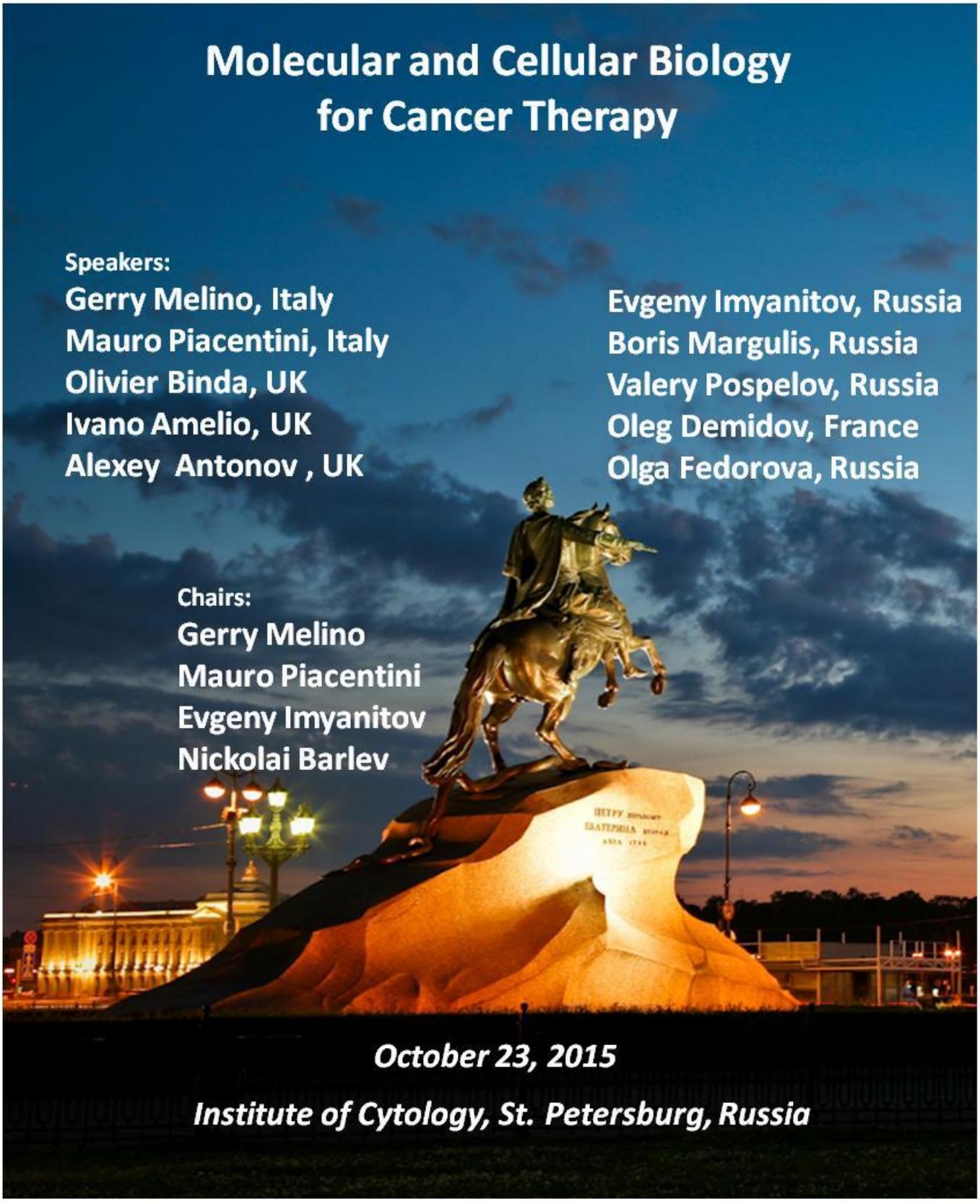
# International Symposium “Molecular oncology”




Russian Science Foundation 14-50-00068

# Welcome Address

Dear Colleagues,

We are delighted to welcome you to the second Mini-Symposium on Molecular oncology held in the Institute of Cytology, Russian Academy of Sciences. This conference is supported by the Grant to the Institute of Cytology from the Russian Science Foundation 14-50-00068 as part of the scientific development of the institute.

The main emphasis of this event is on innovative therapeutic aspects in the broad field of molecular and cellular biology with particular focus on the inducers and mechanisms of cell death, epigenetic modulators, tumor suppressor proteins of the p53 family and functions of the molecular chaperons in tumorigenesis.

This conference that attracted the world leading scientists both from Russia and abroad will provide an opportunity for researchers to share their achievements, discuss pivotal questions in the field and explore potential collaborations.

We very much hope that you have a productive and enjoyable time during the conference!

