# ORAL PRESENTATIONS

# PL1: MOUSE MODELS OF THORACIC CANCERS. WHAT DO THEY TEACH US?

ANTON BERNS

*Oncode Institute; Division of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam, Netherlands (e-mail: a.berns@nki.nl)*

Lung cancers belong to the most lethal human malignancies. In particular, patients with small cell lung cancer (SCLC) and lung squamous cell carcinoma (LSCC) show very poor survival statistics due to the often late detection, early metastatic spread and chemo-resistance of the tumors. We have generated multiple mouse models for the various lung cancer subtypes and studied how closely they resemble their human counterparts, how these tumors develop over time, from which cell type they originate, what additional recurrent genomic alterations are recurrently found, and how the combination of the cell-of-origin and the driver lesions influence the response to single drugs or drug combinations. We observed that the mouse tumors showed remarkable resemblance to their cognate human counterparts. This includes their marker profiles, their specific location within the tissue, their immunophenotypes and their refractoriness to the standard treatments. Remarkably, we also observed substantial intra-tumor heterogeneity and tumor cell plasticity, this in spite of the fact that the mouse tumors are driven by a set of well-defined driver lesions and do not exhibit a high mutation load as is the case for most human lung cancers. Interestingly, it appears that also the cell-of-origin of these mouse tumors can be quite diverse resulting in tumors that significantly differ although carrying the same driver mutations. The lessons learned from studying these models and how this might help designing more effective treatments for patients with lung cancer will be discussed.

*EACR Sponsored Lecture*

## PL2: UBIQUITIN AND AUTOPHAGY NETWORKS IN CANCER PATHOGENESIS

IVAN DIKIC

*Institute of Biochemistry II, Frankfurt Cancer Institute and Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt/Main, Germany*
*(e-mail: ivan.dikic@biochem2.de)*

Autophagy and Ubiquitin proteasome degradation systems are major quality control pathways for the maintenance of cellular homeostasis. As such they provide protection against rapid aging and various human diseases. An increasing number of distinct functions have been assigned to different types of ubiquitin modifications (mono ubiquitin, Lysine-linked and Met1-linked ubiquitin chains). We have cloned a number of Ub receptors that are able to mediate a variety of Ub functions including TLS polymerases in DNA repair, proteasomal degradation, receptor endocytosis and innate immunity. Deregulation of these pathways has been link to the development of variety of human cancers. We have recently identified SPRTN, a novel Ub-binding protein, whose germline mutations causes Ruijs-Aalfs syndrome (RJALS). This syndrome is characterized by early onset hepatocellular carcinoma, genome instability and progeria. Cells derived from patients with Ruijs-Aalfs syndrome are impaired in the resolution of covalent DPCs and exhibit leakage of the G2/M cell cycle checkpoint and proliferation defects. In mice, the complete knockout of Sprtn is embryonically lethal, whereas Sprtn hypomorphic mice are viable despite reduced Spartan expression. Characterization of the Sprtny118c/y118c mouse that contains the mutation that causes early hepatocarcinoma in humans will be presented. Moreover, our current translational activities in the newly established Frankfurt Cancer Institute will be discussed.

## K: THE ROLE OF ENDOCRINE FGFs IN METABOLISM AND CANCER

JOSEPH SCHLESSINGER

*Yale University School of Medicine, New Haven CT, USA*
*(e-mail: joseph.schlessinger@yale.edu)*

Tyrosine phosphorylation of cellular proteins plays an important role in the control of cell proliferation, differentiation, cell metabolism as well as other important cellular processes. Ligand binding to the extracellular ligand binding domain of receptor tyrosine kinases induces receptor dimerization, a step crucial for activation of the catalytic domain and for tyrosine autophosphorylation; both processes are mediated by an intermolecular process. A large family of growth factors such as platelet derived growth factor (PDGF), stem cell factor (SCF), colony stimulating factor (CSF), and nerve growth factor (NGF), among many others are dimeric proteins. These growth factors induce receptor dimerization by virtue of their dimeric nature. Canonical fibroblast growth factors bind to their receptor monovalently, and when added alone are unable to induce dimerization and activation of FGF-receptors. Canonical FGF-induced dimerization of FGF-receptors is mediated by heparin sulfate proteoglycans. Endocrine FGFs, FGF19, FGF21 and FGF23 are circulating hormones that regulate metabolic processes in a variety of tissues. They signal through FGFRs in a manner that requires a Klotho protein. It was proposed that Klotho proteins, which are cell surface proteins with tandem glycoside hydrolase domains, act as co-receptors for FGFR activation by endocrine FGFs, playing roles analogous to heparan sulfate proteoglycans in canonical FGF signaling. By determining crystal structures of free and ligand-bound β-Klotho extracellular regions, we show that β-Klotho in fact serves as a primary high-affinity cell-surface receptor for FGF21, with FGFR1c functioning as a catalytic subunit that mediates intracellular signaling.

## L1: CANCER STEM CELLS: NEW DRIVERS AND MECHANISMS

UGO CAVALLARO

*Unit of Gynecological Oncology Research, European Institute of Oncology, Milano, Italy*
*(e-mail: ugo.cavallaro@ieo.it)*

The cancer stem cell (CSC) theory has revolutionized the tumor biology field, in that it pointed to a subpopulation of malignant cells that accounts for the onset, progression, heterogeneity, metastatic spread and resistance to treatments. Such a theory would explain very well the biological features and the clinical evolution of ovarian cancer (OC), including its peritoneal dissemination, the high rate of relapse in spite of optimal tumor debulking, and the unresponsiveness to chemotherapy, namely the factors that determine the high mortality rate of this neoplasm. Nevertheless, while experimental evidence has supported the existence and the pathogenic function of ovarian CSC (OCSC), this subset of cells has remained elusive, and the contribution of OCSC to specific aspects of OC biology remains to be elucidated.

In an attempt to identify and characterize OCSC, we have opted to rely on the intrinsic biological properties of this cell subpopulation rather than on the expression of markers inferred from either CSC of different solid tumor types or OC cell lines (that often fail to recapitulate the disease). We established a number of primary cell cultures from patient-derived tissue, utilizing not only OC samples but also their normal counterparts, i.e. ovarian surface epithelium and fallopian tube epithelium for the fimbriae. Culturing these cells under conditions that are selectively permissive for the stem cell compartment, we could obtain the molecular portrait of OCSC vs. normal SC.

Our approach has revealed a series of OCSC-specific biomarkers which may offer new strategies for the prospective purification and characterization of this cell subpopulation. In addition, we have identified novel players that not only play a causal role in OC stemness, but also represent potential targets for molecular therapies.

Thus, we present an approach that, by capitalizing on clinically relevant model systems and on the unbiased, function-based definition of OCSC, provided novel insights into the nature and function of this elusive cell subset. These results might set the stage for innovative treatments aimed at the eradication of ovarian cancer through the inactivation of its CSC compartment.

# L2: DNA-PROTEIN CROSSLINK PROTEOLYSIS REPAIR IN PREMATURE AGEING AND CANCER

KRISTIJAN RAMADAN

*Cancer Research UK and Medical Research Council Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, United Kingdom*
*(e-mail: kristijan.ramadan@oncology.ox.ac.uk)*

DNA-protein crosslinks (DPCs) are a specific type of DNA lesion consisting of a protein covalently and irreversibly bound to DNA, which arise after exposure to physical and chemical crosslinking agents. DPCs are bulky and pose a barrier to DNA replication and transcription. Persistence of DPCs during S-phase causes DNA replication stress and genome instability. Recent work from several laboratories discovered a specialized repair pathway for DPCs, namely DPC proteolysis (DPCP) repair. DPCP repair is carried out by replication-coupled DNA-dependent metalloproteases: Wss1 in yeast and SPRTN (Spartan) in metazoans. Mutations in the DPC protease SPRTN cause premature aging and liver cancer in humans and mice; thus, efficient DPC repair has great medical value. Here, I will discuss our recent progress on the regulation of DPCP repair and discuss the relevance of DPCP repair for cancer therapy.

