"HDIR-6: Targeting Cancer"

The 6th Meeting of the Croatian Association for Cancer Research with International Participation

November 10-12, 2022

Hotel International, Zagreb, Croatia

BOOK OF ABSTRACTS

Hrvatsko društvo za istraživanje raka (HDIR)
Croatian Association for Cancer Research (CACR)

"HDIR-6: Targeting Cancer" The 6th Meeting of the Croatian Association for Cancer Research with International Participation – Book of Abstracts

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"HDIR-6: Targeting Cancer" The 6th Meeting of the Croatian Association for Cancer Research with International Participation

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Hotel International, Miramarska cesta 24, HR-10000 Zagreb, Croatia

Dates:
November 10-12, 2022

Scientific Committee:
Sonja Levanat, Andreja Ambriović Ristov, Paola Defilippi, Katja Ester, Maja Herak Bosnar, Dinko Leović, Vesna Musani, Petar Ozretić, Maja Sabol, Maja Sirotković-Skerlev, Neda Slade, Sandra Sobočanec, Ivan Šamija, Engin Ulukaya

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With great pleasure we announce the sixth meeting of the Croatian Association for Cancer Research (Hrvatsko društvo za istraživanje raka, HDIR), a member of the European Association for Cancer Research (EACR).

The HDIR-6 Meeting subtitled "Targeting Cancer" has a program divided into three days and covering the hottest cancer research topics including signaling networks, new therapies and cancer resistance, metastasis, immunology, genomics and epigenomics.

The meeting will be held in Zagreb, Croatia, at the Hotel International (Miramarska 24, Zagreb) from November 10 to 12, 2022.

The goal of the HDIR-6 Meeting is again to promote cancer research in Croatia focused on the cooperation between basic research and clinical cancer management. This meeting will put an emphasis on international communication and cooperation, with speakers from many European countries. The Meeting provides opportunity for cancer researchers and clinicians to gather together and to establish a good working collaborations with each other and with other regional EACR national societies.

Original contributions by researchers and clinicians with an interest in cancer are more than welcome and encouraged. Your contribution to this meeting will mutually benefit all participants and help to make this event successful.

We invite you to join us, and we are looking forward meeting you in Zagreb.

Sincerely,

Prof. Sonja Levanat, PhD
HDIR President

GENERAL INFORMATION

MEETING VENUE

The Meeting will be held at Hotel International, Miramarska 24, Zagreb, Croatia

Congress Hall

Ground floor, Grand Salon

REGISTRATION

Registration desk will be in the lobby in front of the Grand Salon of Hotel International.

Opening hours:

Thursday, November 10th	13:00 – 19:30
Friday, November 11th	8:30 – 18:00
Saturday, November 12th	8:30 – 13:30

Registration fee includes: attendance to the lectures and poster session, certificate of attendance, CME credits, welcome reception on November 10, lunch on November 11, and refreshments during coffee breaks. Presentations (apart from invited speakers) will not be allowed without paid registration fee.

Registration Fees

	HDIR / EACR MEMBERS*	NON-MEMBERS
students	570 kn / 75 €	760 kn / 100 €
researchers/MD/other	760 kn / 100 €	1140 kn / 150 €
*non-HDIR/EACR members will have to show their EACR membership card / number at the registration		

BADGES

All registered participants and exhibitors will receive official Meeting badges.

LANGUAGE

The official language of the meeting is English. All lectures will be in English, and there will be no simultaneous translation.

LECTURES

Windows-based laptop and LCD projector will be available. The speakers are kindly asked to provide their slides in advance before each session.

POSTER PRESENTATIONS

Poster number corresponding to the authors' abstract will already be posted at the top of the board. Posters could be mounted on Thursday, November 10 between 13:00 and 19:30 and Friday, November 11 between 8:30 and 14:30. Poster session will be held after lunch on Friday, November 11 between 14:30 and 15:30. Presenters are kindly asked to check their poster numbers. Poster removal will be possible after the conclusion of the Poster session. Three best poster prizes (sponsored by EACR) will be awarded at the closing ceremony.

CERTIFICATE OF ATTENDANCE

Confirmations of attendance will be issued at the Registration desk.

CME CREDITS AND ECTS POINTS

The Croatian Medical Chamber will award active participants with 15 CME credits, and passive participants with 10 credits. Graduate students from the University of Zagreb could be awarded ECTS points for active participation (poster presentation).

