infection causes a variety of clinical signs varying from fever, rash or headache to significant inflammatory pathologies including severe arthritis and myositis and, in some cases, encephalitis and death.

However, CHIKV, like all arboviruses, can persistently infect the invertebrate vector with no major health consequence and *in vitro*, insect cells can be chronically infected, whereas no vertebrate cell survives the acute infection. The way apoptosis may be triggered in mammals or inhibited in insects is still obscure and unknown host cell factors, as well as viral factors, may regulate cell fate and thus the outcome of the infection in human and mosquito.

Infection-induced cell death is usually associated with major cellular stress (oxidative, endoplasmic reticulum, chromatin stress…) which may directly, or indirectly, involve p53 response. On one hand, in mammals, it has been recently shown that different p53 isoforms

are involved in cell response to infection by Influenza Virus (Terrier *et al.* JVirol, 2012) or Helicobacter Pylori (Wei *et al.* PNAS, 2012) and that they can be modulated by the pathogen itself. On the other hand, in mosquito, recent studies show that a certain degree of cell death in some tissue may correlate with vector competence (Liu *et al.* PlosPatho, 2013) : a rapid and strong activation of p53 is observed in resistant mosquitoes, whereas this response is delayed and necrotic in permissive ones. More recently, in mosquito cells, p53 has been shown to be involved in Dengue virus (*Flaviviridae* family) infection, by reducing infection-induced oxidative stress and death (WJ. Chen *et al.* Virology, 2018).

By studying in parallel CHIKV infection in mammals and insects, our aim is to unveil the host and/or viral factors responsible for pathogenicity or resistance. Focusing on p53 and p53 isoforms, we explore how host response, and especially cell death, may be differently regulated in mammals and insects. To this aim, we compare CHIKV infection in wild type or p53 knocked-out models *in vitro* (human immortalized myoblasts, mosquito cells) and *in insecto* (*Drosophila Melanogaster*).

The data we are presenting here show an opposite effect of p53 on CHIKV replication and production in mammal *versus* insect (unpublished data).

OP11 Identification of novel partners/regulators of p73 proteins in gastric cancer

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Keywords: TP53, TP73, gastric cancer

Gastric cancer is the third in terms of mortality and the fifth in terms of incidence in the world (2018). This situation can be explained by a late diagnosis and a tumor insensibility to the actual treatments. TP53 is mutated in about 50% of the gastric cancers and account in part for a reduced sensibility towards treatment. In addition, alteration of the expression of the TA and ΔN isoforms of TP73 have been described. For instance, ΔNp73 is overexpressed, whereas, the proapoptotic isoform, TAp73, is under-expressed, correlating with a bad prognosis and resistances to chemotherapy. To provide a better understanding of the functions and the regulations of the activity of TAp73 and ΔNp73 in gastric cancers, we have used interactomic approach with gastric cancer cells to identify novel p73 interactants that may regulate their function. We used state of the art and high sensitivity and quantitative mass spectrometry analyses following immunoprecipitation assays to detect partners of TAp73 and ΔNp73 in a gastric cancer cell line. We identified 38 interactants for the ΔNp73 isoform and 31 for TAp73. Some of them were already known (ex. Itch) but most of them were novel interactants. Using a decision diagram, taking in account molecular alterations in gastric cancer inventoried in The Cancer Genome Atlas database, we select the most relevant interactants for functional analysis. Among them, we choose an E3 ubiquitin ligase which was not described in the literature to interact with P73 and that was part of a pathway frequently altered in gastric cancers. First, we validated the interaction between this ubiquitin ligase and our protein of interest. Then, we found that this enzyme plays a role in controlling P73 protein level, but also on its activity by modulating the expression of p73 target genes (ex. *PUMA*, *BAX*). Altogether, our results identified novel interactants of p73 that may play a role in the regulation of p73 activity in gastric cancers. Among them, we described physical and functional interaction with an E3 ubiquitin ligase, presenting alterations in gastric cancer and that may account for gastric cancer aggressiveness.

OP12 Functional analysis of wt and mutant p53 isoforms

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1) NIH/NCI

Keywords: p53 isoforms, mutant p53, cellular senescence

p53 has a key role in the maintenance of the genetic stability and, thus, in preventing tumor development. Consistently, *TP53* is the most frequently mutated gene in human cancers and plays central roles in cancer formation, progression and treatment.

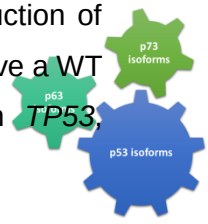
Human *TP53* expresses 12 p53 isoforms which differ in their N- and C-termini, but all share a central domain. Interestingly, the most common *TP53* mutations in human cancers are almost all located within this shared domain, meaning that in most *TP53* mutant cancers, not only is the canonical FLp53 (p53alpha) mutated, but also each of the isoforms of p53.

Importantly, several studies in different human cancers reported that the prognostic value of *TP53* mutation status is improved when combined with p53 isoform expression. These studies also reported that p53 isoforms, particularly p53beta and Delta133p53, are abnormally expressed in different cancer types, and could be involved in resistance to treatment.

Therefore, identifying the functional differences between WT and mutant p53beta and Delta133p53 isoforms is important to understand how they are involved in mutant *TP53* cancers, whether they may be used as prognostic makers, and represent potential therapeutic targets.

These two isoforms have been shown to regulate cellular senescence of primary human astrocytes, a critical barrier to tumor progression. They have also been shown to be involved in the regulation of inflammation, invasion, angiogenesis and cell growth. However, the roles of these isoforms in tumor progression and malignancy are still being investigated, and almost nothing is known about the activities of their mutants.

Glioblastoma (Grade IV Astrocytoma) is the most common primary brain tumor with a high rate of recurrence. Following diagnosis, patients may receive radiation treatment and temozolomide (TMZ) chemotherapy, both of which have been associated with induction of cellular senescence in cancer cells. Although the majority of primary glioblastoma have a WT *TP53*, tumors accumulate mutations following treatment, including mutations in *TP53*.



suggesting that mutant p53 function may be important in TMZ-induced senescence. Because p53 isoforms have been shown to regulate senescence in primary non-tumor cells and being involved in the regulation of tumor progression and resistance mechanisms, our study aims to investigate the roles of p53 isoforms and their mutations in the response of glioblastoma cells to TMZ treatment.

OP13 Quantitation and analysis of alternatively-spliced TP53 RNAs in a NZ breast cancer cohort, using a novel multiplex long amplicon digital PCR method

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Keywords: TP53 isoforms, quantitation, digital PCR, breast cancer,

The *TP53* isoforms differ at the 5' and 3' ends, but have a central region of 618bp common to all. For this reason, the study of *TP53* RNA splice forms has been extremely challenging; the individual isoforms are impossible to specifically detect and quantitate in standard PCR-based methods and short read RNA-sequencing. Exploiting digital PCR technology, we have devised probe-based droplet digital PCR assays allowing the detection and precise quantitation of functionally distinct 0.85-1.85kb *TP53* isoforms in parallel. Multiple modifications to the standard droplet digital PCR assay procedures were required to enable specific co-amplification of these long transcripts and to overcome issues with secondary structures. These assays have also allowed detection of a novel *TP53* transcript in tumour cells, with implications for protein function. Furthermore, for the first time it is possible to quantitate and study the endogenous individual *TP53* splice forms in tumour samples. We have now quantitated eight *TP53* isoforms in a well-characterised cohort of NZ breast cancer samples, to understand the potential role of the individual splice forms in breast cancer biology, and evaluate their role as potential biomarkers of patient prognosis. Our novel assays and results from the application of these will be presented.

This research was supported by the Health Research Council of New Zealand.

OP14 Targeting nonsense-mediated decay and mRNA splicing to activate p53 pathway in p53 mutant and non-mutant cancer cells

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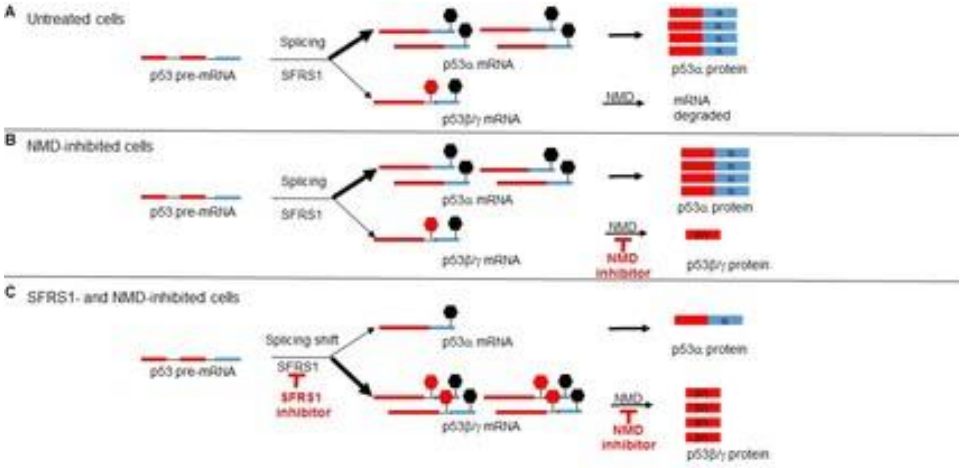
8) University of Dundee, Dundee, Scotland, UK

Keywords: NMD inhibition, p53beta gamma isoforms

TP53 is one of the highly mutated tumor suppressors in cancer. A vast majority of p53 mutations are truncating mutations that trigger mRNA degradation by nonsense-mediated decay (NMD), a regulator of aberrant mRNA stability. Here we show that p53beta and p53gamma isoforms which retain the functional properties of canonical full-length p53 are NMD substrates. Inhibiting NMD rescues nonsense mutation-bearing p53 transcripts as well as p53beta and p53gamma isoforms. NMD inhibition leads to p53 pathway activation in both p53 mutant and non-mutant cancer cells and the relative contribution of p53gamma is greater than that of p53beta in promoting p53 pathway activation. We show that NMD inhibition increased radiosensitivity and inhibited tumor growth. Furthermore, dual inhibition of NMD and the splicing factor SFRS1 shifts p53 mRNA splicing from canonical p53alpha to the p53beta/gamma isoforms, markedly enhancing the effects of NMD inhibition. These results identify a novel therapeutic strategy for restoration of p53 function in p53 deficient cancers.



Combination of NMD inhibition with SFRS1 inactivation increases the expression of p53 functional isoforms beta and gamma

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OP15 The RNA Binding protein PTBP1 controls the alternative splicing of the C-terminal Exons of TP63 in HNSCC.