Yours sincerely,

**The Organizing Committee**

**The Organizing Committee:**

Dr Nickolai Barlev
Prof Boris Margulis
Dr Dmitry Tentler
Dr Olga Fedorova

**Chairs:**

Prof Mauro Piacentini
Dr Nickolai Barlev
Prof Gerry Melino
Prof Evgeny Imyanitov

**PROGRAM OF THE SATELLITE SIMPOSIUM "MOLECULAR ONCOLOGY"**
**AS PART OF THE 2nd INTERNATIONAL MINI-SIMPOSIUM**
**"MOLECULAR AND CELLULAR BIOLOGY FOR CANCER THERAPY"**
**(**в рамках выполнения Комплексной научной программы развития Института цитологии РАН по гранту Российского научного фонда 14-50-00068**)**

**OCTOBER 23, 2015**
**Institute of Cytology of the Russian Academy of Sciences**
**Conference Hall, Tikhoretsky pr., 4, Saint-Petersburg**

09.30-10.00 REGISTRATION
**Chairs: Prof Mauro Piacentini and Dr Nickolai Barlev**
10.00-10.10 WELCOME GREETINGS FROM ORGANIZERS

10.10-11.00 THE KEYNOTE LECTURE © *Prof Gerry Melino, University Tor Vergata, Rome, Italy*

11.00-11.20 CROSSTALK BETWEEN P53 FAMILY AND HYPOXIA INDUCIBLE FACTOR IN CANCER. © *Dr Ivano Amelio MRC Toxicology Unit, Leicester, UK.*

**11.20-11.50 COFFE BREAK**

11.50-12.35 THE REGULATION OF AUTOPHAGY IN MAMMALS: THE ROLE OF AMBRA1. © *Prof Mauro Piacentini, University Tor Vergata, Rome, Italy*

12.35-13.00 THE INHIBITOR OF GROWTH ING3 STIMULATES PROLIFERATION OF PROSTATE CANCER CELLS © *Dr Olivier Binda, University of Newcastle, Newcastle, UK*

13.00-13.45 HEREDITARY CANCER SYNDROMS IN RUSSIA@ *Prof Eugene Imyanitov, Petrov Institute of Molecular Oncology, St-Petersburg, Russia*

**13.45-14.45 LUNCH**

**Chairs: Prof Gerry Melino and Prof Eugene Imyanitov**
14.45-15.15 MODULATION OR TUMORGENECITY BY HDJ2 MOLECULAR CHAPERONE © *Prof Boris Margulis, Institute of Cytology, St-Petersburg, Russia*

15.15-15.45 SIGNALING PATHWAYS INVOLVED IN MAINTENANCE OF SELF-RENEWAL AND PLURIPOTENCY OF MOUSE EMBRYONAL STEM CELLS © *Prof Valery Pospelov, Institute of Cytology, St-Petersburg, Russia*

**15.45-16.20 COFFE BREAK**

16.20-16.50 ESTIMATING THE EFFECT OF P53 MUTATION STATUS ON GENE REGULATION IN CANCER FROM GENE EXPRESSION DATA © *Dr Alexey Antonov, MRC Toxicology Unit, Leicester, UK.*

16.50-17.20 TARGETING P53-NEGATIVE TUMORS WITH ACTIVATION OF STRESS-INDUCED PHOSPHATASE , WIP1© *Dr Oleg Demidov, INSERM, Dijon, France*

17.20-17.40 TALK FROM POSTERS *Olga Fedorova, Institute of Cytology, St-Petersburg, Russia*

17.50-18.20 SPONSOR TALKS

18.20-18.25 CONCLUDING REMARKS

## SPEAKERS

**Prof. Gerry Melino, MD, PhD, DSc hc**

working at the MRC Toxicology Unit (Leicester UK) and at the University Tor Vergata of Rome (Italy), has created the major forum for discussion and innovation in the field of cell death in the last twenty years. In fact, his editorial contribution to the scientific community has been pivotal, being the Founder & Editor-in-Chief of the journals *Cell Death Differentiation*(www.nature.com/cdd) and *Cell Death Disease*(www.nature.com/cddis), of impact factor 8.5 and 5.5 respectively. Recently, he gas launched another journal *Cell Death Discovery*. His scientific interest focuses upon programmed cell death in epidermal and cancer models, and in particular on the p53 family - p63 and p73, where his contribution has been fundamental.