## L3: GENOTYPE-IMMUNOPHENOTYPE CORRELATION IN CANCER

HUBERT HACKL

*Division of Bioinformatics, Biocenter, Medical University of Innsbruck, Innsbruck, Austria*
*(e-mail: hubert.hackl@i-med.ac.at)*

Recent breakthroughs in cancer immune therapy has sparked intense research on tumor- immune system interactions and - at least a part of - patients benefit and show long-term response. Patients with genomic changes in the tumor such as microsatellite instability, hypermutation, and encoded factors involved in MHC processing have been shown to profit from checkpoint blocker immune therapy. One of the mechanisms might involve deficient DNA repair genes raising the chance of release of immunogenic neo-antigens. Resistance to immunotherapy is due to a complex tumor microenvironment that counteracts antitumor immunity through a combination of poorly antigenic tumor cells and an immunosuppressive tumor microenvironment. Using available RNA sequencing and whole exome sequencing data from The Cancer Genome Atlas (TCGA) as well as computational genomics tools we systematically analyzed 20 different solid cancer types from > 8000 patients predicted neo-antigens, estimated tumor-infiltrating lymphocytes (TILs), cytolytic activity, and identified determinants of tumor immunogenicity. In particular we are following up on how tumor-intrinsic factors and oncogenic pathways (involving e.g. MYC) shaping the immune landscape and impact the evasion of antitumor responses or which molecules are involved in the regulation of the expression of PD-L1 and other check points. I will present data addressing the correlation of patients sharing specific tumor genotypes or molecular subtypes with increased expression of MHC related genes, immune modulators, and activating or suppressive cells from the innate/adaptive immune system as evident in BRAF versus RAS genotypes in thyroid carcinoma. Another focus will be on how deficient genes encoding factors of mismatch repair (MLH1) in colorectal cancer or recombinational repair (BRAC1/2) in ovarian cancer are associated with the immune system and finally will discuss how the immune system surveil the tumor and intra tumor heterogeneity (immunoediting).

# L4: THE MUTATION LANDSCAPE OF CANCERS SERVE AS RECORDS OF EARLY MALIGNANT TRANSFORMATION

ROSA KARLIĆ[1], KIRSTEN KUBLER[2,3,4], WILLIAM D FOULKES[5,6], PAZ POLAK[7], GAD GETZ[2,3,4]

[1] *Bioinformatics Group, Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
*(e-mail: rosa@bioinfo.hr)*
[2] *The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA*
[3] *Center for Cancer Research, Massachusetts General Hospital, Charlestown, Massachusetts, USA.*
[4] *Harvard Medical School, Boston, Massachusetts, USA.*
[5] *Department of Human Genetics, McGill University, Montreal, QC, Canada,*
[6] *Lady Davis Institute for Medical Research and Research Institute McGill University Health Centre, McGill University, Montreal, Quebec, Canada.*
[7] *Icahn School of Medicine at Mount Sinai, New York, NY, USA*

A fundamental question in cancer biology is how the cell lineage influences the cell's susceptibility to malignant transformation. Cell properties are encoded in the cell type-specific chromatin structure and we previously demonstrated that the landscape of somatic mutations in cancer is associated with the chromatin marks of the cell-of-origin (Polak et al, Nature, 2015). Many of these mutations accumulate before malignant transformation and serve as a historical record of the original normal cell.

Here, we show that this principle is generalizable to common cancer types and provides new insights into the molecular events of cancer initiation. We extended our analysis to include 2,641 whole genomes across 30 cancer types and epigenetic modifications from 98 normal tissue types. We found that 28/30 cancer types originated from a biologically plausible cell-of-origin. In 25 cancer types, the tumor originated from a cell type that was its direct normal cell counterpart (or a related cell type). In three cancer types (esophageal, pancreatic ductal and biliary adenocarcinomas) the best-matched normal cell type indicated metaplasia. In the remaining two cancer types, we did not find a good match among the normal cell types, due to the lack of relevant epigenetic data.

We analyzed in more detail different breast cancer subtypes. We found that basal-like tumors appear to originate from luminal progenitor cells, while all other subtypes (luminal A, luminal B, and HER2-enriched) arise from mature luminal cells. Furthermore, this association held true when accounting for various gene inactivation events. Irrespective of the exact mechanism of inactivation, all BRCA1/2- and RAD51C-altered basal-like tumors best matched to luminal progenitors while BRCA1/2- and CHEK2 -mutated luminal A/B subtypes best matched mature luminal cells, implying that the inactivation of specific genes was less crucial than the cell-of-origin for driving the formation of subtypes. Taken together, our findings highlight the crucial role of the specific cell type of origin in shaping the mutational landscape and early tumor evolution.

## L5: MICROENVIRONMENTAL REGULATION OF METASTASIS

JONATHAN SLEEMAN

*Microvascular Biology and Pathobiology, European Center for Angioscience, Medical Faculty Mannheim of the University of Heidelberg, Heidelberg, Germany*
*(e-mail: sleeman@medma.uni-heidelberg.de)*

The reception and integration of the plethora of signals a cell receives from its microenvironment is decisive in determining cell behaviour. External signals that regulate cell behaviour and properties include signalling molecules such as growth factors and cytokines, extra cellular matrix (ECM) components, contacts with other cells, mechanical stress, matrix stiffness and roughness, the redox status of the surrounding milieu, and the local oxygen tension. Perturbation of extracellular cues or an inappropriate response or integration of these signals lies at the root of many diseases such as cancer. Accordingly it is increasingly recognised that changes in the extracellular environment can drive tumorigenesis, tumor progression and metastasis, and can act dominantly for example over genetic aberrations in the tumor cells. Focusing on the extracellular matrix and the immune system, in this talk I will discuss some of our recent findings about how the microenvironment regulates tumor metastasis.

## L6: NOVEL MECHANISMS OF TUMOR-STROMA CROSSTALK

ESTHER ARWERT[1], EMMA MILFORD[1], ANTONIO RULLAN[1], ALAN MELCHER[2], KEVIN HARRINGTON[2], ERIK SAHAI[1]

[1] *Sahai Lab, Francis Crick Institute, London, United Kingdom*
*(e-mail: Erik.Sahai@crick.ac.uk)*
[2] *Institute of Cancer Research, London, United Kingdom*

We will present data on the mechanism of cooperation between cancer cells and stromal fibroblasts. Many tumors show an initial response to targeted therapies before genetic resistance emerges, however little is known about how tumor cells tolerate therapy before genetic resistance dominates. We show how the ECM generates a 'safe haven' in which melanoma cells can tolerate targeted therapy. This supports the population of cancer cells from which genetically resistance emerges. These data argue fibroblast – cancer cell cross-talk via the ECM. We have recently uncovered a novel mechanism of tumour – stroma cross-talk involving a pathological heterotypic cell-cell contact. In normal skin, epithelial cells and fibroblasts do not contact because they are separated by a basement membrane. However, a signature feature of tissue damage and invasive squamous cell carcinoma is breakdown of the basement membrane, epithelial cells and fibroblasts can then contact each other. This heterotypic can transmit force and enable invasion. More crucially, it triggers dramatic changes in chemokine, cytokine, and other inflammatory modulators triggering anti-microbial and anti-viral responses. We propose that this represents a tissue level damage sensing mechanism analogous to molecular DAMPs.