PROMOTIONAL EXHIBITIONS

The participants are kindly invited by the Organizing Committee of the HDIR-6 to visit promotional exhibitions of the following companies:

Avantor / VWR
Biosistemi
Biovit
Diagnostica Skalpeli
Jasika
KEFO
Labena
Medic
Vita Lab Nova

INVITED SPEAKERS

Yari Ciribilli
CIBIO Department
University of Trento
Povo (Trento), Italy

Paola Defilippi
Department of Molecular Biotechnology and Health Sciences
University of Torino
Torino, Italy

Vedrana Filić Mileta
Division of Molecular Biology
Ruđer Bošković Institute
Zagreb, Croatia

Bato Korać
Institute for Biological Research "Sinisa Stankovic"
National Institute of Republic of Serbia;
Faculty of Biology
University of Belgrade
Belgrade, Serbia

Mitchell P. Levesque
Department of Dermatology
University Hospital Zurich
University of Zurich
Zürich, Switzerland

Alan Parker
Division of Cancer and Genetics
Cardiff University School of Medicine
Cardiff, UK

Daniel Peeper
Department of Molecular Oncology & Immunology
Netherlands Cancer Institute (NKI);
Vrije Universiteit Amsterdam
Amsterdam, Netherlands
Oncode Institute
Utrecht, Netherlands

Bojan Polić
Department of Histology & Embryology
Faculty of Medicine
University of Rijeka
Rijeka, Croatia

Neda Slade
Division of Molecular Medicine
Ruđer Bošković Institute
Zagreb, Croatia

Sandra Sobočanec
Division of Molecular Medicine
Ruđer Bošković Institute
Zagreb, Croatia

Krisztina Takacs-Vellai
Department of Biological Anthropology
Eötvös Lorand University
Budapest, Hungary

Daniela Taverna
Molecular Biotechnology Center;
Department of Molecular Biotechnology and Health Sciences
University of Torino
Torino, Italy

Iva Tolić
Division of Molecular Biology
Ruđer Bošković Institute
Zagreb, Croatia

Ariana Znaor
Cancer Surveillance Branch
International Agency for Research on Cancer
Lyon, France

PROGRAMME

November 10, 2022 / Thursday

13:00-15:00 *Registration*

15:00-15:30 *Opening ceremony*

15:30-16:20 **PLENARY LECTURE**

(Chair: Sonja Levanat)

PL: Iva Tolić (Croatia): "Chromosome Segregation Apparatus and Cancer"

16:20-17:20 **NON-MAMMALIAN MODELS OF TUMOR**

(Chairs: Maja Herak Bosnar, Krisztina Takacs-Vellai)

16:20-16:50 **L1:** Krisztina Takacs-Vellai (Hungary): "How to Use *C. elegans* as a Tumor Model?"

16:50-17:20 **L2:** Vedrana Filić Mileta (Croatia): "Regulation of Ras Activity in Cellular Feeding"

17:20-17:40 *Coffee break*

17:40-18:30 **KEYNOTE LECTURE [Sponsored by EACR]**

(Chairs: Sonja Levanat, Engin Ulukaya)

KL: Daniel Peeper (Netherlands): "Rational Concepts for Tumor & Immune Cell Therapy Combinations"

18:30-19:30 *Welcome reception*

November 11, 2022 / Friday

9:00-11:00 **CANCER BIOMARKERS AND RESISTANCE**

(Chairs: Neda Slade, Vesna Musani)

9:00-9:30 **L3:** Ariana Znaor (France): "Global Cancer Burden and Cancer Surveillance"

9:30-10:00 **L4:** Mitchell P. Levesque (Switzerland): "ROS Induction as a Strategy to Target Persister Cancer Cells with Low Metabolic Activity"

10:00-10:20 **ST1:** Ignacija Vlašić (Croatia): "Unique Hallmarks of Targeted Therapy Resistant Melanoma Cell Lines"

10:20-10:40 **ST2:** Margareta Pernar Kovač (Croatia): "The mIR-200c/TUBB3 Regulatory Axis is Part of the Cellular Stress Response to Carboplatin in Drug-resistant Ovarian Cancer Cell Lines"

10:40-11:00 **ST3:** Miodrag Vuković (Serbia): "Antimelanoma Potential of New Telmisartan Analogues Without AT1 Receptor Activity"

11:00-11:20 *Coffee break sponsored by Gorea Plus d.o.o.*

11:20-13:20 **CANCER GENOMICS AND EPIGENOMICS**

(Chairs: Chairs: Sandra Sobočanec, Bato Korać)

11:20-11:50 **L5:** Bato Korać (Serbia): "A New Look at the Warburg Effect of Breast Cancer"

11:50-12:20 **L6:** Daniela Taverna (Italy): "miR-214 Mediated Stroma-Tumor Cell Crosstalk During Tumor Progression"