**Prof. Mauro Piacentini**

is a Full Professor at University of Rome "Tor Vergata", Rome, Italy. He is also President of the Biotechnology Program at the university, is on the Board of Directors for the European Cell Death Organization and is Basic Research Director at the National Institute for Infectious Diseases in Rome. Since 1993, he has been the Founder and an Editor of the journal *Cell Death & Differentiation*. He is a journal reviewer for other journals including *Brain Research*, *Cancer Research*, *Cancer Cell*, and *Nature*. Furthermore, Dr Piacentini has organized several international meetings including the 14th Euroconference on "Apoptosis or Programmed Cell Death". He has co-edited a book entitled "Methods in Enzymology: Programmed Cell Death". His research interest is to understand the molecular mechanisms regulating apoptosis and autophagy under both physiological and pathological conditions. In particular, he is interested in the pathogenesis of Huntington's disease with particular regard to the role of TG2 and mitochondria. He is also studying infectious diseases such as HIV and HCV. With autophagy, he is characterizing the role of Ambra1, a key component of the Beclin1 complex.

**Dr Olivier Binda, PhD**

received his PhD in Biochemistry from McGill University, Montréal, Canada in 2007. After that he spent two years at Stanford University, Palo Alto, USA (2007 - 2009) as a post-doctoral fellow. Following this, he returned to Canada to become a postdoctoral fellow at the Lady Davis Institute for Medical Research, Montréal, Canada (2009 - 2012). Currently, he is a group leader and Fellow at the Northern Institute for Cancer Research, Newcastle upon Tyne, UK. His primary research interests concern the role of the lysine methyltransferase SETD6 in breast carcinomas development and metastasis.Also, he is interested in elucidating the role of lysine methylation of histones in carcinogenesis.

**Dr Ivano Amelio, PhD**

is a postdoctoral research fellow at MRC, Toxicology Unit in Leicester (UK). His research interests focus the functions of the p53 family members, p63 and p73. His current research aims the identification of novel p73 targets involved in different aspects of cancer transformation and progression. Ivano is also using selective genetic engineered mouse models to dissect the roles in development and cancer of the different isoforms codified by p63 and p73 genes. He received his PhD in Biochemistry and Molecular Biology from University of Rome, Tor Vergata, (Italy) in 2011, elucidating a novel mechanism, of epidermal differentiation regulation, promoted by a microRNA.

**Prof. Evgeny N. Imyanitov, MD**

He holds positions of the Head of Department of Tumor Growth Biology (N.N. Petrov Institute of Oncology), Head of Department of Medical Genetics (St.-Petersburg Pediatric Medical University), and Professor of Department of Oncology (Medical Academy for Postgraduate Studies) in St.-Petersburg, Russia. He graduated from the I.P. Pavlov Medical School (St.-Petersburg, Russia) in 1989. Evgeny Imyanitov completed his PhD studies in N.N. Petrov Institute of Oncology (St.-Petersburg, Russia) in 1992, and received the IARC Fellowship for post-doctoral training in the Max Planck Institute of

Biochemistry (Munich, Germany). In 1996 he returned to the home institute and established a research group. His current activities are focused on translational aspects of cancer science. In particular, the team of Prof. Imyanitov made a substantial contribution in the studies of hereditary cancer syndromes. Furthermore, Evgeny Imyanitov published a number of articles devoted to molecular-based customization of cancer therapy. In addition to clinically oriented research, the Evgeny Imyanitov's laboratory investigates some basic aspects of cancer pathogenesis; for example, there are systematic investigations on molecular features of bilateral breast cancer. Prof. Imyanitov is an author of over 100 publications in internationally recognized scientific journals.

**Dr Alexey Antonov**, **PhD**

He received his Biophysics Masters degree in 1997 and Applied Math Ph.D. in 2001, both from Moscow Institute of Physics and Technology. He underwent postdoctoral work at Moscow Institute of Physics and Technology but since 2003 was working at the Helmholtz Center Munich as a senior scientist at the Bioinformatics Institute where he developed novel methods for analyses and interpretation of microarray data. In 2011, he joined MRC Toxicology Unit (Leicester, UK). His scientific interests focus on the development of algorithms and software for various areas of molecular biology, chemical biology and biomedical applications. His current projects are centered on high throughput chemical screens to elucidate potential therapeutic mechanisms of complex diseases.

**Dr Oleg Demidov, MD, PhD**

He obtained his MD in 1994 from the Military Medical Academy, St.Petersburg, Russia. In 1999 he obtained PhD in Physiology and Biochemistry from the Military Medical Academy and Institute of Cytology, Russian Academy of Sciences, St.Petersburg, Russia. Dr Demidov has been working in the field of p53 regulation since year 2000 first as a Visiting Fellow at the Laboratory of Cell Biology headed by Dr Fornace at NCI, NIH, Bethesda, MD, USA and then as a research fellow at the Institute of Molecular and Cell Biology in Singapore. In 2009 he became an independent researcher and a group leader at INSERM, University of Burgundy, Dijon, France, where he studies the role of WIP1 phosphatase. In 2014 Dr Oleg Demidov received a prestigious grant award from the Russian Science foundation to run a group in the Institute of Cytology, St-Petersburg, Russia.