## L7: HEDGEHOG/GLI SIGNALING: TARGETS AND TARGETING

PETER G. ZAPHIROPOULOS, YUMEI DIAO, ANI AZATYAN

*Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden*
*(e-mail: Peter.Zaphiropoulos@ki.se)*

Although the Glioma associated oncogene 1 (GLI1) acts as the ultimate effector of Hedgehog signaling little is known on the target genes of this transcription factor. Via RNA sequencing and correlation analysis with FANTOM5, a signature of 29 GLI1 target genes has been identified. One of the prominent novel targets, FOXS1, a transcription factor previously implicated in nervous system development, is highly expressed in the Sonic Hedgehog class of medulloblastoma tumors. Importantly, FOXS1 blocks the capacity of GLI1 to activate target genes and elicits opposite effects on tumor cell proliferation compared to GLI1. These findings suggest that FOXS1 may have tumor suppressive properties and could be a marker of good prognosis [1].

Efforts to target Hedgehog signaling-dependent cancers have focused on the small molecule RITA, which is considered to be an activator of the p53 tumor suppressor. RITA treatment of cancer cells downregulated Hedgehog signaling, however this was independent of p53 and mediated via the MAP kinase JNK. Treatment of rhabdomyosarcoma xenografts with RITA suppressed tumor growth, as did GANT61 a small molecule that is thought to inhibit the GLI factors. RT/PCR analysis of GLI1, GLI2 and PTCH1 expression in the tumor xenografts revealed more pronounced downregulation by RITA than by GANT61. Surprisingly, the combinatorial RITA and GANT61 treatment did not further suppress tumor growth suggesting a certain antagonism of the two drugs [2].

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## L8: TARGETING ONCOGENIC HEDGEHOG SIGNALING BEYOND SMOOTHENED INHIBITORS

ELISABETH PEER[1], WOLFGANG GRUBER[1], FILIP VILOTIC[1], THOMAS PARIGGER[2], SVETLANA HAMM[3], RENE BARTZ[3], FRITZ ABERGER[1]

[1] *Department of Biosciences, Cancer Cluster Salzburg, Paris-Lodron University of Salzburg, Salzburg, Austria (e-mail: fritz.aberger@sbg.ac.at)*
[2] *Salzburg Cancer Research Institute, Cancer Cluster Salzburg, University Clinic Salzburg, Salzburg, Austria*
[3] *4SC AG, Planegg-Martinsried, Munich, Germany*

Aberrantly activated Hedgehog/GLI (HH/GLI) signaling has been identified as driver signal in a number of cancer entities with a critical role in the maintenance of rare and highly malignant cancer stem cells (CSC). Eradicating tumor-initiating metastatic CSCs by targeted inhibition of CSC pathways such as HH/GLI is of high medical need. Clinically advanced inhibitors of the essential HH pathway effector Smoothened (SMO) showed promising efficacy as a first-line therapy in skin cancer, though the rapid development of drug resistance paired with frequent SMO-independent activation of GLI factors limit their applicability. The identification and targeting of further GLI-regulating effectors downstream of SMO is, therefore, an important step towards overcoming SMO-inhibitor (SMOi) resistance and improved disease management.

We will discuss novel druggable targets within the HH/GLI pathway and demonstrate that selective inhibition of these pathway effectors holds promise for future therapeutic applications, particularly for rational combination treatments.

# L9: NON-CANONICAL HEDGEHOG SIGNALLING: IS IT IMPLICATED IN CANCER?

NATALIA RIOBO-DEL GALDO

*School of Cellular and Molecular Biology and Leeds Institute of Medical Research, University of Leeds, Leeds, United Kingdom*
*(e-mail: N.A.Riobo-DelGaldo@leeds.ac.uk)*

Non-canonical Hedgehog (HH) signalling refers to a plethora of cellular responses to HH proteins that are not mediated by stimulation of the GLI family of transcription factors. We have described two types of non-canonical signalling: a Type I Patched1 (PTCH1)-dependent and a Type II that is mediated by Smoothened (SMO). Here I will discuss the recent findings in novel non-canonical HH pathways and evidence of their relevance in cancer, in particular of epithelial cancers with upregulation of HH proteins and with mutations in the C-terminal domain of PTCH1.

## L10: THE GLI CODE IN OVARIAN CANCER

MAJA SABOL, NIKOLINA RINČIĆ, DIANA TRNSKI, VESNA MUSANI, PETAR OZRETIĆ, SONJA LEVANAT

*Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: maja.sabol@irb.hr)*

Ovarian tumors are a heterogenic malignancy, often named "the silent killer" due to lack of symptoms, which leads to late detection of the disease. It is often diagnosed in the advanced stages when the tumor has already spread. Additional complication are the different tissues of origin for different tumor types, or even of the same subtype. The Hedgehog signaling pathway has been implicated in ovarian tumors by several different mechanisms. Hyperactivation of the Hedgehog pathway can occur via hypermethylation of PTCH1 promoter, mutations in PTCH1 gene, microRNA activity, or through cross-talk with other signaling pathways. All these events eventually lead to activation of GLI proteins and to tilting the balance imparted by the GLI code towards activation of the pathway. Expression of GLI1 is usually associated with tumor progression in a clinical setting, but GLI2 and GLI3 also play a role by modifying the activity of GLI1 and transcription of their common transcriptional targets. In ovarian cancer, GLI3 protein is expressed in the full-length activator form, and not the shortened repressor form which is the predominant form for GLI3 protein. CRISPR/Cas9-generated knock-out ovarian cancer cell lines for each of the three GLI proteins show that all three GLI proteins are relevant for the pathway activation in ovarian cancer, including GLI3 which is usually considered a repressor.

# L11: AN INTEGRATED MODEL OF HEDGEHOG-GLI MOLECULAR FUNCTIONS IN CANCER

BARBARA STECCA

*Tumor Cell Biology Unit - Institute for Cancer Research and Prevention (ISPRO), Florenze, Italy (e-mail: b.stecca@ispro.toscana.it)*

The Hedgehog-GLI signaling is a key pathway critical in embryonic development, stem cell biology and tissue homeostasis. Aberrant activation of Hedgehog-GLI signaling has been linked to several types of cancer, including those of the skin, brain, lungs, prostate, gastrointestinal tract and blood. Canonical Hedgehog-GLI pathway activation is triggered by binding of Hedgehog ligands to the transmembrane receptor PATCHED and is mediated by transcriptional effectors that belong to the GLI family, whose activity is finely tuned by a number of molecular interactions and post-translation modifications. The activity of the GLI proteins can be also regulated by numerous proliferative and oncogenic inputs, in addition or independent of upstream HH signaling. In turn, the GLI transcription factors exert their activities in cancer cells through a number of specific targets, including regulators of proliferation and differentiation, survival, angiogenesis, self-renewal, epithelial-mesenchymal transition and invasiveness.

We and others have described a number of targets of the GLI, such as E2F1 and SOX2, but the mechanisms of action seem to be critical to understand how the GLI transcription factors integrate different oncogenic molecular functions and how this information nexus operates. Here we will show how the Hedgehog/GLI-E2F1 axis regulates iASPP, providing an additional mechanism by which Hedgehog signaling restrains p53 pro-apoptotic function in melanoma cells. In addition, we will present recent findings on the positive autoregulatory loop between GLI1 and SOX2 in cancer stem cells and on the identification of novel common targets of GLI1 and SOX2. Finally, we will discuss the implications of the integrated model of Hedgehog-GLI molecular functions for anti-cancer therapy.