12:20-12:40 **ST4:** Hattie Ollerton (UK): "PTCH1 as a Novel B-arrestin Target Suggests a Regulatory Role in Non-canonical Hedgehog Signalling"

12:40-13:00 **ST5:** Gordana Bubanović (Croatia): "Liquid Biopsy for Detection of Resistance Mutation T790M in EGFR Gene From Lung Cancer Patients"

13:00-13:20 **ST6:** Chanchai Boonla (Thailand): "H4K20me3 Upregulated by Reactive Oxygen Species is Associated with Tumor Progression and Poor Prognosis in Patients with Hepatocellular Carcinoma"

13:20-14:30 *Lunch break*

14:30-15:30 **POSTER SESSION**

15:30-16:00 **SPONSORED LECTURE [Sponsored by Labena d.o.o.]**

(Chair: Mario Dananić)

L7: Agnieszka Ciesielska (Poland): "Single-Cell Sequencing"

16:00-18:00 **SIGNALING NETWORKS I**

(Chairs: Petar Ozretić, Yari Ciribilli**)**

16:00-16:30 **L8:** Yari Ciribilli (Italy): "ETV7 as a Mediator of Chemoresistance and Cancer Aggressiveness in Breast Cancer"

16:30-17:00 **L9:** Neda Slade (Croatia): "The Role of p53 Family in Melanoma Development and Therapy Resistance"

17:00-17:20 **ST7:** Anđela Horvat (Croatia): "Reciprocal Regulation of p21 and Chk1 Controls the Cyclin D1-RB Pathway to Mediate Senescence Onset After G2 Arrest"

17:20-17:40 **ST8:** Matea Kurtović (Croatia): "Unique and Overlapping Transcriptional Targets of GLI1, GLI2 and GLI3 in Melanoma Cell Lines"

17:40-18:00 **ST9:** Nives Pećina-Šlaus (Croatia): "Tracking Molecular Actors of Progression in Intracranial Meningioma"

November 12, 2022 / Saturday

9:00-11:00 **SIGNALING NETWORKS II**

(Chairs: Andreja Ambriović Ristov, Sandra Sobočanec)

9:00-9:30 **L10:** Paola Defilippi (Italy): "The Adaptor Protein p140Cap Negatively Regulates the Aggressiveness of Breast Cancer and Neuroblastoma: Molecular and Functional Insights"

9:30-10:00 **L11:** Sandra Sobočanec (Croatia): "Sirtuin 3 Exerts Its Tumour-Suppressive Role by Stabilizing p53 and Attenuating Response to Estrogen in MCF-7 Cells"

10:00-10:20 **ST10:** Anja Rac (Croatia): "Focal and Reticular Adhesions Composition in Melanoma Cell Lines MDA-MB-435S and RPMI-7951"

10:20-10:40 **ST11:** Marija Ljubojević (Croatia): "Sex and Age-related Changes in Kidney and Liver Expression of Inducible Metallothioneins in Correlation with Essential and Toxic Trace Elements Accumulation: Influence of Antioxidants"

10:40-11:00 **ST12:** Marija Tomić (Croatia): "TLN2 and KANK2 Are Potential Targets for Enhanced Sensitivity to Paclitaxel Treatment in MDA-MB-435S Cells"

11:00-11:20 *Coffee break*

11:20-13:20 **CANCER IMMUNOLOGY AND IMMUNOTHERAPIES**

(Chairs: Maja Sabol, Maja Sirotković-Skerlev)

11:20-11:50 **L12:** Alan Parker (UK): "Development of αvβ6 Selective Precision Immunovirotherapies"

11:50-12:20 **L13:** Bojan Polić (Croatia): "The Role of NKG2D in MAFLD Pathogenesis"

12:20-12:40 **ST13:** Beata Halassy (Croatia): "Neoadjuvant Oncolytic Virotherapy of a Localized Breast Cancer Recurrence: A Case Study"

12:40-13:00 **ST14:** Dora Knezović (Croatia): "The Contribution of TLR4 and MYD88 to Bladder Cancer Development"

13:00-13:20 **ST15:** Muhlis Akman (Italy): "TFEB Drives Chemo-immuno-resistance in Lung Cancer"

13:20-13:40 *Closing ceremony and best poster awards [Sponsored by EACR]*

ABSTRACTS

Plenary Lecture PL

Keynote Lecture KL

Lectures L1 – L13

Short Talks ST1 – ST15

Posters P1 – P18

ORAL PRESENTATIONS

PL: Chromosome Segregation Apparatus and Cancer

Iva Tolić

Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: tolic@irb.hr)