**Prof. Boris Margulis, PhD, DSc**

He has started his scientific career as a physicist (Department of Physics, Leningrad State University). Then he worked in Vladivostok for a few years. He obtained his DSc degree in the Institute of Cytology RAS. From 1998 to 2011 Dr Margulis was a Head of the Laboratory of Cell Defense Mechanisms. He is currently an interim Director of the Department of Cell Culturing at the Institute of Cytology, RAS. The major research interest of Dr Margulis is the biology of cell response to heat shock stress and pathogens.

**Prof Valery Pospelov, PhD, DSc**

He graduated from the Biology Department of Saint-Petersburg State University. In 1989, after defending his PhD and DSc degrees, he was appointed as the head of the Laboratory of the Molecular Mechanisms of Cell at the Institute of Cytology. The research interests of Dr Pospelov focus on the cell and molecular biology with a primary focus on pluripotent stem cells, such as embryonic stem (ES), molecular mechanisms of mammalian cell transformation by viral and cellular oncogenes, epigenetic mechanisms of gene regulation in transformed cells, cellular senescence as a powerful antitumor mechanism.

**Dr Olga Fedorova, PhD**

She graduated from Saint Petersburg Polytechnical University, Department of Medical Physics and Bioengineering as Master of Biophysics in 2006. In 2012 she obtained PhD degree in Molecular Biology in the Institute of Cytology, Russian Academy of Sciences. Her project concerned the elucidation of the role of proteasomes in RNA degradation and splicing. Since 2012 she is working in the Laboratory of Gene Expression Regulation in the Institute of Cytology. Her scientific interests are focused on the protein degradation by the ubiquitin-proteasome pathway and new transcriptional targets of p53 in respect to tumor progression.

**Gerry Melino**

## The p53 family in cancer biology

Ivano Amelio[1], Francesca Bernassola[2], Tak Wah Mak[2] and Gerry Melino[1,3]
[1] MRC Toxicology Unit, LeicesterLE1 9HN, United Kingdom
[2] The Campbell Family Cancer Research Institute, Toronto, Ontario M5G 2M9, Canada
[3] University of Rome Tor Vergata, Rome, Italy

p73 and p63 are a members of the p53 family, transcribed as two distinct isoforms TA-isoforms and DN-isoforms, containing or not the N-terminal transactivation domain. Both p63 and p73 are involved in female infertility maternal reproduction (Nature Rev Mol Cell Biol 2011;12,4:259-65) and as well as in cancer formation (TiBS 2014;39(4):191-8). We identified their activation during DNA damage, several transcriptional targets, the mechanisms of regulation of cell death, and the protein degradation pathway.

TAp73 knockout mice show high tumor incidence with hippocampal dysegensis. Conversely, ΔNp73 knockout mice show a very low incidence of cancer, with sign of moderate neurodegeneration with a significant loss of cellularity in the cortex. This indicates a tumor suppressor role for TAp73 and an oncogenic role for ΔNp73. Here, we demonstrate that the transcription factor TAp73 opposes HIF-1 activity through a non-transcriptional mechanism, thus affecting tumour angiogenesis. TAp73-deficient mice have an increased incidence of spontaneous and chemically induced tumours that also display enhanced vascularisation. Mechanistically, TAp73 interacts with HIF-1a, promoting HIF-1a polyubiquitination and consequent proteasomal degradation. In human lung cancer, TAp73 strongly predicts good patient prognosis, and its expression is associated with low HIF-1 activation and angiogenesis. These findings demonstrate a novel mechanism for HIF-1 regulation and provide an additional explanation for the molecular basis of the growth, progression, and invasiveness of human cancers. (PNAS-USA 2015. 112,1:226-31. PMID: 25535359) (TiBS 2015. 40,8:425-34. PMID: 26032560)

P63 is a determinant of skin development. Using a MMTV-ErbB2 murine model, we found that ΔNp63 regulates mammary Cancer Stem Cells self-renewal and breast tumorigenesis via the direct transactivation of Sonic Hedgehog (Shh), GLI family zinc finger 2 (Gli2), and Patched1 (Ptch1) genes. (PNAS-USA 2015. 112,11:3499-504. PMID: 25739959). At least in part, this seems to be exerted by regulation of the metabolism via Hexokinase II (PNAS-USA 2015. 112,37: 11577-82. PMID: 26324887).

**Mauro Piacentini**

## The Regulation of Autophagy in Mammals: the role of Ambra1

Manuela Antonioli[1], Gian Maria Fimia[2,3] and Mauro Piacentini[1,2]
[1]Department of Biology, University of Rome 'Tor Vergata', Rome, 00133, Italy; [2]National Institute for Infectious Diseases I.R.C.C.S. 'Lazzaro Spallanzani' Rome, 00149, Italy; [3]Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce 73100, Italy.