# L12: IMMUNOTHERAPY OF HEMATOLOGICAL MALIGNANCIES: A SUCCESS STORY

IGOR AURER

*University Hospital Centre Zagreb and Medical School, University of Zagreb, Zagreb, Croatia (e-mail: igor.aurer@mef.hr)*

In the last decades immunotherapy of hematological neoplasms became a very important and effective therapeutic approach, substantially increasing response rates, progression-free and overall survival of patients. Non-conjugated monoclonal antibodies (MoAbs), mostly directed against the CD20 antigen, the prime example being rituximab, are used as monotherapy, or more frequently in combination with chemotherapy, in the treatment of mature B-lymphoid neoplasms. Despite decades of use, the mode of action of rituximab is still not completely clear. Proposed mechanisms include complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), induction of apoptosis and increased sensitivity to cytotoxic agents. Rituximab has very little toxicity (except for depletion of normal B-cells which is surprisingly well tolerated) or off-target effects. Obinutuzumab, an anti-CD20 MoAb with more pronounced ADCC and less CDC than rituximab, is more effective than the latter in only a limited number of lymphoma types but causes more frequently neutropenia, making it more difficult to combine with chemotherapy.

Brentuximab vedotin is an example of an effective MoAb conjugated to a cytotoxic agent. It is directed against CD30, an antigen present on Hodgkin and some non-Hodgkin lymphomas. In contrast to CD20, which remains on the cell surface amenable to host effector mechanisms, CD30 is internalized after MoAb binding and cell killing results from the intracellular action of the cytotoxic agent.

Transplantation of allogeneic hematopoietic stem cells (AlloSCT) is an example of cellular immunotherapy of hematologic neoplasia. A significant part of the efficacy of this very effective and very toxic therapeutic option is due to the graft-versus tumor effect mediated by alloreactive immunocompetent donor cells. Advances in AlloSCT include the use of reduced-intensity conditioning and haploidentical related donors, enabling its use in older patients and those with few or no siblings. Immunomodulatory agents, thalidomide, lenalidomide and pomalidomide are a mainstay of multiple myeloma therapy. While their modes of action are multifaceted, stimulating host immune responses seems to be a very important part of it.

Finally, new treatment approaches with exciting preliminary results include the use of bispecific antibodies and chimeric antigen receptor T-cells (CAR-T).

## L13: TARGETING MEDULLOBLASTOMA METASTATIC TUMORS VIA IMMUNOMODULATION THERAPY

VERONICA FERRUCCI[1,2,3], FATEMEH ASADZADEH[2], ROBERTO SICILIANO[2], LUISA DASSI[2], ANTONELLA VIRGILIO[4], ALDO GALEONE[4], LUCIA DE MARTINO[6], LUCIA QUAGLIETTA[6], MARIA ELENA ERRICO[5], VITTORIA DONOFRIO[5], MARC REMKE[7], DANIEL PICARD[7], LOUIS CHESLER[8], FREDRIK J. SWARTLING[9], WA. WEISS[10], PASQUALINO DE ANTONELLIS[11], MICHAEL D. TAYLOR[11,12], GIUSEPPE CINALLI[6], MASSIMO ZOLLO[1,2,3]

[1] *Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Naples, Italy*
[2] *CEINGE Biotecnologie Avanzate, Naples, Italy*
[3] *European School of Molecular Medicine (SEMM), Milan, Italy*
*(e-mail: massimo.zollo@unina.it)*
[4] *Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Naples, Italy*
[5] *Surgical Pathology, Ospedale Santobono-Pausilipon, Naples, Italy*
[6] *Paediatric Neurosurgery, Ospedale Santobono-Pausilipon, Naples, Italy*
[7] *German Cancer Consortium (DKTK), Department of Paediatric Oncology, Haematology, and Clinical Immunology, University Hospital Düsseldorf, Düsseldorf, Germany*
[8] *Division of Clinical Studies, The Institute of Cancer Research, London SM2 5NG, United Kingdom*
[9] *Science for Life Laboratory, Rudbeck Laboratory, Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
[10] *Department of Neurology, University of California, San Francisco, San Francisco, CA, USA*
[11] *Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto ON, Canada*
[12] *Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Ontario, Canada*

Medulloblastoma (MB), an embryonal tumour of the cerebellum, accounts for 25% of childhood central nervous system (CNS) tumors. The molecular classification of MB identified 4 molecular entities (WNT, SHH, Group 3, Group 4) that were further classified into 12 subtypes with distinct transcriptional profiles, somatic mutations and clinical outcomes. MB Group 3 has the worse prognosis due to the higher tendency to metastatize in the leptomeningeal space (40% of children at diagnosis). The clinical management includes surgical resection followed by chemo-radiation regimens. The lack of a targeted-therapy for metastatic MB is also due to the absence of genetically engineered mouse models (GEMMs) resembling metastatic MB Group 3.

The brain tumour microenvironment (TME; tumour associated macrophages, microglia, dendritic cells, T-cells) are emerging as critical regulators of cancer progression also in MB. Immunotherapeutic strategies, based on immunomodulatory drugs pomalidomide or dendritic cells vaccines, are in Phase 1/2 clinical trials.

We identified a new "metastatic axis" in MB Group 3 driven by PRUNE-1. We found the overexpression of Prune-1 enhances TGF-beta pathway thought is binding to NME-1, thus leading to OTX2 up-regulation, EMT, and PTEN inhibition. We identified a non-toxic small molecule (pyrimido-pyrimidine derivative, AA7.1) with the ability to impair MB Group 3 metastatic dissemination in vivo by targeting Prune-1. Of importance, through WES applied to primary human metastatic MB cells, we identified 'non-synonymous homozygous' deleterious variants affecting immune cells activation/differentiation, taking part to a protein network of relevance for metastatic processes, thus highlighting a role for immune cells in metastatic MB. Recently, we developed a GEMM of PRUNE-1-driven-metastatic MB Group 3. Anatomo-path-

ological analyses performed in our model and in a cohort of MB Group 3 patients show the presence of FOXP3 infiltrating cells in the brain TME, thus suggesting a role for Regulatory T-cells in MB Group 3.

Our in vitro data measuring the proliferation of MB Group 3 cells show the anti-Prune-1 drug to act synergistically with chemotherapeutics currently used in clinics for MB. The anti-Prune-1 drug also exerts immunomodulation in vitro and in vivo by reducing inflammatory cytokines and the immune cells in TME. To our knowledge, this is one of the first immunotherapeutic approach in a preclinical model of metastatic MB Group 3.