Do all chromosomes segregate with the same accuracy? Does it matter where they come from? We found a "danger zone" behind the spindle pole, which puts the chromosomes with this unfavorable starting position at risk of mis-segregation. These polar chromosomes often remain unaligned or acquire merotelic attachments in chromosomally unstable cancer cells, creating a bias in mis-segregation that may contribute to the distinct aneuploid karyotypes in cancers and during embryonic development. Chromosomes ride out of the danger zone by the rotation of the kinetochore-associated astral microtubules around the spindle pole. Once the kinetochores reach the spindle body, they stimulate microtubule bundling by lateral binding via CENP-E in an Aurora B-regulated manner. This leads to the formation of bridging fibers, which promote kinetochore congression at the spindle midplane by driving flux of kinetochore fibers in such a manner that the longer sister kinetochore fiber fluxes faster that its shorter counterpart. We propose a model in which the congression of polar chromosomes is critical for error-free segregation, and depends on the pivoting movement of microtubules around the spindle pole together with length-dependent poleward flux of kinetochore fibers.

KL: Rational Concepts for Tumor & Immune Cell Therapy Combinations [Lecture sponsored by EACR]

Daniel Peeper[1,2,3]

[1]Department of Molecular Oncology & Immunology, Netherlands Cancer Institute (NKI), Amsterdam, Netherlands; [2]Vrije Universiteit Amsterdam, Amsterdam, Netherlands; [3]Oncode Institute, Utrecht, Netherlands (e-mail: d.peeper@nki.nl)

Notwithstanding clinical advances, unfortunately many patients are not experiencing durably benefit from targeted and immunotherapies, mostly because of early or late resistance. We employ function-based, genome-wide screens and other strategies to develop rational combinatorial cancer treatment, targeting both cancer and immune cells.

On the one hand, we're increasing our understanding of how cancer cells rewire their signaling networks, to expose and exploit new pharmacologically tractable tumor susceptibilities, particularly in the context of immunotherapy. On the other hand, we're manipulating various cell types from the patient's own immune system to revert their dysfunction and boost their specific cytotoxicity towards tumor cells. We complement these studies with analyses of clinical samples in collaboration with our clinical colleagues.

L1: How to Use *C. Elegans* as a Tumor Model?

Krisztina Takacs-Vellai

Department of Biological Anthropology, Eötvös Lorand University, Budapest, Hungary
(e-mail: takacsk@caesar.elte.hu)

Caenorhabditis elegans is known as a model for tumor genetics[1, 2]. The *C. elegans* genome encodes 19000 genes, 40 % of them have highly conserved human homologs[3]. Analyzing the worm homologs of cancer related genes helps to better understand their biological functions and reveal signaling networks. In addition, processes influencing tumor progression like cell motility, apoptosis, phagocytosis are conserved and can be studied in this model organism.

The first identified metastasis suppressor gene NM23 (non-metastatic clone 23) or NME1, which displays NDPK (nucleoside-diphosphate kinase) activity, is a negative regulator of cell motility. First, we investigated the function of NDK-1, the sole *C. elegans* homolog of group I NDPKs in distal tip cell (DTC) migration. We found that NDK-1 regulates DTC migration in a dose-dependent manner and overexpression of NDK-1 results in reduced migration. As DTC migration and engulfment of apoptotic corpses are analogous processes, we investigated defects of apoptosis in ndk-1(-) mutants. Embryos and germ cells defective for NDK-1 showed an accumulation of apoptotic cell corpses. NDK-1::GFP is expressed in gonadal sheath cells, specialized cells for engulfment and clearance of apoptotic corpses in the germ line, indicating a role for NDK-1 in apoptotic corpse removal. We revealed by IP-MS and Duolink proximity ligation assay that NDK-1/NME1 works in a complex with DYN-1/Dynamin, which is essential for engulfment and phagosome maturation. Silencing of NM23-M1 in mouse bone marrow-derived macrophages resulted in decreased phagocytosis of apoptotic thymocytes. In human macrophages, NME1 and Dynamin are corecruited at sites of phagosome formation in F-actin-rich cups. Together, our data demonstrate that NDK-1/NME1 is an evolutionarily conserved element of successful phagocytosis.

Nematodes can serve as a model to better understand some aspects of metabolic switches in cancer. Pheochromocytomas and paragangliomas (PCCs) are neuroendocrine tumors and in many cases mutations in subunits of the SDH (succinate-dehydrogenase) enzyme lead to early cancer. The Arg230His mutation in the SDHB subunit causes a familial form of malignant PCC. To see the consequences of the Arg230His mutation, we first analyze the effects of the corresponding mutation in a worm model at the level of metabolomics and transcriptomics.