Autophagy is a basic cellular phenomenon essential for maintaining cellular homeostasis by the lysosomal degradation of protein aggregates, damaged organelles, and recyclying their components. It is involved both in physiological and pathological conditions, including cancer. The role of autophagy in cancer is controversial due to its known participation both in tumor suppression and tumor survival. Thus the comprehension of the molecular mechanisms regulating this important intracellular event represents the basis for its application in cancer therapy. One of the key and not fully understood aspect of the autophagy response in mamalian cells is represented by its rapid and transient induction. We have recently demonstrated that the E3 ubiquitin ligases Cullin-5 and Cullin-4 act as key regulators of the induction and termination of autophagy by their dynamic interaction with AMBRA1, an essential element of the Beclin1 complex regulating autophagy. Under steady state physiological conditions, Cullin-4 binds to AMBRA1 leading to its degradation by the proteasome. By contrast, autophagy

induction promotes AMBRA1 stabilization by its release from Cullin-4 mediated by ULK1. Interestinglly, the Cullin-4/AMBRA1 dissociation is transient, and the re-established interaction triggers AMBRA1 degradation, terminating the autophagy response. Furthermore, upon the Cullin-4 release, AMBRA1 interacts and inhibits Cullin-5, thus promoting the accumulation of the mTOR inhibitor DEPTOR that ensures the rapid onset of autophagy. These findings show that Cullin-mediated modulation of the levels of autophagy key regulators such as Ambra1 and DEPTOR dynamically controls the autophagy response. Considering that the dysregulation of cullin activity has been shown to contribute to oncogenesis our findings open a new avenue for their application in cancer therapy by the regulation of autophagy.

**Ivano Amelio**

**Crosstalk between p53 family and hypoxia inducible factor in cancer progression**

Ivano Amelio and Gerry Melino;
[1]Medical Research Council, Toxicology Unit, Leicester, UK.

HIFs have long been associated with resistance to therapy, metastasis, and poor survival rates in cancer patients. In parallel, although the tumor-suppressor p53 acts as the first barrier against tumor transformation, its inactivation also appears to be crucial for enabling cancer progression at advanced stages. Crosstalk between HIFs and the p53 family might act as a determinant of cancer progression through regulating angiogenesis, the tumor microenvironment, dormancy, metastasis, and recurrence. Our data demonstrates that the tumor suppressor TAp73, a member of the p53 family of genes, opposes HIF-1 activation in cancer cells, resulting in reduced angiogenesis and tumor progression. TAp73-depleted mice show increased tumorigenicity, associated with increased HIF-1 signaling and angiogenesis. Expression of TAp73 in human cancers predicts good survival outcome and retrocorrelates with HIF-1 expression and activation. In addition, expression of mutant forms of p53 in cancer cells affects p73 antagonism on HIF-1 expression, thus promoting cancer progression. We suggest that a complex interfamily crosstalk p53 family/HIF plays a critical role in cancer pathogenesis.

**Olivier Binda**

**The inhibitor of growth ING3 stimulates proliferation of prostate cancer cells**

Urszula McClurg[1], Svitlana Korolchuk[1], Byron Matthiopoulos[1], Craig N Robson[1], Rémy Pedeux[2], Olivier Binda[1]
[1]Newcastle Cancer Centre at the Northern Institute for Cancer Research, Newcastle University, Paul O'Gorman Building, Medical School, Framlington Place, Newcastle upon Tyne, England, NE2 4HH;
[2]INSERM U917, Microenvironnement et Cancer, Université de Rennes 1, Établissement Français du Sang Bretagne, Rennes, France

Despite their fundamental importance in cancer, the molecular mechanisms that regulate the access to genetic information remain incompletely defined. One recently identified mechanism is based on the association of proteins (readers) with the scaffolding histone proteins that condense the genome within the nucleus of the cell. These readers recruit enzymes to open or close the structure of the genome, thereby regulating access to genetic information. Aberrant access to genetic information leads to human pathologies, including cancer.

Members of the inhibitor of growth (ING1-5) family of histone mark readers associate with methylated histones to regulate chromatin signalling. As reported for other ING protein, ING3 regulates p53-dependent transcription. In additon, as a subunit of the TIP60 histone acetyltransferase complex and an essential component for effective TIP60 activity on nucleosomes, ING3 may also regulate androgen receptor AR-dependent transcription and play a role in prostate physiology.