## L14: CHARACTERISATION OF INTEGRIN αV-DEPENDENT ADHESOME IN TUMOR CELLS

ANDREJA AMBRIOVIĆ-RISTOV

*Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
*(e-mail: Andreja.Ambriovic.Ristov@irb.hr)*

Integrins are heterodimeric glycoproteins that bind cells to extracellular matrix proteins. The connection between integrins and the cytoskeleton is mediated by a dynamic integrin adhesion complex (IAC), which transduces chemical and mechanical signals to control a multitude of cellular functions, including sensitivity to antitumor drugs, migration and invasion. Methods based on proteomics enable the analysis of IAC, the composition of which has been termed adhesome. Our recent focus has been the characterisation of integrin αV-dependent adhesome in three tumor cell models. The first one is tongue squamous carcinoma Cal27 cell model composed of Cal27 cells and a cell clone obtained by stable transfection of integrin subunit β3 cDNA that led to de novo expression of integrin αVβ3 conferring decreased sensitivity to cisplatin, mitomycin C, doxorubicin and 5-fluorouracil, and increased cell migration and invasion. The other two cell models were selected based on our recently published data showing that knockdown of integrin αV sensitizes melanoma cell line MDA-MB-435S and triple negative breast carcinoma cell line MDA-MB-231 to vincristine and paclitaxel. Therefore, the second model involves cell line MDA-MB-435S and corresponding cell clone isolated by stable transfection of integrin αV-specific shRNA expressing plasmid demonstrating increased sensitivity to vincristine and paclitaxel, and decreased migration, which is consistent with transient transfection data. Interestingly, sensitivity pattern of the MDA-MB-231 cell model was not in line with transient transfection data whereas MDA-MB-231 cell clone with decreased amount of integrin αV demonstrated decreased sensitivity to paclitaxel and vincristine, but still, decreased migration. To precisely understand the role of integrins αV, preferentially αVβ3 and αVβ5, in regulating IAC in tumor cells, we characterised by mass spectrometry (MS), the adhesome of Cal27, MDA-MB-435S and MDA-MB-231 cell lines grown in standard cell culture conditions and compared it to corresponding stable cell clones expressing altered amount of integrin αV. MS data enables the assessment of main integrins used by tumor cells and the analysis of the composition of IAC recruited by integrins αV. These data represent a valuable resource for improving our understanding of the composition of IAC and of the mechanisms involved adhesion control of cell sensitivity to antitumor drugs, migration and invasion.

# L15: TARGETED THERAPIES FOR HER2 POSITIVE METASTATIC BREAST CANCER PATIENTS

PAULA PODOLSKI, NERA ŠARIĆ

*Department of Oncology, University Hospital Centre Zagreb, Zagreb, Croatia*
*(e-mail: podolski.paula@gmail.com)*

The amplification of the human epidermal growth factor receptor 2 (HER2) is observed in 20-25% of all breast cancers. Targeting HER2 receptor has led to improved outcomes for patients with metastatic breast cancer, and is considered as major progress in breast cancer therapy. The HER2-targeted agents currently approved for treatment of metastatic breast cancer are trastuzumab, pertuzumab, trastuzumab-emtansine and lapatinib. They are used in different combinations with each other and with other chemotherapeutic agents. Patients with hormone receptor-positive (ER and/or PgR) and HER2-positive disease, should receive HER2-directed therapy as a component of their first-line treatment, while endocrine therapy is administrated following induction chemotherapy plus HER2-directed therapy. Although these drugs improve progression-free survival and overall survival, they are generally not curative in the metastatic setting in the majority of patients, due to several mechanisms of anti-HER2 therapy resistance. There are a few prognostic biomarkers, but still no predictive biomarkers to guide the treatment selection of metastatic HER2 targeted therapy. Important is to continue research into the biology of HER2-positive breast cancer, including investigation of resistance pathways and their interaction with our immune system. The wide range of the next generation of therapies are under development. They include: new tyrosine kinase inhibitors (TKI) targeting HER2 receptors and inhibitors of their down-stream signaling (neratinib, afatinib, tucatinib); inhibitors of the cell cycle (CDK4/6 inhibitors), HSP90 and angiogenesis; HER2 targeted antibody-drug conjugates (ADC) and immunotherapy aimed at HER2-positive breast cancer (magretuximab, ertumaxomab, checkpoints inhibitors).

# SL: DROPLET DIGITAL PCR (ddPCR) AND THE DETECTION OF SOMATIC MUTATIONS FROM LIQUID BIOPSIES OF COLORECTAL CANCER PATIENTS

ROK KOŠIR[1], URŠULA PROSENC ZMRZLJAK[1], ZORAN KRIVOKAPIĆ[2,3], DRAGICA RADOJKOVIĆ[4], ALEKSANDRA NIKOLIĆ[4]

[1] *Molecular Biology Laboratory, Bia Separations CRO, Labena d.o.o., Ljubljana, Slovenia (e-mail: rok.kosir@labena.si)*
[2] *Department of Colorectal Diseases, Clinic for Digestive Surgery, First Surgical Clinic, Clinical Center of Serbia, Belgrade, Serbia*
[3] *Medical Faculty, University of Belgrade, Belgrade, Serbia*
[4] *Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia*

Tumor mutation status has become an important part of molecular diagnostics that enables optimal treatment response. Genetic screens for somatic mutations are performed on genes including KRAS, BRAF and NRAS that are known to be frequently mutated in CRC tumors and confer resistance to therapies directed against EGFR signaling pathway. Considering that these mutations provide a selective advantage for tumor cell survival, detecting somatic mutations present in wild type background with as low as < 0.1% is of potential diagnostic utility. Currently, the main source of tumor DNA for mutation detection is FFPE. However, in recent years liquid biopsy has become a promising alternative. While the use of liquid biopsy is not included in current protocols for mCRC patient management, it has been extensively studied as a non-invasive method for both treatment response and patient monitoring.

The aim of the study was to determine the cfDNA levels and the KRAS and BRAF mutation status in patients who underwent surgical removal of primary CRC. Since cfDNA in the bloodstream can be of various origins (different necrotic / apoptotic processes), we compared cfDNA concentrations of CRC patients to two additional groups (hemorrhoid patients and healthy individuals). cfDNA was isolated from serum and its concentration measured with our custom designed human gDNA ddPCR assay which enables efficient and specific measurement of low concentrations of human DNA. The highest concentration of cfDNA was measured in CRC patients group (average 0.44 ng/ul), while both hemorrhoid patients and healthy individuals had significantly lower average concentrations, 0.25 ng/ul (* $p = 0.01$) and 0.08 ng/ul ($p = 0.0001$), respectively. Elevated cfDNA concentration must be interpreted with caution since other factors in addition to cancer can be the cause of higher cfDNA serum concentrations. All patients were previously screened for somatic mutations in the 12 and 13 KRAS codons and the BRAF V600E mutation using the SNaPshot Multiplex System. Mutations were confirmed with Sanger sequencing. Mutations were detected in some of the primary tumors, but their presence in the serum was not confirmed with the use of the above mentioned method. In the current study, we detected the same mutations using BioRad's ddPCR mutation assays and showed that ddPCR is the method of choice when more sensitive measurements are required in molecular diagnostics in oncology.

*Labena d.o.o. Sponsored Lecture*

# ST1: THE EFFECT OF SIRT3 EXPRESSION ON HUMAN BREAST CANCER CELLS IN NORMOXIA AND HYPEROXIA

IVA I. PODGORSKI[1], MARIJA PINTERIĆ[1], SANDRA SOBOČANEC[1], MARIJANA POPOVIĆ HADŽIJA[1], MLADEN PARADŽIK[2], ANA DEKANIĆ[2], MAJA MARINOVIĆ[2], MIRNA HALASZ[2], ROBERT BELUŽIĆ[1], GRAZIA DAVIDOVIĆ[1], ANDREJA AMBRIOVIĆ RISTOV[2], TIHOMIR BALOG[1]

[1] *Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: iskrinj@irb.hr)*
[2] *Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*