[1]Kirienko *et al*. (2010) Dev Dyn, 239:1413-1448.
[2]Stuelten *et al.*, (2018) Nat Rev Cancer, 18(5):296-312.
[3]Shaye and Greenwald (2011) Plos One, 6(5):e20085.

L2: Regulation of Ras Activity in Cellular Feeding

Vedrana Filić Mileta

Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: vfilic@irb.hr)

Cellular feeding and proliferation are major processes required for the growth of an organism. Nevertheless, uncontrolled cell growth is a hallmark of cancer. Macropinocytosis or "cellular drinking" is nonselective, bulk uptake of large volumes of extracellular fluid. For unicellular organisms like an amoeba, macropinocytosis is a way of nutrient uptake. In a metazoan organism, cells preferentially import nutrients via cell surface transporters or by receptor-mediated endocytosis, while macropinocytosis is used by specialized cells for other purposes. Although all tested mammalian cell lines are capable of performing stimulated macropinocytosis in cell culture, it is still not clear whether and to what extent is macropinocytosis used by mammalian cells as a nutrient uptake pathway. However, tumor cells often exploit macropinocytosis to obtain macromolecules as a nutrient source and thus thrive in the microenvironment that is often scarce in glucose and amino acids due to poor tumor vascularization. In particular, malignant cells harboring mutated oncogenic Ras proteins perform constitutive macropinocytosis similar to amoeba *Dictyostelium discoideum*. Hence, mammalian and *D. discoideum* cells in culture are the most widely used models to study macropinocytic uptake.

We investigated D. discoideum protein IqgC and showed that it negatively regulates macropinocytosis by inhibiting Ras activity. IqgC acts as a RasGAP (Ras GTPase activating protein) that specifically deactivates RasG, a GTPase crucial for efficient fluid uptake in D. discoideum. The negative effect was more pronounced in wild-type *D. discoideum* strain that contains another RasGAP, NF1, a homolog of a human RasGAP neurofibromin 1, and thus has more suppressed macropinocytosis. Deletion of IqgC in this genetic background induced more elevated fluid uptake compared to NF1 deficient strain.

IqgC strongly accumulates at forming and nascent macropinosomes where it colocalizes with the active Ras probe. However, Ras dissociates from the internalized vesicle prior to IqgC. We demonstrated that RasG is indispensable for the recruitment of IqgC to the forming cup, but other proteins seem to be required for its retention during early macropinosome maturation. One candidate is another GTPase from the Ras superfamily, Rab5A which is a direct interactor of IqgC. The biological significance of this interaction is currently under investigation.

L3: Global Cancer Burden and Cancer Surveillance

Ariana Znaor

Cancer Surveillance Branch, International Agency for Research on Cancer, Lyon, France
(e-mail: znaora@iarc.who.int)

In 2020, there were 19 million new cancer cases and 10 million cancer deaths globally. While the highest incidence rates are observed in high income countries, mortality is highest in the low- and middle-income countries (LMIC). Currently, 70% cancer deaths occur in LMIC, where we expect the highest increase in cancer burden in the next 20 years, both due to population aging and changes towards Western lifestyles. The patterns of most common cancers are changing as well, and vary according to the development level. Presented global estimates will be from the International Agency for Research on Cancer (IARC) GLOBOCAN 2020 database (https://gco.iarc.fr/today/home) and IARC Global Cancer Observatory, Cancer Tomorrow website (https://gco.iarc.fr/).

In view of the increasing cancer burden, high quality cancer registry data are critical for cancer control planning. The Cancer Surveillance Branch of IARC supports cancer surveillance at global, regional and local level via its Global Initiative for Cancer Registry Development program (GICR, https://gicr.iarc.fr/).

While the pandemic has shown the importance of global and local data and action, it has also disrupted the work of cancer surveillance systems worldwide, with a negative impact more marked in LMIC. As a silver lining, there has been a move toward electronic reporting systems, broader dissemination and outreach of training and education materials via e-learning and increased use of telemedicine.