Unexpectedly for a candidate tumour suppressor, we observed that silencing of ING3 expression by siRNA inhibits the proliferation of several human prostate cancer cell lines, independently of AR status. The reduced proliferation was characterized by increased $G_1$/S ration, sub-$G_1$ population, and apoptosis. Generally, ING family members function by bridging HDAC or HAT complexes to chromatin by associating with histone H3 trimethylated on lysine 4 ($H3K4^{me3}$) via the carboxy terminal plant homeodomain (PHD). Thus, to dissect the unexpected ING3-mediated growth stimulation, we have predicted the structure of the PHD domain of ING3 and determined that the conserved tyrosine 362 (Y362) and tryptophane 385 (W385) are involved in the association with $H3K4^{me3}$. Indeed, mutation of either Y362 or W385 greatly reduced the association of ING3 with methylated histones. To understand how ING3 regulates cellular proliferation, we conducted a gene expression analysis and uncovered a network of cell cycle genes that are regulated ING3. Specifically, silencing of ING3 expression led to decreased cellular proliferation marked by increased expression of genes including BAX, p21 (CDKN1A), and TMPRSS2, and reduced expression of AURKA, CCNA2, and CCNB2, while exogenous expression reduced their expression in a PHD-dependent fashion.

Together, our results demonstrate that ING3 expression in prostate cancer cell lines stimulates cellular proliferation by controlling the expression of cell cycle, androgen receptor-dependent, and p53-dependent genes.

**Evgeny N. Imyanitov**

**Hereditary cancer syndromes in Russia**

Evgeny N. Imyanitov
N.N. Petrov Institute of Oncology, St.-Petersburg, Russia

Russia had turbulent and complex history in the past, which resulted in the formation of truly multiethnic society. Nevertheless, Slavic population in Russia preserved unexpectedly high level of genetic homogeneity. Eastern Slavs residing in Russia, Poland, Ukraine and Belarus represent apparently the largest founder community in the world, being an attractive resource for efficient genetic research. Studies of hereditary cancer syndromes in Poland and Russia revealed a number of recurrent germ-line mutations in BRCA1, CHEK2, NBS1 and BLM genes, thus permitting rapid identification of thousands of mutation carriers. This allowed to run some practice-changing clinical trials on the therapy of hereditary breast cancer. Platinum containing compounds, e.g. cisplatin, turned out to be specifically efficient for the treatment of BRCA1-driven tumors, while these and related drugs have limited potential for the therapy of CHEK2-, NBS1- and BLM-related malignancies. Unexpectedly, even a short-term exposure to cisplatin (~2 months) resulted in a robust selection of BRCA1-proficient tumor cells; these data should be taken into account for the long-term planning of clinical management of patients with hereditary cancer disease. “Founder effect” provides unique opportunities for a rapid discovery of novel tumor-predisposing genes. Use of whole exome sequencing led to the identification of a new modifier of BRCA1 penetrance, GPRC5A. Germ-line truncating mutations in GPRC5A occur 10 times more frequently in breast cancer patients carrying BRCA1 mutations than in controls. Furthermore, BRCA1 and GPRC5A genes show strong evidence of coordinated expression. Search for other novel breast cancer predisposing genes in currently underway.

**Boris A. Margulis**

**Modulation or tumorgenecity by hdj2 molecular chaperone**

Meshalkina D, Guzhova I, Shevtsov M, Margulis B. A.
Institute of Cytology of Russian Academy of Sciences, StPetersburg, Russia

Elevated expression of molecular chaperones in tumor cells is known to correlate with poor prognosis in most of cancers. Heat shock protein 70, Hsp70, provides the correct folding of newly synthesized or

damaged polypeptides while Hdj1, Hdj2 and relative proteins assist to major chaperone in ATP-dependent manner. We studied how the knock-down of the abundant Hdj2 co-chaperone affect physiology of highly malignant C6 rat glioblastoma cells. To shift the level of the protein down we employed shRNA technology coupled with lentivirus infection. Knock-down of Hdj2 caused considerable changes in C6 cell physiology. C6shHdj2 cells demonstrated high migration activity and the ability to detach from substrate and form new colonies. These cells lose intercellular contacts mediated by N-cadherin as well as increased level of matrix metalloproteases in the cell medium in comparison with the parent C6 cells. Being injected into rat brain C6shHdj2 cells demonstrated elevated invasiveness and metastatic activity as well as dramatically reduced survival of tumor-bearing animals. Taking together our data prove the significant role of Hdj2 in tumorigenecity and metastasis.