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Keywords: Squamous carcinoma, splicing regulation, RBP, PTBP1, p63 isoforms

Head and neck squamous cell carcinomas (HNSCC) are one of the most prevalent types of cancer worldwide, and are frequently lethal (1). Treatment strategies combine surgery and either radiotherapy or chemotherapy, to which HNSCC cells often show resistance. So far, poor molecular understanding of HNSCC translates to a lack of personalised treatment options. Gaining better insight into the molecular mechanisms that govern HNSCC could therefore provide useful diagnostic tools, and improve the specificity of treatment options. One clue may reside in the frequent amplification of the TP63 gene. Three protein isoforms, p63α, β and γ, stem from its alternative splicing. On one hand it has been proposed that the pro-proliferation effect of the most abundant isoform, ΔNp63α, could stimulate tumour growth, while on the other hand the downregulation of TP63 expression is observed in advanced stage carcinoma of various origins. By analysing RNA sequencing data of HNSCC patients included in the TCGA database, we have determined that patients with a higher proportion of ΔNp63β or ΔNp63γ have a decreased overall survival. This could indicate that beyond the abundance of p63, it is also the fine balance between its isoforms that determines its functions. Working with several HNSCC derived cell lines, we have identified an RNA binding protein, PTBP1, capable of repressing the production of p63γ and promoting the production of p63β. This paves the way towards understanding how, by modulating the pattern of expressed RNA binding proteins, HNSCC cells could alter their p63 isoform profile, and perhaps shift between phenotypes.

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OP16 Exosomal ΔNp73, TAp73 and Δ133p53 isoforms as early diagnosis markers in colorectal cancer

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Keywords: p73 isoforms; Δ133p53; exosomal content; colorectal cancer; early diagnosis

The detection of soluble biomarkers from biological fluids — referred to as liquid biopsy — is an appealing approach to explore for the early diagnosis of cancer. The plasma amount and content of exosomes, a type of extracellular microvesicles, have been described altered in many cancers. Since the content of exosomes may represent the state of the disease it may serve as a biomarker of the carcinogenesis process.

The screening tools for the detection of colorectal cancer (colonoscopy and Fecal Occult Blood Test) are invasive and/or detect the tumor in advanced stages. The identification of biomarkers through a non-invasive approach that can identify the disease at the premalignant stage could significantly improve the management of the disease.

Here, we studied the plasma-derived exosomes mRNA content of ΔNp73, TAp73 and Δ133p53 from i) healthy subjects (n=29), ii) individuals with premalignant lesions (low and high grade adenomas, n=49) and, iii) CRC patients diagnosed at different stages of the disease (n=42). Our main goal is to determine the early diagnosis potential of the altered content of these 3 isoforms.

We have observed that ΔNp73 was significantly higher in premalignant lesion subjects and CRC patients than in controls. Regarding Δ133p53, its levels were significantly increased in CRC patients respect to the other two groups. Remarkably, TAp73 mRNA was not detected in any group.

Our preliminary data support ΔNp73 as a putative biomarker for the early diagnosis of colorectal cancer through a non-invasive approach since it is already elevated in those patients with premalignant lesions. Respect to Δ133p53, its content is over-represented in CRC patients, what may indicate its plausible used as a diagnosis marker of the disease although could not identify those patients at the very early stages of the disease. Tumor cells package specific cargo in their exosomes what may facilitate their progression and metastasis formation. In this sense, sending abroad a tumor suppressor gene such as TAp73 may not

provide the tumor with a selective advantage. Although preliminary, our results are promising. Thus, p53 family members content in plasma-derived exosomes from cancer patients, may emerge as an interesting area to explore.

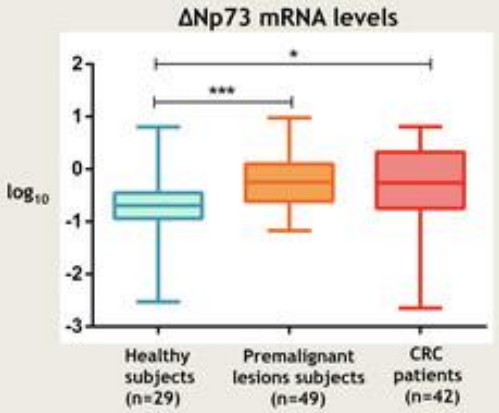

Figure 1. Quantitative real-time PCR analysis of ΔNp73 mRNA levels in serum-derived exosomes relative to exosome concentration of healthy group, premalignant lesions group and CRC patients. *** P <0,001 and * P <0,05 using Mann-Whitney U-test.

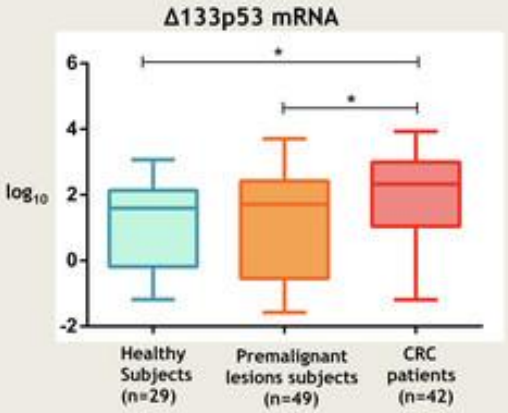

Figure 2. Quantitative real-time PCR analysis of Δ133p53 mRNA levels in serum-derived exosomes relative to exosome concentration of healthy group, premalignant lesions group and CRC patients. * P <0,05 using Mann-Whitney U-test. .

This research was supported by FIS PI18/00473, FEDER funds and Cátedra UAM-ROCHE

OP17 The E3 ubiquitin-protein ligase Mdm2 is a novel interactor of the von Hippel-Lindau tumor suppressor

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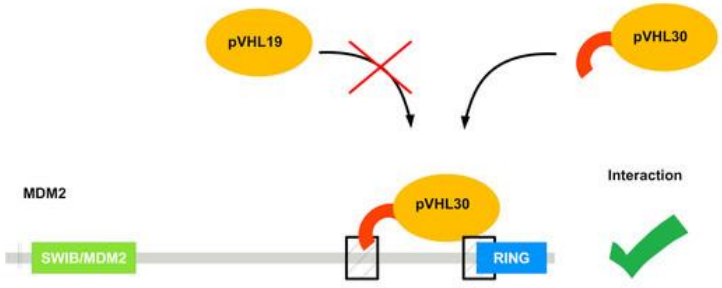
Keywords: pVHL, von Hippel-Lindau, hypoxia, MDM2, yeast, pVHL isoforms

Mutations of the von Hippel-Lindau tumor suppressor (pVHL) are causative of a familiar predisposition to develop different cancers. pVHL is mainly known for its role in regulating hypoxia-inducible factor 1-α (HIF-1α) degradation, thus modulating the hypoxia response. Previous studies however suggested that isoform-specific specializations can be associated with human pVHL. Here, we present a novel interaction between pVHL and Mouse double minute 2 homolog (MDM2). Integrating *in silico* predictions with *in vivo* assays we found that the N-terminal acidic tail of pVHL30 is required for its association with MDM2. Further, we demonstrate that an intrinsically disordered region upstream the tetramerization domain of MDM2 is responsible for its isoform-specific association with pVHL30. This region is mostly conserved in higher mammals, including human, similarly to what already proposed for the N-terminal tail of pVHL30. Collectively, our data support the idea that the isoform pVHL30 may play a role in MDM2 regulation, suggesting a wider interplay among hypoxia sensing and cell cycle regulation.



Schematic representation of pVHL30 specific association with MDM2.

This work was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC) grant IG17753 to ST.

OP18 DAZAP2 acts as phosphorylation-dependent specifier of the p53 response controling cancer cell chemosensitivity

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Keywords: DAZAP2, HIPK2, nuclear translocation, p53 target genes, chemosensitivity

Massive genome damage activates the tumour suppressor p53 which facilitates elimination of damaged cells. Protein kinase HIPK2 plays a fundamental role in specifying p53 function by stimulating proapoptotic target gene expression upon phosphorylation of p53 at Serine 46. Here we identify the evolutionarily conserved adaptor protein DAZAP2 as a phosphorylation-dependent specifier in the p53 response. In unstressed cells, DAZAP2 interacts with HIPK2 and promotes HIPK2 degradation by potentiating complex formation between HIPK2 and the ubiquitin ligase Siah-1, thereby supporting HIPK2 degradation. Upon DNA damage, HIPK2 phosphorylates DAZAP2 at a set of Ser/Thr residues including Serine 77. DAZAP2 phosphorylation suppresses its interaction with HIPK2 and Siah-1 and inhibits its HIPK2-degrading activity upon stress. Moreover, upon DNA damage DAZAP2 accumulates in the cell nucleus, which is stimulated by its phosphorylation. Genome-wide RNA Seq analysis revealed that DAZAP2 specifies p53 target gene expression upon DNA damage by controlling a distinct subset of cell death-regulatory target genes. Accordingly, nuclear DAZAP2 colocalizes and interacts with the alpha-isoform of p53. Our in vitro interaction analysis indicates that DAZAP2 contacts the DNA-binding domain of p53, suggesting a potential interplay with several p53 isoforms. Knock-down of DAZAP2 or its genetic deletion potentiates induction of cell death of cancer cells upon chemotherapeutic treatment both in vitro and in a mouse xenograft model. Collectively, our findings identify DAZAP2 as a novel, phosphorylation-dependent specifier of the p53 response regulating cancer cell chemosensitivity.

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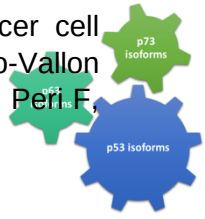

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Posters

P1 Neuron Navigator-3 is a novel p73 transcriptional target and inhibits colon cancer cell migration, invasion and metastasis.

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Keywords: p73, Neuron Navigator-3, genotoxic stress, migration, metastasis

p73 is a member of the p53 tumor suppressor family, which transactivates p53-responsive genes and mediates DNA damage response. Recent evidences suggest that p73 exerts its tumor suppressor functions by suppressing metastasis, but the exact mechanism remains unknown. Here, we identify Navigator-3 (NAV3), a microtubule-binding protein, as a novel transcriptional target of p73, that can be induced by DNA damage in a p73-dependent manner and plays a role in p73-mediated inhibition of cancer cell invasion, migration and metastasis. Through bioinformatics analysis, we identified two p73-binding sites in NAV3 promoter. Consistent with this, p73 binding to NAV3 promoter was confirmed through luciferase reporter assays and Chromatin Immunoprecipitation (ChIP) assays. Site-directed mutagenesis of both the binding sites totally abrogated p73 responsiveness, indicating that both the sites are equally responsible and essential for p73 binding. In addition, Real-time quantitative PCR and western blot analysis demonstrated a rapid increase in endogenous NAV3 mRNA and NAV3 protein upon induction of p73. Furthermore, knockdown of NAV3 and p73 significantly increased the invasion and migration rate of colorectal cancer cells as confirmed by wound-healing, cell invasion and cell migration assays. Also, knockdown of NAV3 decreased the expression of E-cadherin, a cancer metastasis suppressor, and increased the expression of other prominent EMT markers such as N-cadherin, Snail, Vimentin and Fibronectin. Immuno-histochemistry analysis further revealed down-regulation of p73 and NAV3 expression in metastatic colon cancer tissues as compared to non-metastatic cancer tissues. Taken together, we provide evidence that Navigator-3 is a direct transcriptional target of p73 and p73 exerts its anti-metastatic function by inducing the expression of Navigator-3 in response to genotoxic stress.