L4: ROS Induction as a Strategy to Target Persister Cancer Cells with Low Metabolic Activity

Ossia Eichhoff[1], Corinne I. Stoffel[1], The Tumor Profiler Consortium[2], Mitchell P. Levesque[1]

[1]Department of Dermatology, University Hospital Zurich, University of Zurich, Zürich, Switzerland; [2]The Tumor Profiler Consortium Switzerland
(e-mail: Mitchell.Levesque@usz.ch)

Metabolic reprogramming is an emerging hallmark of resistance to cancer therapy but may generate vulnerabilities that can be targeted with small molecules. Multi-omics analysis revealed that NRAS-mutated melanoma cells with a mesenchymal transcriptional profile adopt a quiescent metabolic program to resist cellular stress response induced by MEK-inhibitor resistance. However, as a result of elevated baseline ROS levels, these cells become highly sensitive to ROS induction. *In vivo* xenograft experiments and single-cell RNA sequencing demonstrated that intra-tumor heterogeneity requires the combination of a ROS-inducer and a MEK-inhibitor to target both tumor growth and metastasis. By *ex vivo* pharmacoscopy of 62 human metastatic melanomas, we found that MEK-inhibitor resistant tumors significantly benefitted from the combination therapy. Finally, we profiled 486 cancer cell lines and revealed that oxidative stress responses and translational suppression are biomarkers of ROS-inducer sensitivity, independent of cancer indication. These findings link transcriptional plasticity to a metabolic phenotype that can be inhibited by ROS-inducers in melanoma and other cancers.

L5: A New Look at the Warburg Effect of Breast Cancer

Andjelika Kalezic[1], Aleksandra Jankovic[1], Aleksandra Korac[2], Bato Korac[1,2]

[1]Institute for Biological Research "Sinisa Stankovic", National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia
[2]Faculty of Biology, University of Belgrade, Belgrade, Serbia
(e-mail: b.korac@bio.bg.ac.rs)

Metabolic reprogramming is a universal evolutionary conserved response of tissues and organs to adapt to different physiological and pathological conditions. Metabolic reprogramming refers to cancer cells' ability to respond to the demands for energy, biomass, redox regulation, and cellular communication. Originally, the metabolic properties of cancer cells were viewed through the lens of the Warburg effect: they were thought to display high glycolysis rates with lactate production even in the presence of oxygen. Warburg views the tumor as a new, pseudo-organ. Currently, metabolic reprogramming underscores immense plasticity that enables cancer cells to combine glycolytic and oxidative metabolism or switch between carbohydrate, lipid, and amino acid energy substrates in response to changing oxygen and nutrient conditions. Recent advances consistently point to the tumor microenvironment as a powerful tool to control tumor progression. In breast cancer, the most prevalent malignancy in women in the world, the principal cellular component of the tumor milieu is the adipose tissue. Cross-talk between cancer and adipose tissue leads to mutual metabolic reprogramming that contributes to cancer progression. Hence, we view breast cancer as a unique, complex pseudo-organ with a huge capacity for metabolic reprogramming of tumor and adipose tissue during adaptations in response to the selective pressures imposed by the tumor microenvironment. This is a review of integrated metabolic orchestration in breast cancer.

Acknowledgment: This research was supported by the Science Fund of the Republic of Serbia, #7750238, Exploring new avenues in breast cancer research: Redox and metabolic reprogramming of cancer and associated adipose tissue - REFRAME.

L6: miR-214 Mediated Stroma-Tumor Cell Crosstalk During Tumor Progression

F. Orso[1,2], F. Virga[1,2,10], D. Dettori[1, 2], A. Dalmasso[1,2], M. Paradzik[1,2], A. Savino[1,2], S. Cucinelli[1,2], M. Coco[1,2], I. C. Salaroglio[3], J. Kopecka[3], M. A. C. Pomatto[4], G. Camussi[4], K. Mareschi[5,6], L. Salmena[7], P. Provero[8,9], V. Poli[1,2], C. Riganti[3], M. Mazzone[1,2,10], P. P. Pandolfi[1,2,11], D. Taverna[1,2]

[1]Molecular Biotechnology Center (MBC); [2]Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy; [3]Department of Oncology, University of Torino, Italy; [4]Department of Medical Sciences, University of Turin, Turin, Italy; [5]Paediatric Onco-Haematology Division, Regina Margherita Children's Hospital, City of Health and Science of Turin, Italy; [6]Department of Public Health and Paediatrics, University of Turin, Italy; [7]Princess Margaret Cancer Centre, University Health Network, Toronto, Canada; [8]Center for Omics Sciences, IRCCS San Raffaele Scientific Institute, Milan, Italy; [9]Department of Neurosciences "Rita Levi Montalcini", University of Turin, Turin, Italy; [10]Center for Cancer Biology (CCB), VIB, Leuven, Belgium; [11]Renown Institute for Cancer, Nevada System of Higher Education, Reno, NV, USA
(e-mail: daniela.taverna@unito.it)