**Valery A.Pospelov**

**Signaling pathways involved in maintenance of self-renewal and pluripotency of mouse embryonal stem cells.**

I. I.Suvorova, M.Y.Cherepkova, B.B.Grigorash and V.A.Pospelov
Institute of Cytology, Russian Academy of Sciences, St. Petersburg Russia

Study on the molecular mechanisms underlying self-renewal, pluripotency and differentiation of mouse embryonal stem cells (mESCs) identified several key signaling pathways: LIF/STAT3, FGF/Ras/MAPK, Wnt/β-cathenin etc. Unlimited self-renewal of mESCs is provided by a partial dysfunction of p53/p21Waf1 pathway that allow them do not stop in G1 and thereby to avoid an impetus to differentiation. In spite of the lack of G1-checkpoint control, the program for recognizing and repairing DNA defects (DDR signaling) is functional in mESCs. We used several agents with different mechanisms of p53 and/or p21Waf1 activation to study a transition from self-renewal to early differentiation: gamma-irradiation, nutlin, histone deacetylasel inhibitors, AICA-riboside. The data obtained show that only those agents, which reactivate p53 and cause accumulation of its target p21Waf1protein, are capable of restoration of G1/S checkpoint and induction of differentiation that in turn is accompanied by down-regulation of pluripotency genes oct-4 and nanog and up-regulation of endoderm-specific genes.

We found that the LIF/STAT3 signaling pathway can contribute to the maintenance of self-renewal and pluripotency of mouse ESCs by suppressing mTOR pathway.The mTOR signaling is known to be involved in proliferation, cell growth, translation regulation and cell metabolism. When LIF ligand is withdrawn from culture medium, the mTOR activity rapidly increases as detected by phosphorylation of its targets - ribosomal protein S6 and translation factor 4EBP1. In turn, suppression of STAT3 phosphorylation on Tyr-705 by a specific small molecule WP1066 also activates phosphorylation of the mTOR target S6 ribosomal protein. LIF removal strongly activates ERK activity indicating that ERK can be involved in either direct phosphorylation of mTOR or phosphorylation of an upstream negative regulator of mTOR - TSC2 protein. mTOR activation is accompanied by a decrease of pluripotent gene expression Oct-4, Nanog, Sox2 and by an increase of fgf5 gene expression – an early marker of neuronal commitme Together, these data indicate that upon LIF withdrawal mouse ESCs undergo a transition from LIF/STAT3-supported pluripotency to FGF/Erk-committed differentiation mediated through activation of mTOR signaling.

Supported by RSF grant 14-50-00068 (VAP and MYC) and SPbSU grant 1.38.247.2014 (BBG and IIS)

**Oleg N Demidov**

**Targeting p53-negative tumors by activation of stress-induced phosphatase, Wip1**

Demidov O.N.[1,2], Clausse V.[2] Uyanik B.[2], Kochetkova E[1], Pospelov V.A.[1], Goloudina A.R.[2]
[1]Institute of Cytology, Russian Academy of Sciences, St. Petersburg Russia;
[2]INSERM U866, University of Burgundy, Dijon.

One of the major pathways activated by anti-cancer therapy is p53 tumor suppressor pathway, which is very efficient in induction of cell death in cancer cells. Unfortunately, p53 is one of the most frequently mutated genes in tumors. To improve the treatment of p53-negative tumors we proposed activation of stress-induced phosphatase, Wip1, which potentiates toxicity of chemotherapeutic drugs towards tumor cells with inactivated p53 tumor suppressor. At the same time, Wip1 protects sensitive normal tissues from anti-cancer drugs side effects by decreasing cell death signaling in normal cells with preserved p53 functions.

We performed high-throughput screening of chemical libraries and found several activators of Wip1 expression that could improve efficiency of anti-cancer treatment for tumors with mutated p53 tumor suppressor. Alternatively, we screened siRNA kinome library for kinases, which modulate DNA damage signaling. Phosphorylation levels of histone H2AX -Ser139 as a target site for Wip1 phosphatase activity and at the same time a quantitative marker of DNA damage signaling was used as a read-out. Several positive hits are currently studied for cumulative effects in ”bringing down” tumor cells with mutated p53, but without increasing cell death in normal tissues at the same time.

By targeting Wip1 network, we expect to establish novel improved anti-cancer strategies modulating multiple aspects of tumor behavior: tumor microenvironment, anti-tumor immunity and responsiveness of tumor cells to the therapy.

**Alexey Antonov**

## Estimating the effect of p53 mutation status on the gene regulation in cancer from gene expression data

Ivano Amelio, Gerry Melino, Alexey Antonov,
MRC Toxicology Unit, Leicester, UK.