Sirtuin 3 (Sirt3), a major mitochondrial NAD+-dependent deacetylase, has bifunctional role in cancer tumorigenesis, acting as both oncogene and tumor suppressor, depending on the tissue and cancer-type specific metabolic programs. Changes in its expression are associated with the excessive production of reactive oxygen species (ROS), thus contributing to mitochondrial dysfunction and age-related pathologies. Hyperoxic treatment (i.e. generator of ROS) was shown to support some tumorigenic properties, but finally suppresses growth of certain mammary carcinoma cells. 70% of all breast cancer cases are estrogen receptor (ER) positive and express ER-alpha (ER-$\alpha$). While ER-$\alpha$ positive cancers are more receptive to hormonal therapy, triple negative breast cancers (TNBC) are characterized by an aggressive behaviour and the lack of targeted therapeutic strategies. Due to strikingly reduced Sirt3 level in many breast cancer cells, we aimed at deciphering the effect of de novo Sirt3 expression in normoxic and hyperoxic conditions in the human breast cancer cells. Although we have recently shown that Sirt3 acts as a tumor suppressor in non-invasive (ER-$\alpha$ positive) breast cancer cells, it remains unclear whether this effect is mediated through ER-$\alpha$ signalling pathway. Therefore, we stably transfected both MCF-7 (ER-$\alpha$ positive) and MDA-MB-231 (TNBC) cells with Flag-tagged Sirt-3 plasmid and characterized Sirt-3 overexpressing clones in normoxic and hyperoxic conditions. To further characterize clones and decipher the cause of the observed differences in their response, we used combination of treatments to alter the expression and activity of different proteins. We monitored the expression of proteins involved in mitochondrial biogenesis, glycolysis, metabolic regulation and antioxidant defense. Furthermore, we compared the growth rate, metabolic activity, mitochondrial ROS production and the cell cycle of the clones. Initial findings showed enhanced susceptibility of MCF-7 cells to hyperoxia and decreased cellular growth upon de novo Sirt3 expression. On the other hand, Sirt-3 markedly promoted growth of highly invasive MDA-MB-231 cells. Collectively, results suggested that Sirt-3 may either have a tumor suppressing or tumor promoting role in breast cancer cells depending on their invasiveness, thus giving us a rationale for further studies on Sirt3 and hyperoxia as an adjuvant tumor therapy in breast cancer malignancies.

# ST2: DIGITAL LOCALIZATION AND QUANTIFICATION OF WNT-3A SIGNALLING-EDITED TARGET APPROACH IN A 21 DAY MURINE MODEL OF IDIOPATHIC PULMONARY FIBROSIS

ŽELJKA ANZULOVIĆ, SNJEŽANA ČUŽIĆ, ANJA OGNJENOVIĆ, MAJA ANTOLIĆ, ANAMARIJA MARKOTA, BOŠKA HRVAČIĆ, INES GLOJNARIĆ

*Fidelta Ltd., Zagreb, Croatia*
*(e-mail: Zeljka.Anzulovic@glpg.com)*

Idiopathic pulmonary fibrosis (IPF) is an irreversible disease, with a rising incidence in the world. Characteristics of the disease are distorted lung architecture and progressive loss of functional tissue. Although the complete pathogenesis of the disease is still unclear, the hypothesis of alveolar epithelium micro-injuries and abnormal tissue repair has been frequently reported. Due to the limits of approved therapies in IPF, emerging need for the new therapeutic targets and the effective compounds is present. Wingless/integrase-1 (Wnt) signalling pathway recently gained emerged attention in the pathogenesis and progression of IPF. Many studies have reported the implication of dysregulated Wnt signalling in lung remodelling, pulmonary myofibroblast proliferation and epithelial to mesenchymal transition. In line with this, an increased expression of the Wnt signalling pathway related genes was observed in the lung tissue of diagnosed individuals. Wnts, in particular, Wnt 3α protein play a key role in matrix remodelling, tissue repair and fibrogenesis. The aim of the present study was to detect the localisation of the Wnt-3α protein in the lung tissue of the murine bleomycin-induced lung fibrosis.

In short, C57Bl/6 mice were administered intranasally with bleomycin (BLM) or saline (vehicle). Animals were sacrificed on day 21 post-challenge and the lungs were paraffin embedded. The expression of Wnt-3α was evaluated by immunohistochemistry on lung tissue slides. Quantification of Wnt-3α expression was analysed in digital pathology imaging software (Calopix, TRIBVN) on scanned slides (AxioScan. Z1, Zeiss) using immuno-object protocol. At day 21 post IN administration, the percentage of the Wnt-3α positive objects on slides was statistically increased in animals challenged with BLM. In naïve murine lungs, Wnt-3α was strongly expressed by bronchial epithelial cells. In BLM challenged animals, strong Wnt-3α expression was observed within the cytoplasm of a subset of bronchial epithelial cells. Honeycomb epithelial cells were Wnt-3α positive, as well as a proportion of cells in the fibro-inflammatory area.

In summary, our study shows a strong expression of the Wnt-3α protein in the lungs of BLM-challenged mice. It implicates that our 21-day model of BLM induced lung fibrosis is a good basis for the further evaluation and can be a suitable platform for the investigation of the potent Wnt/β-catenin target molecules.

## ST3: DNA METHYLATION IN HEAD AND NECK CANCER AND ORAL LESIONS

NINA MILUTIN GAŠPEROV, KSENIJA BOŽINOVIĆ, IVAN SABOL, MAGDALENA GRCE

*Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*
*(e-mial: nmilutin@irb.hr)*

Head and Neck Squamous Cell Carcinoma (HNSCC) is the 7th most frequent malignancy in the world, with a very low 5-year survival. It is known that the main risk factors associated with this carcinoma are excessive tobacco and alcohol consumption together with high-risk types of human papillomavirus (HPV). Still, there is a strong need to find new, good biomarkers of this disease. The most appropriate biomarkers would be those that would point out changes in epithelial cells before carcinoma occurrence. DNA methylation is irreversible change that inhibits gene expression. Altered DNA methylation is one of the possible factors associated with the HNSCC development. The presumption is that epigenetic biomarkers, such as methylated genes could point to changes even before they can be clinically detected. The model on which we explore those changes of DNA methylation are normal oral mucosa tissue, potentially malignant lesions of the oral mucosa, oral lichen planus (OLP) and oral lichenoid lesions (OLL) and HNSCC tissue. OLP and OLL are difficult to distinguish clinically and histopathologically, so early detection and distinguishing by epigenetic tools would be of great importance in further treatment. Herein, we will discuss DNA methylation profiling of different diagnosis by Infinium MethylationEPIC BeadChip array and pyrosequencing methods. The main goal was to reveal DNA methylation differences in normal oral samples, possible in oral lesions, and in HNSCC samples. The results obtained with Infinium MethylationEPIC BeadChip array showed a panel of gene promoters significantly methylated in HNSCC samples in comparison to normal samples, mainly of genes related with receptor and transmembrane functions and transcription activity. Some of them are included in cell cycle, cellular growth, transformation, apoptosis and autophagy. The group of hypomethylated gene promoters in HNSCC, in comparison to normal oral mucosa, and lesions, was mainly involved in immune response and transcriptional regulation.