This research was supported by Department of Biotechnology (DBT), India and Department of Science and Technology (DST), India.

P2 The role of the p53 isoform Δ133TP53 in treatment resistance and immune infiltration.

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Keywords: Δ133TP53, glioblastoma, treatment resistance, immune infiltration

Tumour protein 53 (TP53) has many isoforms including the Δ133TP53 isoform, which has been associated with inflammation, angiogenesis, migration, and invasion. Recent work has shown an association between increased Δ133TP53 expression and a poorer prognosis for those with glioblastoma. To investigate the possible association between Δ133TP53 and treatment resistance and increased immune infiltration in glioblastoma, in vitro experiments using a mouse embryonic fibroblast cell line (10.1) that was engineered to express either the mouse mimic of the Δ133TP53 isoform, known as Δ122TP53, or an empty control vector (p53 null) were done. Cells were treated with temozolomide, a chemotherapeutic drug used to treat glioblastoma and tert-butyl hydrogen peroxide (tBHP), a compound that causes oxidative stress. Results from these experiments showed Δ122TP53 cells showed improved cell survival in response to both temozolomide and oxidative stress when compared to p53 null cells, suggesting Δ122TP53 conferred resistance to some forms of cellular stress [1]. To investigate the potential role of Δ133TP53 in immune infiltration, immunohistochemistry and RNAscope was done. Staining for the macrophage chemoattractant marker, CCL2, showed positive staining for CCL2 in Δ122TP53 compared to p53 null 10.1 cells, which showed no positive staining for CCL2 [1]. Staining in glioblastoma sections showed an association between areas with Δ133TP53 and CCL2 expression and CD163 positive macrophages [1]. Results from these experiments suggest a possible association between Δ133TP53 isoform and treatment resistance and immune cell infiltration in glioblastoma.

References: Kazantseva et al., J. Pathol. (2018) 246(1):77-88.

P3 Regulation of expression of the oxidoreductase STEAP4 by the delta133p53beta isoform: An approach to develop a targeted therapy for prostate cancer

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Keywords: Prostate cancer (PCa)

Prostate cancer (PCa) is the second most common cancer in men and the fourth most common cancer worldwide. Despite this there are no reliable prognostic markers or effective therapies to treat the individuals that relapse. Hypoxia and elevated serum levels of IL-6 in castration-resistant prostate cancer (CRPC) have been linked with aggressive disease. Data published by our group has shown that elevated levels of the delta133p53beta isoform in a subgroup of PCa is associated with shorter patient disease-free survival. Preliminary data suggested hypoxia may be a regulator of the delta133p53beta. In addition to hypoxia, we have demonstrated that IL-6 signalling is important for the delta133p53 to promote tumour progression. Interestingly both IL-6 and hypoxia activate six-transmembrane protein of prostate 2 (STAMP2 or STEAP4) which catalyses hypoxia activated prodrugs (HAPs). However, the mechanism regulating either delta133p53beta or STEAP4 and whether delta133p53beta regulates STEAP4 in prostate cancer remains unknown. To investigate whether hypoxia can co-induce delta133p53 isoforms and STEAP4, three PCa lines with wild type p53 (LNCaP) or different p53 mutations (22Rv1, DU145) were treated with hypoxia for 24 and 48 hours. Unsupervised clustering of data collected at different time points showed an association between the expression of delta133p53, p53beta and STEAP4 at mRNA level in LNCaP and 22Rv1 compared to DU145, irrespective of hypoxia. This suggests that delta133p53 expression depends on the presence or absence of p53 mutation. Furthermore, reducing levels of delta133p53 using siRNAs resulted in reduced expression of both STEAP4 and IL6-ST (a subunit of the IL-6 receptor) in all three PCa lines. Taken together our results suggest delta133p53 regulates STEAP4 and IL6-ST expression in PCa. We will now determine

whether STEAP4 is directly regulated by delta133p53 in PCa and whether this regulation is dependent on IL-6 signalling. The findings from this project may allow us to use HAPs to target delta133p53 expressing tumours.

P4 p73 coordinates with Δ133p53 to promote DNA double-strand break repair

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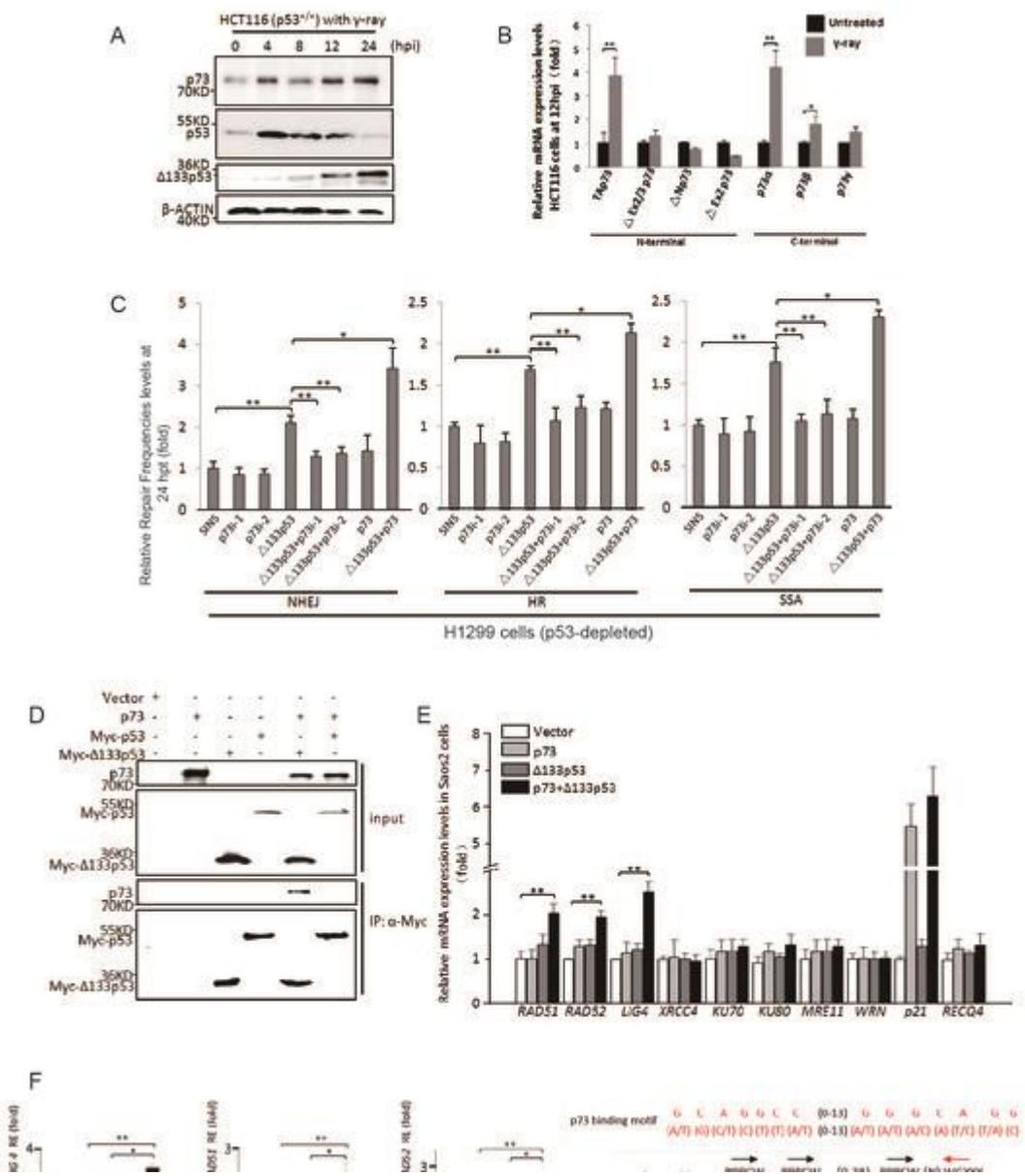
Tumour repressor p53 isoform Δ133p53 is a target gene of p53 and an antagonist of p53-mediated apoptotic activity. We recently demonstrated that Δ133p53 promotes DNA double-strand break (DSB) repair by upregulating transcription of the repair genes RAD51, LIG4 and RAD52 in a p53-independent manner. However, Δ133p53 lacks the transactivation domain of full-length p53, and the mechanism by which it exerts transcriptional activity independently of full-length p53 remains unclear. In this report, we describe the accumulation of high levels of both Δ133p53 and p73 (a p53 family member) at 24 h post γ-irradiation (hpi). Δ133p53 can form a complex with p73 upon γ-irradiation. The co-expression of Δ133p53 and p73, but not either protein alone, can significantly promote DNA DSB repair mechanisms, including homologous recombination (HR), non-homologous end joining (NHEJ) and single-strand annealing (SSA). p73 and Δ133p53 act synergistically to promote the expression of RAD51, LIG4 and RAD52 by joining together to bind to region containing a Δ133p53-responsive element (RE) and a p73-RE in the promoters of all three repair genes. In addition to its accumulation at 24 hpi, p73 protein expression also peaks at 4 hpi. The depletion of p73 not only reduces early-stage apoptotic frequency (4–6 hpi), but also significantly increases later-stage DNA DSB accumulation (48 hpi), leading to cell cycle arrest in the G2 phase and, ultimately, cell senescence. In summary, the apoptotic regulator p73 also coordinates with Δ133p53 to promote DNA DSB repair, and the loss of function of p73 in DNA DSB repair may underlie spontaneous and carcinogen-induced tumorigenesis in p73 knockout mice.



AB Full-length p73 is activated.C p73 and Δ133p53 act synergistically to promote DSB repair.D P73 forms a complex with Δ133p53.E p73 coordinates with Δ133p53 to promote repair genes expressio.F Δ133p53 and p73 join together to bind to the promoters.

P5 Interference with host cell DNA repair pathways by Chlamydia: contribution to carcinogenesis

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Cervical cancer remains a major cause of death and virtually all cases are thought to result from high-risk human papillomavirus infections. While 80% of women contract HPV infection in a lifetime, only 1-2% of them develop cervical cancers. *C. trachomatis* infection is recognized as one of the significant risk factors for cervical carcinogenesis, however, the molecular mechanisms involved are not clear. *C. trachomatis* has evolved various strategies to establish and promote infections within its target niche and plays an active role in damaging the host genome. Failure of effective DNA damage response (DDR), a set of sophisticated genome surveillance and repair mechanisms, leads to the accumulation of mutations and genomic instability. We developed a 2D and 3D culture system that supports long-term expansion of primary human ectocervical cells from healthy donors. These human primary ectocervical cell models were used to investigate the effects of *C. trachomatis*, HPV E6E7 and co-infection on host cell. In line with previous reports, *C. trachomatis* infection-induced DNA breaks, while simultaneously enhancing host cell proliferation. Interestingly, HPV E6E7 expression enhanced the host cell proliferation and was found to interfere with normal *C. trachomatis* development. Further, our data revealed a significant suppression of mismatch repair (MMR) and base excision repair (BER) by *C. trachomatis,* while HPV was found to induce these pathways. Additionally, by employing a plasmid-based in vitro MMR and BER repair assays we confirmed that *C. trachomatis* infected cells have reduced ability to repair mismatches and oxidative base lesions irrespective of HPV status. Proteasomal degradation and p53-MDM2 pathway were found to be the major mechanisms involved in MMR and BER suppression by *C. trachomatis*. Thus, we provide first insights into seemingly opposite influences of *C. trachomatis* and HPV E6E7 on host cell MMR and BER pathway, placing the host cell genome at the risk of accumulating mutations. The data also implies that *C. trachomatis* might have a direct role in causing a subset of cervical squamous cell carcinomas in addition to being a co-factor for HPV.