Significant efforts are still underway to understand the complexity of gene regulation in mut-p53 cancer. In a majority of cases, initial evidence of novel gene regulatory patterns is commonly discovered in vitro or in animal models. The current in-vitro/animal experimental pipeline is based on experimental validation of an abnormal control by mut-p53 of putative target proteins (such as transcription factors, TF), supported by experimental evidence of abnormal regulation of downstream players (such as TF targets). However, all these experimental settings have significant limitations in the degree to which they reproduce human cancer pathology and still demand extensive validation in the clinical setting. Statistical validation of gene regulatory patterns identified in vitro and in animal models can be performed by mining clinical gene expression datasets. In clinical gene expression data, statistically significant differences between cohorts of mut-p53 and wt p53 samples in the correlation of TF and TF target pairs would represent a signature of this regulatory model.

To address the wide interest of the scientific community on mut-p53-mediated gene regulation, we developed p53MutaGene, an online data mining tool for testing the effect of p53 mutational status on gene regulation in cancer. The tool is based on several clinical gene expression datasets annotated with p53-mutational status, and currently covers breast, colon cancers and lung cancer. p53MutaGene detects a shift in the correlation between mut-p53 and wt-p53 sample cohorts for user specified genes. The tool can be used in “single mode” to test a specific pair of genes for sensitivity to p53 mutation**,** or in “discovery mode” to screen genes in order to identify candidates whose regulation might be sensitive to p53 mutational status. To demonstrate the potential utility of p53MutaGene, we have provided multiple examples of application of p53MutaGene to validate well known p53-dependent gene regulatory models. We have also demonstrated the utility of p53MutaGene to identify potential candidates implicated in p53 regulatory programs from the large list of candidate genes.

**Olga Fedorova**

**Novel small-molecule inhibitors of the mdm2-p53 protein-protein interaction**

Olga Fedorova[1,2], Alexandra Daks[1,2], Pavel Davidovich[1,2], Alexey Petukhov[1,2], Oleg Shuvalov[1], Elena Vasileva[1], Gerry Melino[2] and Nickolai Barlev[1,2].

[1]Institute of Cytology RAS; [2]St.Petersburg State Institute of Technology, Saint-Petersburg, Russia

The major tumor suppressor protein p53 is a transcriptional master regulator that controls cell cycle arrest, senescence, apoptosis, autophagy, DNA repair and metabolism (Vousden and Prives, 2009). In the absence of stress signals, p53 is inactivated by E3 ubiquitin ligase MDM2 that targets p53 for degradation (Momand et al., 1992; Oliner et al., 1993; Kubbutat et al., 1997). Thus, p53 stabilization and activation in malignant cells is a very promising target for anticancer therapy. Small molecule candidates selected from *in silico* analyses were screened for the ability to stabilize p53 in cancer cells using a GFP-based p53-responsive reporter plasmid. Two isogenic human osteosarcoma cell lines $U2OS^{p53+}$ and $U2OS^{p53-}$ (stably expressing shRNA knockdown plasmid of p53) were used for evaluation of specificity of small molecules towards p53. To assess the effect of compounds on p53 stabilization the protein levels of p53 in cell lines after treatment with chemical compounds were analyzed by western blot. Experiments have shown that t least three selected compounds stabilized p53 comparable to Nutlin-3, a well known p53 activator. We simultaneously examined the p53 mRNA level after treatment with small molecule activators of p53 and Nutlin-3, which was used as control. Real-time PCR assay showed equal levels of p53 mRNA expression after treatments, indicating that the compounds affect p53 on the protein level likely by modulating its protein stability. The tumor suppressor p53 functions as a transcription factor by modulating the expression of several target genes (p21, PUMA, Bax etc), whose products, in turn, regulate cell cycle, apoptosis, etc. Real-time PCR data have shown that two compounds induced the expression of p53 target genes (p21, PUMA and BAX). A cytotoxic effect of these compounds was evaluated based on the colony formation assay using $U2OS^{p53+}$ and $U2OS^{p53-}$ cell lines. Collectively, our results demonstrated that two compounds specifically activate p53 on the protein level and have no cytotoxic effect on $U2OS^{p53-}$ cell line. In sum, these two compounds are chosen for in vivo preclinical studies.

This work was supported by the RFBR (No. 13-04-01024A, 12-04-01397A and 14-04-32242 mol-a); and RSF 14-15-00816.

# CONTACT LIST OF SPEAKERS


Professor Gerry Melino
gm89@le.ac.uk

Professor Mauro Piacentini
mauro.piacentini@uniroma2.it

Dr Ivano Amelio
ia119@leicester.ac.uk

Dr Olivier Binda
Olivier.Binda@newcastle.ac.uk

Professor Evgeny Imyanitov
evgeny@imyanitov.spb.ru

Professor Boris Margulis
margulis@incras.ru

Professor Valery Pospelov
pospelov_v@mail.ru

Dr Oleg Demidov
oleg.demidov@u-bourgogne.fr

Dr Alexey Antonov
aa668@leicester.ac.uk

Dr Olga Fedorova
fedorovaolgand@gmail.com

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