Cancer and stroma cells continuously interact during tumor progression and influence each other. Secreted microRNAs (miRNAs) have recently been implicated in the tumor-stroma crosstalk. Here, we show that miR-214 is highly expressed in stromal cells and that it correlates with stromal signatures in human breast cancers and melanomas. Upon tumor cell signals, stroma miR-214 is released via Extracellular Vesicles (EVs) and is instrumental for cancer cells to promote metastasis formation through the activation of a pro-metastatic pathway which involves the protein-coding genes TFAP2C, ITGA5 and ALCAM and the anti-metastatic small non-coding RNA, miR-148b. Metabolic rewiring and, in particular, reprogrammed glucose metabolism is a hallmark of cancer, crucial for tumor progression. We analyzed the impact of stroma-derived miR-214 on the metabolic status of tumor cells and we provide evidence for a glycolysis enhancement and an Oxidative Phosphorylation (OXPHOS) impairment linked to metastatic traits. Our results underline the relevance of "stroma miR-214" for tumor dissemination and metastasis formation and suggest the possibility of a double-edge therapeutic approach based on the targeting of miR-214 and of major metabolic players in tumor and/or stroma cells.

L7: Single-Cell Sequencing [Lecture sponsored by Labena d.o.o.]

Agnieszka Ciesielska

10x Genomics, Science and Technology Advisor, Central Eastern & Southern Europe & Middle East & Africa (e-mail: agnieszka.ciesielska@10xgenomics.com)

Developing treatments for complex diseases requires building a complete understanding of both disease and treatment-response mechanisms. As we navigate a century where transformative advances in biology will reshape the way we deliver human health, translational and clinical researchers need approaches that provide actionable insights that can, ultimately, be leveraged to improve how diseases are diagnosed and treated.

Join us to learn how single cell, spatial, and *in situ* technologies from 10x Genomics can help you push the boundaries of your translational and clinical research. Discover novel therapeutic targets, explore how therapeutics modulate disease-associated cell populations and states, gain insights into mechanisms governing therapeutic toxicity, and understand resistance mechanisms governed by transcriptomic and epigenetic remodeling. Enabling deeper insight into cancer, immunology, neuroscience, and immuno-oncology, 10x Genomics gives researchers the ability to see biology in new ways.

L8: ETV7 as a Mediator of Chemoresistance and Cancer Aggressiveness in Breast Cancer

Erna Marija Meskyte[1], Laura Pezze'[1], Mattia Forcato[2], Federica Alessandrini[1], Stefano Pontalti[1], Irene Adelaide Bocci[1], Kalina Aleksandra Badowska[1], Dario Rizzotto[1], Alessandra Bisio[1], Ira Skvortsova[3], Silvio Bicciato[2], Daiva Baltriukiene[4], Yari Ciribilli[1]

[1]CIBIO Department, University of Trento, Povo (TN), Italy; [2]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy; [3]Medical University of Innsbruck, Austria; [4]Life Sciences Center, Vilnius University, Vilnius, Lithuania (e-mail: yari.ciribilli@unitn.it)

Breast cancer (BC) treatment often includes Doxorubicin and/or other DNA damaging agents as adjuvant or neoadjuvant chemotherapy. Despite their cytotoxicity, cancer cells can develop drug resistance to these drugs. Uncovering pathways and mechanisms involved in drug resistance is an urgent and critical aim for breast cancer research oriented to improve treatment efficacy. We have recently demonstrated that different chemotherapeutic drugs, and particularly Doxorubicin, induce the expression of ETV7, a poorly studied transcriptional repressor member of the ETS family. We generated MCF7, MDA-MB-231 and T47D BC-derived cells stably over-expressing ETV7 and we tested the sensitivity of these cells to the chemotherapeutic drugs Doxorubicin and 5-Fluorouracil (5-FU). We observed a reduction in the sensitivity of these BC cells over-expressing ETV7 to both the drugs.
We have also demonstrated that ETV7 led to down-regulation of DNAJC15, a co-chaperone protein whose low expression was previously associated with drug resistance in breast and ovarian cancer. We identified the binding site for ETV7 within the promoter of DNAJC15 and we also found that DNA methylation may be a factor in ETV-mediated transcriptional repression at the DNAJC15 promoter. Moreover, we demonstrated that ETV7-mediated Doxorubicin resistance involves increased Doxorubicin efflux via nuclear pumps, which could be rescued in part by DNAJC15 up-regulation. Consistent with this observation, we could appreciate an increase in ABC transporters and the BCL2 anti-apoptotic protein expression following ETV7 over-expression. These effects were also accompanied by the observation that alteration of ETV7 expression could significantly affect the population of breast cancer stem cells (CD44+/CD24low cells) in different BC cell lines. By transcriptome profiling, we identified a signature of Interferon-responsive genes significantly repressed in cells over-expressing ETV7, which could be responsible for the increase in the breast CSCs population, as this could be partially reverted by the treatment with IFN-beta.
With this study, we propose a novel role for ETV7 in breast cancer stem cells plasticity and associated resistance to conventional chemotherapy. We, therefore, suggest that an in-depth investigation of this mechanism could lead to the identification of novel breast CSCs vulnerabilities and the improvement of combinatorial regimens with the aim of avoiding resistance and relapse in breast cancer.