P6 Targeting the Cancer Stem Cell Population in Head and Neck Squamous Cell Carcinoma

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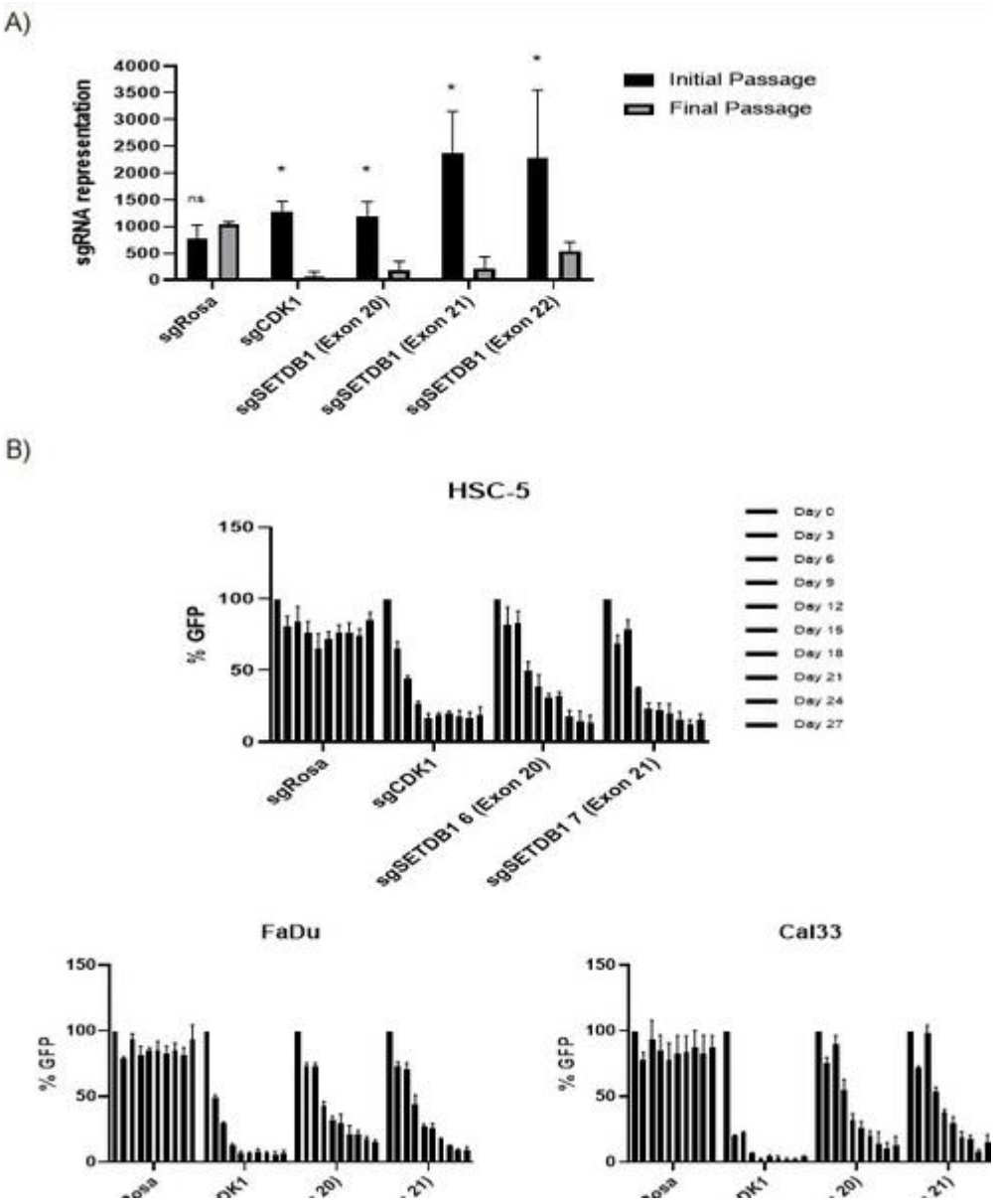
Head and neck squamous cell carcinoma (HNSCC) is a pervasive and dangerous disease with a 5 year survival rate of only 40-50% [1], demonstrating the need for improved therapeutics. The cancer stem cell (CSC) population in HNSCC has become an emerging area of study given its involvement in treatment failure, metastasis, and overall tumorigenesis [2]. Therefore, the HNSCC CSC population is an essential target for new treatments. Our work has connected the transcription factor, p63, to the maintenance of this cell population, as well as to its increased aggressiveness. p63 is a p53-related transcription factor that regulates a number of important cell functions, including proliferation, apoptosis, and differentiation state. ΔNp63α is the primary isoform expressed in SCCs. It has an oncogenic role and has been linked to cancer stemness. One particular mode of action for ΔNp63α in cancer involves epigenetic regulation of target genes via association with chromatin remodeling proteins. Previous literature also links chromatin modifications to stem-like properties in squamous cell carcinomas. Consequently, we employed a CRISPR-Cas9 screen targeting over 200 chromatin remodelers in order to uncover vulnerabilities in HNSCC, and devise new targets for CSC based therapies. Among the top targets was the H3K9 tri-methyltransferase, SETDB1. Further characterization of this target has allowed us to confirm it as an essential gene in multiple HNSCC cell lines, and then uncover links to numerous CSC properties, such as spheroid formation, cell invasion, and cell migration. In addition, we have shown association of SETDB1 with ΔNp63α in our CSC population. This signaling complex, previously identified in breast cancer [3], drives numerous biological hallmarks of our HNSCC CSC population. Furthermore, we have identified critical downstream targets that mediate the effects of SETDB1/ΔNp63α on the CSC population, such as EGFR. Based on the strong connection to the CSC phenotype, and the viability of chromatin remodelers as therapeutic targets, we predict that this signaling pathway can be exploited to target the CSC population in HNSCC.



SETDB1 is essential in HNSCC. A) sgRNAs targeting SETDB1 from a pooled library were quantified in HSC5 cells before and after 8 passages. B) Individual GFP-tagged sgRNAs were tracked for 27 days in 3 cell lines, confirming SETDB1 as essential.

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P7 Unprecedented p53 oddities in long-living organisms

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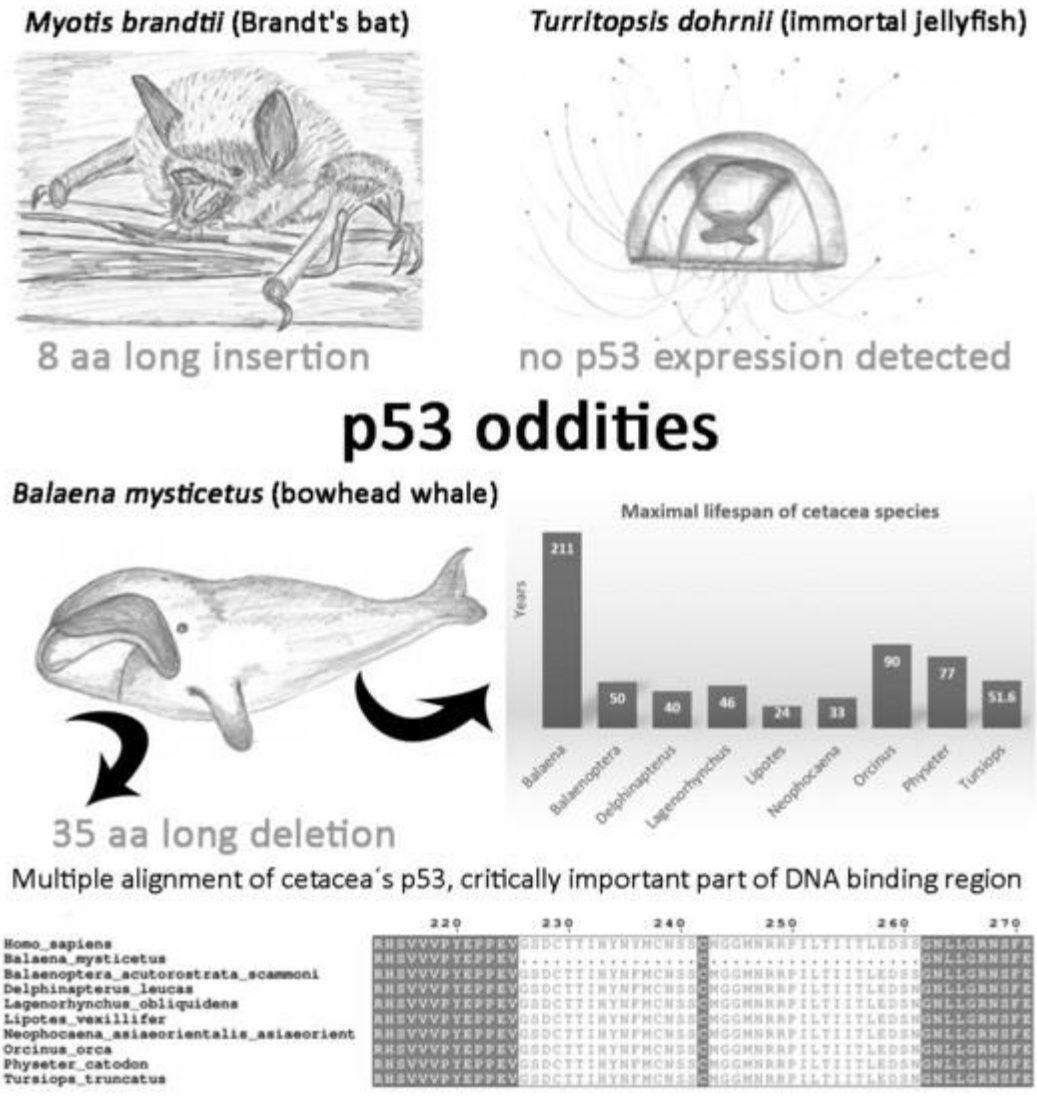
Keywords: p53, ageing, longevity, database, protein alignment