# ST4: CUCURBITACIN B LOADED LIPID POLYMER HYBRID NANOCARRIERS INDUCED A SIGNIFICANT DECREASE IN MITOCHONDRIAL MEMBRANE POTENTIAL IN BREAST CANCER CELLS

FILIZ BAKAR-ATES[1], ERVA OZKAN[1], CEYDA TUBA SENGEL-TURK[2]

[1] *Department of Biochemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey (e-mail: flzbakar@yahoo.com)*
[2] *Department of Pharmaceutical Technology, Faculty of Pharmacy, Ankara University, Ankara, Turkey*

Breast cancer is the second cause of mortality among women. Due to insufficiency of existing therapies, novel therapeutic agents need to be developed in cancer treatment. Cucurbitacin B (CuB) is a triterpenoid compound isolated from Cucurbitaceae plants. The anticancer potential of this compound has shown in various cancer cells. Because of poor solubility of CuB, the extensive use of this potent anticancer compound is prohibited. Among various approaches nanocarriers such as lipid polymer hybrid nanoparticles appear to be a more promising strategy for the delivery of CuB. This study is designed to optimize and investigate the effect of various hybrid nanocarriers of CuB by using a DoE approach. The effects of these nanocarriers on alteration of mitochondrial potential have been investigated in estrogen +/- breast cancer cells. The hybrid nanocarriers were produced by one step self-assembly approach and the optimization studies were performed by a two-factor, three-level full factorial design with a center point (32). The effects of nanocarriers on cell proliferation were determined by MTT assay and cell cycle analysis was performed subsequently in MCF-7 and MDA-MB-231 breast cancer cell lines. The apoptosis was evaluated by annexin-V binding assay with flow cytometry and fluorescence imaging studies. The alteration on mitochondrial membrane potential has been determined by mitopotential assay that detects the amount of live and depolarized cells. In this research, CuB-encapsulated lipid-polymer hybrid nanocarriers were successfully produced. The encapsulation efficiency of nanocarriers ranged from 49.35% to 80.00%. The nanocarriers significantly inhibited cell proliferation at 0.1 μM and higher concentrations in both cell lines ($p<0.01$). The results showed that MCF-7 cells were more prone to apoptotic induction in that the apoptotic cell population % was significantly elevated ($p<0.05$). The results also indicated that the nanocarriers induced a significant decrease in mitochondrial membrane potential in both cell lines. In conclusion, this work has demonstrated that CuB loaded lipid polymer hybrid nanocarriers significantly induced apoptosis in both estrogen +/- breast cancer cells through triggering the downstream events in the apoptotic cascade and should be considered as potential candidate drug carriers for further investigations.

This work was supported by The Scientific and Technological Research Council of Turkey with 117S131 project number.

## ST5: DECIPHERING DNA-PROTEIN CROSSLINK REPAIR *IN VIVO* USING ZEBRAFISH MODEL

MARTA POPOVIĆ, CHRISTINE SUPINA

*Division of Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: mpopovic@irb.hr)*

DNA-protein crosslinks (DPC) are type of DNA lesions where a protein becomes irreversibly covalently bound to DNA upon exposure to endogenous or exogenous crosslink inducers. DPCs present a physical blockage to all DNA transactions: replication, transcription, recombination and repair and therefore the consequences of impaired DNA-Protein Crosslink Repair (DPCR) are severe. Considering their frequent occurrence and detrimental effect on all DNA transactions, it is not surprising that DPCs are implicated in aging, cardiovascular diseases, neurodegeneration and cancer. On a cellular level, aberrant DPC repair leads to the formation of DSBs, genomic instability and/or cell death, while on the organismal level impaired DPCR was so far shown to cause premature aging phenotypes and cancer. The discovery of proteolysis-coupled DPC repair centred on SPRTN and Wss1 proteases led to recognition of DNA-protein crosslink repair as a separate DNA damage repair pathway. However, we currently do not know how is the pathway orchestrated and which factors besides proteases are involved, while almost nothing is known of DPCR mechanism in vivo. Therefore, within this project we aim to unravel the orchestration of the DPCR pathway in vivo using zebrafish (Danio rerio) as a well-characterized vertebrate model. Zebrafish has been increasingly recognized as a valuable cancer model in studying melanoma and metastatic behaviour of various human cancers including glioblastoma and aggresive forms of breat cancer. We use CRISPR/Cas9 gene manipulations to knock-out or mutate specific gene of interest in zebrafish followed by analysis of DPC levels and wide range of phenotypes in zebrafish embryos and adults. We aim to show unequivocal link between DPCR and cancer emergence on the organismal level, with the aim of developing more efficient cancer therapies target.

# ST6: TAKING TUMOR CELLS TO THE THIRD DIMENSION: 3D MODELS TO STUDY TUMOR INVASION AND METASTASIS

JULIENNE K. MUENZNER[1,2], PHILIPP KUNZE[1,2], RAPHELA A. RANJAN[1,2], MARKUS ECKSTEIN[2], CAROL I. GEPPERT[2], PITHI CHANVORACHOTE[3], PABLO LINDNER[1,2], SANDRA POLASCHEK[1,2], KIRA MENKE[1,2], TOBIAS BAEUERLE[4], ARNDT HARTMANN[2], REGINE SCHNEIDER-STOCK[1,2]

[1] *Experimental Tumor Pathology, Institute of Pathology, Friedrich-Alexander University of Erlangen-Nuremberg, Erlangen, Germany*
[2] *Institute of Pathology, Friedrich-Alexander University of Erlangen-Nuremberg, Erlangen, Germany (e-mail: julienne.muenzner@uk-erlangen.de)*
[3] *Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand*
[4] *Preclinical Imaging Platform Erlangen (PIPE), Institute of Radiology, University Hospital Erlangen-Nuremberg, Erlangen, Germany*

Metastasis formation represents one of the well-known hallmarks of cancer and is one of the leading causes of cancer-related deaths. It starts with the dissemination of cancer cells from the primary tumor site and a local invasion of the surrounding tissue. This is followed by intravasation of the tumor cells, their survival in the circulatory system, extravasation and eventually re-colonization at a distant organ, thus generating a secondary tumor. All of these individual steps require specific features of tumor cells, which are largely connected to the epithelial-to-mesenchymal transition and cancer stem cell (CSC) phenotypes. Mostly due to their ease of use and low costs, two-dimensional cell culture systems are commonly applied in cancer research. However, these systems can only partially capture processes or characteristics associated with the formation of metastases in patients. For this reason, various three-dimensional experimental model systems have been established that are able to better mimic the in vivo situation. In our studies, we are applying several of these 3D cell culture and spheroid-based models in order to investigate the invasiveness of tumor cells, e.g. dependent on gene-specific knockouts or their CSC potential. Additionally, we are utilizing the in vivo chorioallantoic membrane assay as an alternative to animal experiments. Within this model system, we are not only able to elucidate the role of new potential metastatic players or CSC sub-populations during the individual steps of the metastatic cascade in more detail, but we can also investigate further hallmarks of cancer that contribute to tumor aggressiveness such as cell proliferation and tumor angiogenesis.

## ST7: ANTICANCER EFFECT OF BERBERINE AGAINST 2D AND 3D CELL CULTURE MODELS

ANA DAMJANOVIĆ, MARIJA ĐORĐIĆ CRNOGORAC, IVANA Z. MATIĆ, TATJANA P. STANOJKOVIĆ

*Institute of Oncology and Radiology of Serbia, Belgrade, Serbia*
*(e-mail: zaanu011@gmail.com)*