Live forever or be forever young, this is a man's lasting desire. Although our average lifespan is constantly growing, it is evident, that our maximal life expectancy is physiologically and genetically limited. There are many ageing theories, for example, The Free Radical Theory or Telomere Shortening Theory. It is well known that p53 plays a regulatory role in key molecular and biological processes. It was shown that all p53 family members have an important role in ageing process regulation [1]. Mutations found in human p53 usually cause severe phenotypes or lead to oncogenic processes. It is expected, that proteins from p53 family led to development of multicellularity and complexity, therefore it is found in all of organisms on the Holozoa branch of the tree of life. Mainly central “core” domain of p53 is very well conserved across most animals. We have inspected p53 proteins of newly sequenced long-living animals to find if there is a link between longevity (maximal lifespan) and p53 protein sequence “oddities”. To access data about longevity and particularly maximal life span we have used AnAge Database [2]. First, we have identified the longest living mammal, bowhead whale (*Balaena mysticetus*) with a maximal lifespan of 211 ± 35 years. We have done multiple alignments of all to date sequenced cetacea and found that only bowhead whale p53 has large 35 amino acid residues long deletion of a critically important part of central DNA binding domain. In all other sequenced cetacea (which live significantly shorter time), none deletion or insertion occurs. The second example is extremely long-living Brandt's bat (*Myotis brandtii*), maximal documented lifespan is 41 years. It is a very small mammal (body length 1.5-2 inches) and according to its bodyweight, life expectancy of this species is enormously high compared to other species. We have found that p53 of this bat has 7 amino acid residues long insertion in its central DNA-binding region. The last (and probably the most important) presented example is the “immortal” jellyfish Turritopsis, which has in animal kingdom an unprecedented lack of p53 expression, based on RNAseq data of Dr. Shin Kubota.

et al. [3]. For graphical summary, see figure below. Overall, this study reveals unexpected variation in p53 primary amino acid sequences across various species of Animal kingdom. Interestingly the most significant differences in p53 sequences were found in long-living animal species, where both deletions, insertions as well as complete lack of p53 expression in “immortal” jellyfish Turritopsis are present. Increased activity of p53 is one of the main causes of ageing [1]. Therefore, our final hypothesis is, that organisms with p53 oddities, probably leading to a decrease of p53 activity, are extremely long-living.



Proposed hypothesis of p53 oddities and related extreme longevity in selected animals. Three question marks express other molecular and physiological animal-specific factors, which play a role in longevity

References: [1] E. Sahin et al., Nat Rev Mol Cell Biol. 13 (2012) 397-404. [2] J. P. De MagalhÃes et al., J Evol Biol. 22 (2009) 1770-1774. [3] Y. Hasegawa et al., Zoological Science. 33 (2016) 366–372. This research was supported by project SGS/09/PrF/2019 financed by

University of Ostrava. We would like to express our gratitude to M.Sc. Alena Volná and Mr. Milan Bolek for their illustrations.

P8 p73 regulates core-PCP proteins localization by modulating actin and microtubule cytoskeleton dynamics.

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Planar Cell Polarity (PCP) refers to the asymmetric and coordinated distribution of cellular components in the direction of the tissue plane. PCP establishment depends on the polarization of cytoskeleton and requires the asymmetric localization of *core*-PCP proteins, including membrane proteins like Vangl1/2, Frizzled and Celsr1/3. However, the molecular mechanisms that govern these processes are not fully understood. The p73 transcription factor belongs to the p53 family, together with p53 and p63. They comprise a central node of interconnected pathways controlling cellular responses to pathological and physiological cues. The *TP73* gene has a dual nature that resides in the existence of TA and DNp73 variants; therefore the outcome of TP73 activation will depend upon the differential expression of these isoforms. We have unveiled novel roles of p73 as a regulator of the cytoarchitectural organization, being required for multiciliogenesis in mouse ependymal cells. Additionally, p73 plays a fundamental role in PCP establishment. Thus, the aim of this study was to elucidate the molecular basis of this regulation. Wild type and p73 knockout induced pluripotent stem cells (WT-iPSCs and p73KO-iPSCs) were used to study the localization of *core*-PCP proteins using immunofluorescence and confocal microscopy. To investigate whether p73 could induce transcriptional programs involved in cytoskeleton dynamics, we performed a Gene Onthology analysis from previously published TAp73β-ChIP-seq/RNA-seq data (GSE15780) (Koeppel et al., 2011) using DAVID Bioinformatics Resources and PANTHER™ GO slim. Our data demonstrate that TAp73 regulates PCP, at least in part, through the modulation of non-muscle myosin-II (NMII) activity *via* MLCK (myosin light chain kinase). In addition, TAp73 modulates cytoskeleton dynamics and is necessary for PCP-core proteins asymmetric membrane localization. GO-analysis confirmed TAp73 role as modulator of transcriptional programs regulating actin and microtubules dynamics and Golgi organization signaling pathways.

References: Koeppel, M. et al. Crosstalk between c-Jun and TAp73alpha/beta contributes to the apoptosis-survival balance. Nucleic Acids Res. 39 (2011) 6069–6085.

P9 TAp73, but no DNp73 or p53, is required for ependymal cell ciliogenesis and establishment of planal cell polarity

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Keywords: Ependymal cell, Planar Cell Polarity, p73, Non-muscle myosin II

Planar cell polarity (PCP) establishes tissue structure and coordinated behaviours across epithelial sheets. In multiciliated ependymal cells (EC), rotational and translational PCP coordinate cilia beating and direct cerebrospinal fluid circulation. Thus, PCP disruption results in ciliopathies and hydrocephalus. The causal relationship between ciliogenesis and PCP signalling is not fully deciphered. In this regard, the Trp73 gene is a key player in the organization of ciliogenesis and planar cell polarization of ECs. Trp73 belongs to the p53 family of transcription factors and generates functionally different TA and DNp73 isoforms. Recent studies from our group have unveiled new insights into the mechanisms underlying p73 regulation of PCP. In this work, we study the role of the different p73-isoforms in this process. Ependymal cells with total lack of p73 (p73KO-ECs), but not p53-deficient ECs (p53-KO-ECs), have severe ciliary defects, with many cells lacking ciliary axoneme and others displaying disorganized cilia of different lengths. Interestingly, cells that only lack the TAp73 isoform (TAp73KO-ECs), which is essential for NMII activation and the organization of actin and microtubule cytoskeleton, had defective BB docking and show ciliary defects, but most of them display a milder ciliary-phenotype than p73KO-ECs, suggesting that the DNp73 isoforms could have a compensatory role in absence of TAp73. However, analysis of the DNp73KO ependymal cells did not reveal any cilia defects, indicating that in the presence of TAp73, DNp73 is not necessary for ciliogenesis regulation. We also studied the role of DNp73 in the

regulation of PCP and observed that DNp73KO EC displayed well-oriented basal bodies and well organized and parallel cilia bundles; in addition, we did not find alterations in NMII activation and localization. Our data suggests that, in the presence of TAp73, DNp73 is not necessary for the translational and rotational PCP establishment and correct ciliogenesis in EC. Nevertheless, they also suggest that compensatory and redundant ciliary programs are induced in the absence of TAp73 when DNp73 is present, but not with total p73-deficiency. Thus, one might speculate that, in this scenario, compensatory DNp73 upregulation could induce, directly or indirectly, key ciliogenesis regulators, downstream of TAp73 function.

P10 Consequences of Tumor Suppressor p73-Deficiency on Cell Development and Pathogenesis

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The p73 tumor suppressor protein is a member of the p53 tumor suppressor family. It often functions to induce cell cycle arrest and cell death in response to DNA damage or conditions of cellular stress. In contrast, publically available data indicate the importance of p73 for initiating changes in gene expression that drive the early phases of tissue development and organogenesis. Specifically, p73 has been proven to activate maturation pathways during which a cell acquires functional and morphological specificity for its destined tissue, a pivotal biological dogma known as cell differentiation. Differentiation processes are frequently implicated in tumor formation and metastasis, for this reason, current biomedical studies endeavor to better understand the molecular regulation of cell development or lack thereof. The contributions of p73 to normal cell differentiation have yet to be examined in great depth and pathologies associated with the attenuation of *TP73* remain poorly defined.

The study of adipose tissue and its regulation have gained importance because of the high prevalence of obesity and its comorbidities in the developed countries. Adipose tissue is mainly composed of adipocytes, whose generation requires two process: adipogenesis and lipogenesis. Adipogenesis is the differentiation program in which mesenchymal progenitors become adipocytes. This differentiation program is tightly regulated by many transcription factors. It has been reported that Trp53 negatively regulates the process of white adipose tissue adipogenesis while it is a positive regulator of brown adipose tissue generation. However, there is no evidence regarding the role of its homologue TP73. In this work, our aim was to analyze the effect of p73 deficiency on adipogenesis *in vitro* employing a 3D differentiation cell culture protocol using p73-deficient embryonic stem cells. This innovative protocol presents an *in vitro* platform for modeling p73 function in human cell development.

Our results reveal that the lack of p73 affects the adipogenesis program. Resulting in reduced expression of markers and key genes of the mesenchymal stage of differentiation and in the terminal phase of adipocyte formation.

P11 Regulation of Δ133p53β isoform activity through an aggregation-dependent mechanism

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Keywords: Δ133p53β, aggregation, invassion

p53 isoforms and particularly Δ133p53β play a critical role in cancer progression. However mechanism of action and regulation of their activity is completely unknown. We demonstrated that wtΔ133p53β activity is regulated through a reversible aggregation-dependent mechanism. Namely, wtΔ133p53β is able to create aggregates in cancer cells due to the disruption of secondary structure. Absence of the N-terminal domain in wtΔ133p53β results in destabilisation and unfolding of the remaining DNA binding region. This event liberates amyloid-prone sequences that tend to stack wtΔ133p53β protein molecules between themselves. Creation of wtΔ133p53β aggregates depends on associations with specific interacting partners and was observed both in cancer cell lines and in human tumour biopsies. Interestingly, aggregate-forming capacity of wtΔ133p53β was independent of wt or mutated p53 since these proteins are not associating between themselves. Depletion of CCT chaperone complex promotes strong accumulation of Δ133p53β aggregates and loss of Δ133p53β dependent cancer cell invasion. In contrast, association with ΔNp63α significantly

reduced Δ133p53β aggregates resulting in an increase of invasiveness. This study shows that wtΔ133p53β aggregation capacity inversely correlates with its activity. Δ133p53β oscillates between non-aggregated, active, and aggregated, non-active states, which critically influence cancer cell features like migration and invasion. Our data lead us to conclude that the invasive activities associated with wtΔ133p53β isoform are not carried out by dominant negative activity on wtp53. This constitutes an original and undescribed mechanism of cancer progression that is basically different from conventional mutant p53 mechanism of action. This study introduces a new concept of oncogene reversible aggregation and represents a turning point in the understanding of the p53 signalling and more largely of the mechanisms that cause cancer.

P12 The E3 ubiquitin-protein ligase Mdm2 is a novel interactor of the von Hippel-Lindau tumor suppressor

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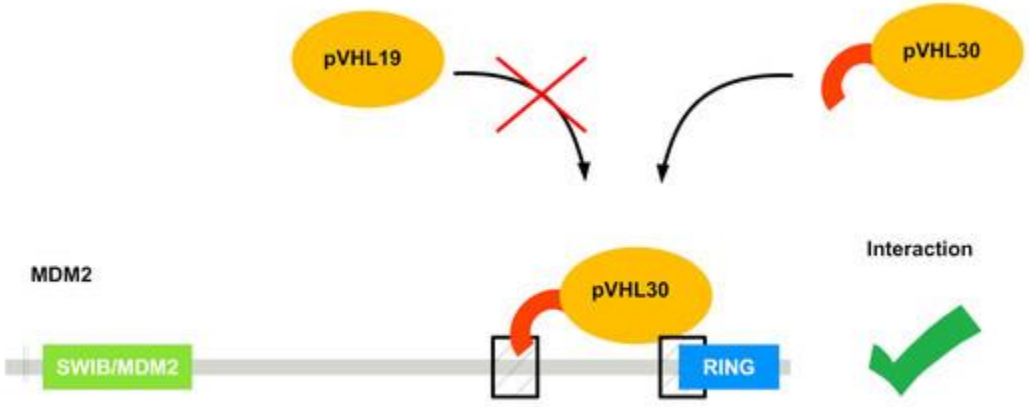
Keywords: pVHL, von Hippel-Lindau, hypoxia, MDM2, yeast, pVHL isoforms

Mutations of the von Hippel-Lindau tumor suppressor (pVHL) are causative of a familiar predisposition to develop different cancers. pVHL is mainly known for its role in regulating hypoxia-inducible factor 1-α (HIF-1α) degradation, thus modulating the hypoxia response. Previous studies however suggested that isoform-specific specializations can be associated with human pVHL. Here, we present a novel interaction between pVHL and Mouse double minute 2 homolog (MDM2). Integrating *in silico* predictions with *in vivo* assays we found that the N-terminal acidic tail of pVHL30 is required for its association with MDM2. Further, we demonstrate that an intrinsically disordered region upstream the tetramerization domain of MDM2 is responsible for its isoform-specific association with pVHL30. This region is mostly conserved in higher mammals, including human, similarly to what already proposed for the N-terminal tail of pVHL30. Collectively, our data support the idea that the isoform pVHL30 may play a role in MDM2 regulation, suggesting a wider interplay among hypoxia sensing and cell cycle regulation.



Schematic representation of pVHL30 association with MDM2

References: This work was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC) grant IG17753 to ST.

P13 BRD4 driven p63 regulates the cancer stem cell phenotype in head and neck squamous cell carcinoma

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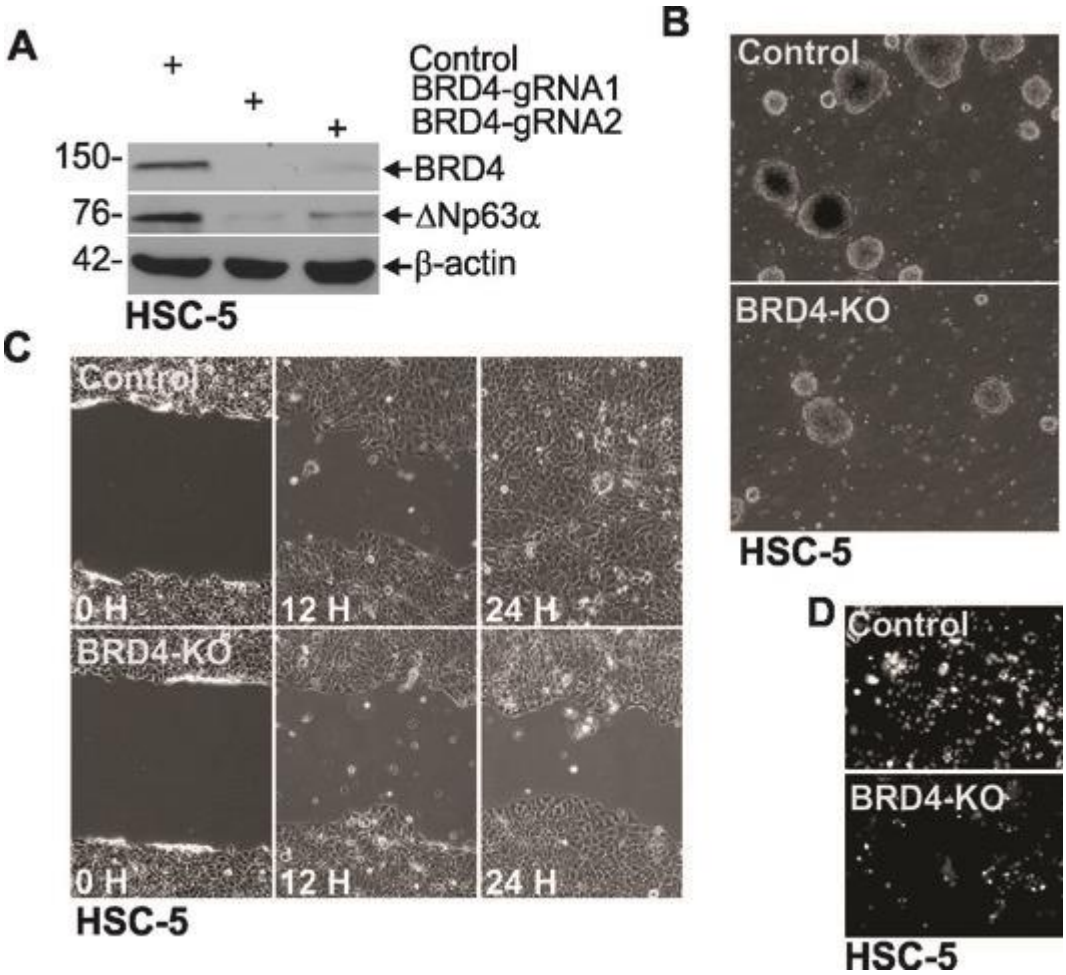
Keywords: BRD4, p63, cancer stem cells

Bromodomain containing protein 4 (BRD4) plays a critical role in mediating the expression of genes involved in development and cancer (1). Inactivating BRD4 inhibits cancer growth, making it a promising anticancer drug target. The cancer stem cell population is a key driver of recurrence and metastasis in cancer patients (2). We show that the cancer stem cell population of Head and neck squamous cell carcinoma displays enhanced migration, invasion, tumor growth and metastatic spread. We show that BRD4 is highly elevated in this aggressive subpopulation of cells, and its function is critical to the CSC phenotype. Moreover, BRD4 regulates □Np63□ a key transcription factor that is essential for epithelial stem cell function, and often overexpressed in cancers. Targeting BRD4 reduces □Np63□, leading to reduced spheroid formation, migration, invasion and tumor growth. Induced expression of □Np63□ following BRD4 Knockout or inhibition rescues the aggressive phenotype of HNSCC CSC. These studies show that BRD4 driven □Np63□ is a critical regulator of spheroid formation, migration, invasion and tumor growth in HNSCC CSC. Furthermore, this work identifies a novel BRD4 regulated signaling network in CSCs that represents a possible avenue of therapeutic intervention in HNSCC.



Caption: BRD4 regulates p63 and the cancer stem cell phenotype. A BRD4 knockout reduces p63 protein expression B spheroid formation C migration and D invasion

References: Donati B et al., Mol Cancer 16 (2018) 164 Pattabiraman DR, Weinberg RA Nat Rev Drug Discov. 7 (2014) 497-512

P14 Association of deltaNp73 isoform expression with centrosome amplification in tumor cell lines of neurogenic origin

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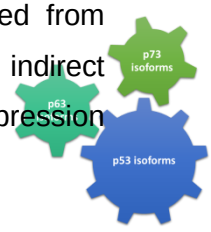
Keywords: deltaNp73, centrosome amplification, tumors of neurogenic origin

The centrosome is a microtubule-organizing center (MTOC) composed of two centrioles surrounded by a matrix of pericentriolar material. The centrosome is copied only once per cell cycle so that each daughter cell inherits one centrosome. The presence of more than two centrosomes in one cell is termed as centrosome amplification, which is very often linked to the appearance of genome instability. The p73 protein has a fundamental role in neuronal development, differentiation, and neurodegeneration. The expression of the N-terminal isoforms, TAp73 and deltaNp73, has been described in many cancer types including tumors of neurogenic origin. The TAp73 isoform co-localizes with the mitotic spindle and interacts with the spindle assembly checkpoint (SAC) proteins [1]. Loss of the TAp73 isoform impairs SAC function and promotes aneuploidy via a direct decrease in the efficiency and accuracy of mitosis. Our project is focused on the expression of p73 N-terminal isoforms and their effect on centrosome number in tumors of neurogenic origin. The special attention was paid to the increased expression of the deltaNp73 isoform in association with centrosome amplification. Our pilot study described a possible role both of p73 N-terminal isoforms in the process of centrosome amplification in cell lines derived from two types of brain tumors, glioblastoma multiforme and medulloblastoma [2]. Our present research verified the association of the deltaNp73 overexpression and centrosome amplification in eight cell lines derived from glioblastoma, medulloblastoma, and neuroblastoma. The cells were analyzed using indirect immunofluorescence to determine the TAp73 and deltaNp73 localization and expression



patterns, as well as the number of centrosomes. Overexpression of both p73 isoforms was used for verification of the relation between higher expression of deltaNp73 and centrosome amplification. The p73 protein isoforms demonstrated nuclear localization with the specific non-random accumulation in the cytoplasm near the cell nucleus. Based on evaluation of the centrosome amplification and expression pattern of the TAp73 and deltaNp73 isoforms, we found that increased expression of the deltaNp73 isoform is associated with the occurrence of abnormal number of centrosomes in the particular cell. However, these results were not confirmed using stable transfection of TAp73 and deltaNp73 isoforms in the selected cell lines.

References: 1. Rufini et al., Genes Cancer., 2 (2011) 491-502. 2. Mikulenkova et al., Tumor Biol., 36 (2015) 7483–91. This study was supported by the project No. LQ1605 from the National Program of Sustainability II (MEYS CR).

P15 Mutant p63: Loss and gain of function in genetic disorders lead to distinct phenotypes depended on the isoform context

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Keywords: p63, syndrome, aggregation, GOF, LOF

Cancer-associated mutations found in the DNA binding domain (DBD) of the tumour suppressor p53 result in its loss of function (LOF) by impairment of DNA binding. A subset of mutations acts by thermodynamic destabilization of the metastable DBD resulting in its unfolding. Subsequent unmasking of aggregation-prone regions (APRs) in the DBD core leads to its aggregation (gain-of-function; GOF).

The DBDs in the p53 family share a high sequence homology. The same mutations in the p53 DBD found in cancer, are also present in p63 in the context of the genetic disorders EEC, LMS and ADULT (combined ELA). While the p53 DBD is only metastable at physiologically temperature, the p63 DBD is highly stable. Therefore mutations only lead to impaired DNA binding by loss of direct DNA contact residues or disruption of the folded and rigid DNA interaction surface needed for specific binding to p63 promoter sequences. While latter, depended on the exact mutation, can be accompanied by the loss of the structural important zinc ion, in all cases the well folded core of the p63 DBD remains intact. Hence, in contrast to mutp53, all p63 DBD mutations are merely loss-of-function mutations, which do not unfold and subsequently aggregate.