Berberine is an isoquinoline alkaloid extensively used for centuries in the traditional medicine. Recent studies showed that berberine possesses good anticancer therapeutic potential. The objectives of our study were to elucidate effect of berberine against 3D cell culture model, and to compare berberine activity against 2D and 3D models. IC50 value of berberine in 2D model was obtained using MTT test during 72h incubation, against human cervix adenocarcinoma cells (HeLa). The concentrations (IC50, 3IC50, 5IC50, 10IC50) were tested on 3D spheroids of HeLa cells formed by Hanging drop method during 72h. Morphological changes in cells after 72h, in both models, were analyzed by fluorescence microscope. The treated cells were stained with a mixture of acridine orange and ethidium bromide dyes. The positive control was cisplatin. IC50 values for berberine and cisplatin were 2.5 and 1.2 μg/mL. The concentrations of berberine (2.5, 7.5, 12.5, and 25 μg/mL) and cisplatin (1.2, 3.6, 6, 12 μg/mL) were added to cell spheroids. After 24h we observed spheroid contraction and diameter reduction in all samples treated with berberine or cisplatin. After 72h the trend of spheroid reduction was continued in cells treated with cisplatin or berberine in lower concentrations (IC50 and 3IC50). Samples treated with higher concentrations of berberine (5IC50 and 10IC50) showed loss of density and compactness, and consequently increase in the diameter. In control sample diameter increased during 24 and 72h. These results showed different influences of berberine and cisplatin on extracellular matrix and cell tight junctions in 3D model, which is important for further investigation of berberine impact on cancer migration and invasiveness. As expected, morphological characteristics typical for cells in apoptosis were detected in treated 2D models with IC50 concentration of berberine or cisplatin. With 3IC50 concentrations all cells were dead. In treated 3D models after 72h ratio between corrected total cell green and red fluorescence showed more viable than dead cells in all samples except in samples treated with 10IC50 concentrations of both agents. This result suggested that more complex 3D models require higher concentrations of active principles than 2D models. Our study showed that berberine has antitumor potential, which should be subject of further research, and that combination of 2D and 3D models gives better information of anticancer mechanism of this agent.

# ST8: NEUTROPHIL ELASTASE INDUCES METASTATIC PHENOTYPE *IN VITRO* AND *IN VIVO*

JULIA KARGL[1,2], GRACE YANG[2], HEATHER METZ[2], HOUGHTON McGARRY[2]

[1] *Medical University Graz, Otto Loewi Research Center, Unit: Pharmacology, Graz, Austria*
[2] *Fred Hutchinson Cancer Research Center, Clinical Research Division, Seattle, USA*
*(e-mail: julia.kargl@medunigraz.at)*

Neutrophil elastase (NE) is a neutrophil-derived serine protease with broad substrate specificity. We have previously shown that NE promotes lung tumor growth in vitro and in vivo and that NE is capable of entering tumor cell endosomes in a clathrin-dependent fashion.

Epithelial-mesenchymal transition (EMT) is a fundamental event for primary tumors to metastasize to distant sites. Altered gene and protein expression causes changes in cell morphology and behavior, resulting in the loss of attachment to neighboring cells, intravasation, and migration into distant tissue. However, the entities residing within the tumor microenvironment that drive EMT are poorly understood.

We identified NE, expressed and released by tumor-associated neutrophils, as such an entity. We found that NE induces EMT-like morphology and behavior in several cancer cell lines as well as upregulates proteins (N-cadherin, $\alpha$-SMA, Fibronectin and Vimentin) associated with EMT. Furthermore, NE provokes tumor cell migration and invasion in vitro and is required for metastasis formation in vivo. Besides known EMT markers we identified significant upregulation of inhibitor of DNA binding 1 (ID1) in tumor cells in the presence of NE. Increased ID1 expression was previously shown to correlate with tumor progression and metastasis. Clathrin-dependent NE internalization is required for both ID1 expression and the EMT-like phenotype. We localized NE in different compartments of tumor cells and found it predominantly in the nuclear fraction; more specifically, NE is located in the chromatin-bound fraction, indicating a potential role in chromatin remodeling and transcriptional regulation. Additionally, the proteolytic activity of NE was measured in nuclear and chromatin-bound fractions and is required for induction of ID1 expression and EMT-like behavior. Taken together, these data suggest the importance of NE-induced ID1 expression in metastasis formation.

# ST9: REPURPOSING OF THE ANGIOTENSIN INHIBITOR TELMISARTAN FOR TREATMENT OF PANCREATIC DUCTAL ADENOCARCINOMA AND MALIGNANT MELANOMA

JELENA GRAHOVAC[1], YVES BOUCHER[2], SINIŠA RADULOVIĆ[1]

[1] *Laboratory for Experimental Pharmacology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia (e-mail: jelena.grahovac@ncrc.ac.rs)*
[2] *Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston MA, USA*

Development of new cancer therapeutics is expensive and the approval and translation into the clinic often take between 10 and 15 years. In contrast, repurposing of drugs already approved for other uses (that have been tested in humans, and for which information is available on pharmacology, formulation and potential toxicity) enables quick translation into clinical trials and their integration into health care. Recently, it has been recognized that therapy for chronic diseases can have an impact on the progression and outcome in cancer patients. We examined the effects of antihypertensive telmisartan on pancreatic ductal adenocarcinoma (PDAC) and metastatic melanoma (MM) cell survival and tumor progression. Telmisartan is an angiotensin receptor 1 (AT1R) antagonist and a partial agonist of the peroxisome-proliferator activated receptor γ (PPARγ). In silico analysis showed that both PDAC and MM tumors express AT1R and PPARγ receptors, and in vitro both PDAC and MM cell lines were sensitive to telmisartan. In PDAC sensitivity to telmisartan inversely correlated with the PPARγ expression level, and telmisartan targeted mesenchymal cells more potently. In metastatic melanoma sensitivity correlated with the BRAF mutation status and treatment synergized with vemurafenib, BRAF inhibitor used in the clinic. In both cancer types telmisartan had extra-receptor effects causing metabolic perturbations in glucose consumption, mitochondrial fission and ultimately apoptosis. In vivo, in orthotopically implanted PDAC model, telmisartan inhibited the growth of primary tumors, decreased the incidence of liver metastasis and improved mouse survival. Taken together, telmisartan showed prominent anti-tumor activity. The effective doses of telmisartan examined in our study can be achieved in patients. Given that telmisartan is widely used and safe antihypertensive drug, our findings provide the scientific rationale for testing its efficacy in the chemoprevention of PDAC and melanoma progression.

## ST10: ANTICANCER EFFECTS OF APIGENIN IN HUMAN BREAST CANCER CELLS

JOSIP MADUNIĆ[1], IVANA VRHOVAC MADUNIĆ[2], MAJA ANTUNOVIĆ[1], MLADEN PARADŽIK[3], VERA GARAJ-VRHOVAC[4], DAVORKA BRELJAK[2], INGA MARIJANOVIĆ[1], GORAN GAJSKI[4]

[1] *Department of Molecular Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
*e-mail: jmadunic@biol.pmf.hr*
[2] *Molecular Toxicology Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia*
[3] *Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
[4] *Mutagenesis Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia*

Apigenin is a dietary flavonoid found in several types of vegetables and fruits. Its potential anticancer properties were investigated in two types of human breast cancer cells: ER-positive MCF-7 and triple-negative MDA MB-231 cells. Human peripheral blood lymphocytes were used to elucidate the apigenin's toxicological safety regarding the normal cells. MTT, comet and lipid peroxidation assays were used to evaluate cyto- and genotoxicity of apigenin towards cancer cells. Furthermore, the type of apigenin-induced cell death was analysed using several biomarkers.

Our results revealed that the treatment with apigenin caused changes in cell morphology, in a dose- and time-dependent manner. This was followed with apoptosis as a dominant type of cell death in both cell lines. Moreover, apigenin exhibited genotoxicity towards cancer cells by inducing oxidative damage. Importantly, cell viability and comet assays showed that apigenin was not cytogenotoxic to normal cells.

The observed anticancer activities of apigenin accompanied by its low toxicity towards normal cells indicate the possibility of using this dietary flavonoid as an anticancer agent. Even though the beneficial effects of apigenin are promising, further in vitro and in vivo studies are needed to enable its translation from bench to bedside.