The C-Terminus of p63α consists of a sterile alpha motif (SAM) and TI domain, where the mutations causing the Ankyloblepharon-Ectodermal defects-Cleft lip/palate (AEC) syndrome cluster. A conditional knock-in mouse model carrying the AEC mutation L514F in the SAM domain shows skin erosion and scaling accompanied by reduced epidermal resilience. This phenotype, representing the one of human patients, is unique to the AEC and not found in the

EEC syndrome. AEC mutations have a reduction in DNA binding and are impaired in activation or suppression of p63 target genes, although the mutations are located in domains not associated with DNA binding and transactivation. These findings emphasize a different molecular mechanism compared to the EEC syndrome: AEC mutations cause either the unmasking or generation of APRs in the SAM and TI domain, respectively. Latter is achieved by point mutations as well as frame shifts. Subsequent formation of large protein aggregates, which have only been described for mutp53, results in inactivation of p63 function as well as a dominant negative effects on WT p63 and p73, but not p53. Importantly, p63 variants able to abolish aggregation rescue the activity. These studies reveal the AEC syndrome as a protein aggregation disorder and open new avenues for therapeutic intervention.

So far the reported phenotypes of the patients almost exclusively originate from the malfunction of the isoform ΔNp63α, playing an important role in limb and epidermal development. In contrast, the second major isoform of p63, the inactive dimeric TAp63α, is highly expressed in resting oocytes and functions as a genomic quality control factor. Naturally, as all p63 isoforms are encoded on the same gene, TAp63α is mutated in those syndromes as well. The previously described point mutations in the DBD and SAM domain will lead to a loss-of-function and could consequently reduce or impair genomic quality control, while frameshift mutations will result in an active open tetramer by destabilization of the inhibitory complex. The constitutive active p63 could then kill the resting oocytes even without DNA damage. The potential primary ovarian insufficiency (POI) of female patients involves early entry of menopause accompanied by disturbance of essential hormonal functions.

References: 1. Coutandin, D. et al., ELife (2016) 5, e13909 2. Kehrloesser, S. et al., Cell Death Differ. (2016) 23, 1952–1960 3. Russo, C. et al., Proc. Natl. Acad. Sci. (2018) 201713773

P16 Single cell approaches to unravel the cell fate determination of epidermal keratinocytes and limbal stem cells.

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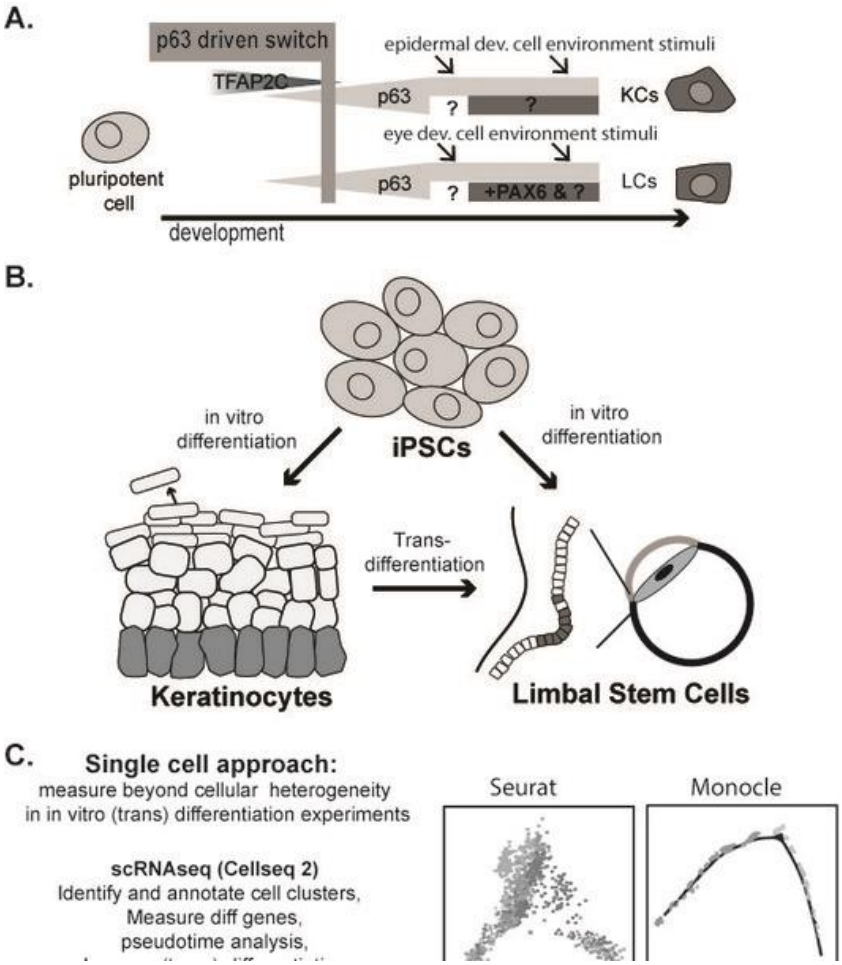
Keywords: p63, Limbal Stem Cells, Keratinocytes, single cell RNA, single cell ATAC

Over 60% of differentiated tissues in humans are epithelia, however the exact mechanisms governing the cell fate determination of these tissues are still unknown. For example, epidermal keratinocytes (KCs) and limbal stem cells (LSC) share many similarities including marker gene expression, but they are functionally distinct. We hypothesize that cell fates of KCs and LSCs are controlled by overlapping yet distinctly different transcriptional networks. In both cell types the transcription factor (TF) p63 (isoform ΔNp63α) plays a vital role, however the TFs cooperating with p63 in determination of epidermal KCs and LSCs are still mostly unknown. In order to look into TF hierarchy we will measure both the transcriptome using RNAseq for gene expression changes over time, while also measuring gene accesibility using ATAC sequencing. This will provide us with genomic accesibility changes, using motif enrichment analysis to predict TF activity.The biological model used to dissect the TF hierarchy will contain two differend aproaches. First, we will use in vitro iPSC differentiation to generate KCs and LSCs. This will provide insights into the temporal expression and activity of TFs during commitment towards these two different cell types. The second approach will be direct transdifferentiation from KCs toward LSCs. This transdifferentiation will be performed using PAX6, an important TF for eye development. We expect to find TFs that can cooperate with both p63 and PAX for the specification of LSCs. A challenge using these approaches is cellular heterogeneity. To tackle this issue, we will use single-cell methods including single cell RNA sequencing for differential gene expression and single cell ATAC sequencing for differential genomic accessibility and TF prediction based on motif enrichment. Both techniques are well established in our lab. These single-cell datasets can be analysed to characterize the molecular events and mechanisms governing the cell fate determination of epidermal KCs and LSCs. Unravelling cell fate determination and TF hierarchy can improve transdifferentiation for treatment of limbal stem cell deficiency, and provide insights into disease mechanisms.



Overview epithelial development project, A: Transcription factor Hierarchy during Keratinocyte and Limbal Stem cell differentiation, B: Overview cell types and (trans)differentiation experiments. C: Used single cell approaches.

References: Fang, Rongxin, et al. “Fast and Accurate Clustering of Single Cell Epigenomes Reveals Cis-Regulatory Elements in Rare Cell Types.” bioRxiv, 13 May 2019, p. 615179, doi:10.1101/615179. Soares, Eduardo, et al. “Single-Cell RNA-Seq Identifies a Reversible Mesodermal Activation in Abnormally Specified Epithelia of p63 EEC Syndrome.” Proceedings of the National Academy of Sciences of the United States of America, vol. 116, no. 35, Aug. 2019, pp. 17361–70. Stuart, Tim, et al. “Comprehensive Integration of Single-Cell Data.” Cell, vol. 177, no. 7, June 2019, pp. 1888–902.e21. This research is supported by the Netherlands Organisation for Scientific Research(NWO/ALW/Open Competition/ALWOP 376)

P17 TA*p63 and GTAp63 achieve tighter transcriptional regulation in quality control by converting an inhibitory element into an additional transactivation domain

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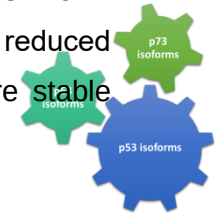
Keywords: TA*p63, GTAp63, dimer, p300, breast cancer

For the transcription factor p63, there exist several isoforms originating from alternative splicing, resulting in different C-termini (α-ε), or different promotor usage, yielding in TAp63 or the N-terminal truncated ΔNp63. While ΔNp63α is located in stem cells in the basal layer of stratified epithelia and is responsible for maintaining the proliferation capacity of the epidermal stem cells, TAp63α is highly expressed in primary oocytes and protects the genomic integrity of the female germline.

Besides to the N-terminal elongated TAp63, there exist two related isoforms with an extended N-terminus: GTAp63 and TA*p63. In contrast to the major isoforms ΔNp63α and TAp63α, the occurrence and function of GTAp63α and TA*p63α are poorly understood up to this point.

GTAp63α is expressed in spermatogenic precursors and is proposed to be the male counterpart of TAp63α for germ cell quality control in humans and great apes (*Hominidae*), while TA*p63α is not described in literature. GTAp63α and TA*p63α have an unique N-terminal elongation of 37 and 39 amino acids (AA), respectively, with the last 18 AA being shared by both.

To elucidate the function of these N-terminal elongations, the “novel” isoforms were characterized in various biochemical and biophysical assays. TA*p63α and GTAp63α are located in the nucleus like their p63 family members. They form inactive dimers as it is known for TAp63α. However, the dimers show a higher kinetic stability, resulting in reduced unintended tetramerization and pro-apoptotic activity in the cell. Despite the more stable



dimeric conformation, both are activated via the same phosphorylation mechanism, as known for TAp63α in oocytes, adopting active tetrameric conformations. In the open, tetrameric state, the two N-terminal extensions expose additional transactivation motifs similar to the FWL (F16, W20, L23) motif in the TA domain able to interact with the co-activator p300. Through this mechanism, the difference in transcriptional activity between the repressed and the active state of the proteins gets enhanced compared to TAp63α.

Finally, we show by MS analysis that TA*p63α is expressed in the breast cancer cell line Sum159 at the protein level together with a mutant p53 form. Upon doxorubicin treatment, TA*p63α gets activated and apoptosis is induced. Thus, TA*p63α is a potential new tool to fight cancer.

References: S. Pitzius et al, Cell Death and Disease (2019) 10:686

P18 Generation of mouse embryonic stem cell lines lacking p73 isoforms using the CRISPR/Cas 9 gene editing system.

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Human lifespan has significantly increased during the last decades in our society. Aging is usually associated with conditions such as cancer, a disease whose incidence is increasing, bringing up the need for new therapeutic alternatives. As cancer stem cells arise from normal cells in different stages of differentiation, their biogenesis can be viewed as a reprogramming process. Indeed, the acquisition and maintenance of full tumorigenic potential and the stemness phenotype are highly comparable processes with striking similarities. For example, mesenchymal to epithelial transition (MET) is required to fully reprogram fibroblasts to induced pluripotent stem cells (iPSC); its reverse process, epithelial to mesenchymal transition, is a genetic program active during embryonic development and demonstrated to potentiate features of cancer cells, like invasion and metastasis. In addition, both processes require the expression or activation of oncogenes or the inactivation of tumour suppressor genes. Several discoveries have already demonstrated the involvement of the p53 family members in cell reprograming. p53 and p63 seem to play opposites roles in mouse embryonic fibroblasts reprogramming: while p53 acts as a barrier, p63 has been reported to act as an enabling factor. The information regarding p73 role in this process is contradictory. Our group has demonstrated that p73 attenuates reprogramming efficiency by abating BMP induced MET, even in the absence of p53. p73 deficiency results in an impairment of the initial steps of the process, generating iPSCs with self-renewal capacity but overall stemness alterations. Thus, the goal of this study is to develop cellular models that will allow us to untangle the mechanisms of p73 function in the maintenance of cell stemness. Using the CRISPR/Cas9 gene editing system we have generated different mouse embryonic stem cell lines specifically lacking the p73 isoforms TAp73 or DNp73 that will be used to characterize the stemness phenotype in the different cell microenvironments.

References: Martin-Lopez et al. Cell Death and Disease, 2017. 8(9):e3034

P19 p53 in resistance to targeted therapy in metastatic melanoma

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Keywords: p53 isoforms, metastatic melanoma, treatment resistance, vemurafenib

Malignant melanoma is the most aggressive form of skin cancer with increasing incidence. Recent advances in melanoma therapy that improve overall patient survival are held down by rapid and pervasive treatment resistance. Targeted therapy, like BRAF inhibitor (BRAFi) therapy for melanoma patients harboring the V600E mutation, is initially highly effective, but a majority of the patients develop resistance and relapse within a few months. The tumor suppressor protein p53, mutated in more than 50% of human cancers, is rarely mutated in metastatic melanoma, but, nevertheless it fails to execute its tumor suppressor activity.

To better understand the mechanisms of resistance to BRAFi targeted therapy, and to reveal the potential role of p53 isoforms in the process of acquisition of resistance, we generated vemurafenib-resistant metastatic melanoma cell lines by growing the A375M and WM793B cells in the vemurafenib-enriched medium and confirmed resistance by MTT assay. Vemurafenib is a BRAFi, used for the treatment of late-stage melanoma with the common BRAFV600E mutation. We analyzed the expression profile of p53 genes and proteins in control and resistant cell lines. Morphological change of newly generated resistant cell lines led us to test EMT (epithelial-mesenchymal transition) markers and we discovered partial EM transition. It is known that partial EMT increases invasive cell properties and promotes resistance to anti-cancer drugs. Furthermore, we determined transcriptional and quantitative gene expression by mass-based parallel cell mRNA sequencing (RNA-seq). The data obtained from RNA-seq showed that a mechanism of resistance differs between two cell lines. Activation of different signaling pathways presents promising results and sets up a path for further research.

P20 Characterisation of transcriptional targets regulated by Δ40p53 in response to DNA damage in breast cancer cells

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Keywords: Breast cancer, Δ40p53, RNA-seq, DNA damage

Breast cancer is the most commonly diagnosed malignancy among women worldwide and the second leading cause of cancer-related deaths. p53 is a critical tumour suppressor that is not commonly mutated in breast cancer (< 25%), indicating compromised canonical function of the p53 protein through other mechanisms. We have previously reported that Δ40p53, one protein variant of full-length p53 (p53α), is highly expressed in breast cancers and related to worse disease-free survival, suggesting a negative role for Δ40p53 in treatment response and tumour progression (1,2). Δ40p53 lacks the N-terminal transcriptional activation domain and studies utilising overexpression vectors have demonstrated that its transcriptional activation capacity is distinct from that of the full-length p53 protein. However, there is limited knowledge regarding the transcriptional targets regulated by Δ40p53 endogenously. The aim of this study was to define the transcriptional targets regulated by Δ40p53 in breast cancer cells at the basal level and in response to DNA damage. The Δ40p53/p53 ratio was modified through Δ40p53-overexpression and Δ40p53-/p53-knockdown and sublines derived from these breast cancer cells (MCF-7, ZR-75-1) were left untreated or treated with doxorubicin (DOX) for 24 hours prior to performing RNA-Seq analysis. Differentially expressed genes (DEGs) were identified between sublines and the corresponding control sublines at the basal level, and between untreated and DOX-treated conditions for each of the sublines. The results showed, that at the basal level, there was very little overlap in the transcriptional targets regulated by either Δ40p53 or full-length p53; and the genes regulated were cell-line dependent. Additionally, endogenous Δ40p53 regulated a similar number of transcripts as p53 in MCF-7 cells, but almost double the number of transcripts when compared to p53 in ZR-75-1 cells. Interestingly, following DNA damage the targets regulated by Δ40p53 or full-length p53 remained distinct.

Hierarchical clustering demonstrated that knockdown of Δ40p53 in MCF-7 cells resulted in more repressed transcription of target genes, whilst overexpression resulted in less repressed transcription of target genes by DOX. In contrast, knockdown of p53 resulted in suppression of DNA-damage induced gene transcription in ZR-75-1 cells, whilst Δ40p53 had limited effect on the same set of target genes. These results suggest that the target genes regulated by Δ40p53 are distinct from p53 both before and following DNA damage and that the impact of endogenous Δ40p53 expression is context dependent.

References: (1) Avery-Kiejda KA et al; Carcinogenesis 2014; 35(3), 586-596. doi:10.1093/carcin/bgt411 (2) Morten BC et al; Carcinogenesis 2016; 37(1): 81-6. doi: 10.1093/carcin/bgv164 This work was supported by grants from the Bloomfield Group Foundation and Pink Frangipani Ball through the Hunter Medical Research Institute (HMRI) (to KAK); the Australian Postgraduate Award (to XZ) and the MM Sawyer Scholarship through HMRI (to BCM). KAK is a Cancer Institute NSW Career Development Fellow.

P21 Bemcentinib Modulates p53 Isoforms, Axl and FLT3 Expression in Acute Myeloid Leukemia (AML)

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Recent studies in solid cancers have indicated an association between the receptor tyrosine kinase Axl – involved in enhanced proliferation and metastasis – and p53. Our aim was to study p53 isoform expression following treatment with clinically relevant concentrations (< 700 nM) of the drug bemcentinib – a kinase inhibitor of the receptor tyrosine kinase Axl currently in clinical trials for the treatment of acute myeloid leukemia (AML). In AML, TP53 is rarely mutated (5-10%) compared to internal tandem duplications in fms-like tyrosine kinase receptor (FLT3-ITD, 25%), both associated with a bad prognosis. In AML patient-derived cells we previously demonstrated a correlation between expression of full-length p53 alpha and mutated FLT3.

The AML cell line MV4-11 (p53wt, FLT3-ITD, Axl expressing) was treated with vehicle control (Ctr) or increasing concentrations of bemcentinib (100, 250 and 500 nM) for 24h cell and 72h. This treatment resulted in reduced cell proliferation, G0-G1 cell cycle arrest and decreased cellular size, however, no significant cell death was observed. By immunoblotting we found that the expression of full-length p53 alpha and full-length p53 beta/gamma expression was decreased, particularly following treatment with 250 nM and 500 nM bemcentinib. Preliminary results using p53 isoform specific antibodies for the expression of Delta133p53beta and Delta160p53beta showed an oscillating expression pattern after 24h and 72h drug exposure. We also found an increased expression of MDM2 and the fully glycosylated cellular receptors Axl and FLT3 whereas as the autophagic marker LC3B was reduced. Studies are currently

ongoing to further map the expression levels (protein and RNA) and subcellular localization of the p53 isoforms and to characterize the molecular pathways involved.

P22 TP53 mutations as drivers of tumorigenesis – in vitro models for ovarian cancer

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Keywords: ovarian cancer, p53-singnature, organoids, CRISPR/Cas9

Mutations in the tumour suppressor gene *TP53* are frequently associated with tumorigenesis. High Grade Serous Ovarian Cancer (HGSOC) is commonly predisposed by mutations in *TP53* and in BRCA1 and BRCA2, two genes involved in DNA-damage repair mechanisms (Karst *et al.*, 2011). HGSOC is proposed to originate in the fallopian tube epithelia, due to a continuous inflammation in the fallopian tube, as a consequence of ovulation and chronic infections. Tubal epithelial cells seem to undergo genetic and molecular transformation, after which they infiltrate the oviductal tissue, promoting the development of the adenocarcinoma (Quartuccio *et al.*, 2015). Infection of tubal epithelial cells with *Chlamydia trachomatis* has shown proteolytic degradation of p53, which seemed to promote the intracellular development of the bacteria through a still unclear mechanism (Gonzalez *et al.*, 2014). To investigate the function of mutant p53 proteins or loss of p53 in the fallopian tube epithelium I began generating tubal organoids carrying the most common point mutation of *TP53* (*R273H*) using CRISPR-Cas9 technology. After characterizing the phenotype of these mutants, we aim to assess the survival of wild-type and p53-mutant organoids through *in vitro* competition assays and to evaluate the molecular niche upon infection with *C. trachomatis.* Currently, I am trying to optimize the culture conditions for the mutated tubal organoids, as I observed that the stemness of these organoids is impaired upon transfection. In parallel, I am doing a complementary study of mouse-derived fallopian tube organoids carrying another gain-of-function mutation in *TP53 – R248Q*.

References: Karst, A.M., Levanon, K., and Drapkin, R. (2011). Modeling high-grade serous ovarian carcinogenesis from the fallopian tube. Proceedings of the National Academy of Sciences of the United States of America 108, 7547-7552. Kessler, M., Hoffmann, K., Brinkmann, V., Thieck, O., Jackisch, S., Toelle, B., Berger, H., Mollenkopf, H.J., Mangler, M., Sehouli, J., et al. (2015). The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. Nat Commun 6, 8989. Quartuccio, S.M., Karthikeyan, S., Eddie, S.L., Lantvit, D.D., E, O.h., Modi, D.A., Wei, J.J., and Burdette, J.E. (2015). Mutant p53 expression in fallopian tube epithelium drives cell migration. International journal of cancer 137, 1528-1538.

P23 p53 isoforms integrate signals in HCT116 cells after treatment with oxaliplatin

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Keywords: p53 isoforms, cell signaling, post-translational modifications, 2D gel electrophoresis

In the past decades data have been gathered leading to the understanding that p53 functions as a multi-protein system composed by twelve isoforms and that the p53-mediated response is the sum of the intrinsic activities of all the co-expressed isoforms.

In order to have a specific biological response signals need to be integrated by the cells and the best way in which they integrate signals is by addition of post-translational modifications on proteins.

By performing western blotting and two-dimensional gel electrophoresis of HCT116 cells treated with oxaliplatin we investigated whether the α and beta isoforms integrate signals in response to the treatment.

We show that different isoforms are co-expressed in absence of treatment and that their expression levels are modulated in association with post-translational modifications after the treatment.

p53α is not the only post-translationally modified protein of the p53 multi-protein system: other isoforms, such as Δ40p53α and Δ160p53β, are modified and therefore integrate signals. Further investigation will be performed to decipher the cell signaling pathways.wqe

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