L9: The Role of p53 Family in Melanoma Development and Therapy Resistance

Neda Slade[1], Ana Tadijan[1], Petar Ozretić[1], Nikolina Hanžić[1], Martina Radić[1], Maja Jazvinšćak Jembrek[1], Francesca Precazzini[2], Yari Ciribilli[2], Ignacija Vlašić[1], Bastien Proust[1], Lidija Škreblin[1], Ivan Milas[3], Zvonimir Puljiz[3]

[1]Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia; [2]Department of Cellular, Computational, and Integrative Biology (CIBIO), University of Trento, Italy; [3]Sestre milosrdnice University Hospital , Zagreb, Croatia
(e-mail: slade@irb.hr)

Cutaneous melanoma is the most aggressive form of skin cancer. Despite the significant improvements in its treatment, the majority of patients develop resistance whose mechanisms are still not completely understood. The mutations of TP53 gene are a crucial driver of cancer development, but in melanoma, p53 is more often present as a wild type (wt). However, wt p53 fails to function as tumor suppressor and regulate its target genes. One reason for this observation is that in addition to the canonical, full-length p53 with tumor suppressive functions, the TP53 gene possesses two distinct promoters, alternative translation initiation sites, and undergoes alternative splicing, giving rise to 12 diverse isoforms. Additionally, p53 family members, TP73 and TP63, which share high structural and functional similarity with TP53, generate multiple isoforms with different functions. Shorter isoforms of the p53 family, whose significance has only recently become apparent, may act as modifiers of p53-dependent responses, including its tumor suppressor functions. We have analyzed the expression profile of p53 and p73 isoforms in a panel of human melanoma cell lines, in normal conditions or after the treatment with common DNA-damaging agents or targeted therapy, as well as in clinical specimens. Our results show that human melanoma cells express a wide array of p53/p73 isoforms, with Δ160p53α being the most variable. Noteworthy, higher Δ133p53β and p53α mRNA had a negative impact on the overall patients' survival. Furthermore, we generated two melanoma-derived cell lines, primary WM793B and metastatic A375M, with acquired resistance to BRAF inhibitor vemurafenib after prolonged exposure to the drug. The BRAFi-resistant cells showed an altered expression of p53 and p73 isoforms, namely an increased expression of potentially pro-oncogenic Δ40p53β and a decrease in tumor-suppressive TAp73β. Lastly, we checked the expression profile of the p53/p73 isoforms in a panel of five patient-derived melanoma cell lines that harbor mutations in BRAF and show different sensitivity to BRAFi and/or MEKi. We have found that increased levels of p53 isoforms (p53α, p53β, and Δ40p53β) and lower levels of tumor-suppressive TAp73β isoform could correlate with acquired resistance to BRAFi/MEKi and/or BRAFi targeted therapy. We, therefore, propose that p53 family isoforms can play a role in melanoma cells' aggressiveness and could be a potential marker and target for melanoma therapy.

L10: The Adaptor Protein p140Cap Negatively Regulates the Aggressiveness of Breast Cancer and Neuroblastoma: Molecular and Functional Insights

Vincenzo Salemme[1], Jennifer Chapelle[1], Alessio Piccolantonio[1], Giorgia Centonze[1], Andrea Guala[1], Costanza Angelini[1], Alessandro Morellato[1], Dora Natalini[1], Laura Conti[1], Daniela Tosoni[2,3], Giovanni Bertalot[2], Stefano Ugel[4], Vincenzo Bronte[4], Davide Cangelosi[5], Federica Cavallo[1], Daniela Taverna[1], Emilia Turco[1], Salvatore Pece[2,3], Paola Defilippi[1]

[1]Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy; [2]Novel Diagnostics Program, IEO, European Institute of Oncology IRCCS, Milano, Italy; [3]Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milano Italy; [4]Immunology Section, Department of Medicine, University of Verona, Verona, Italy; [5]Laboratory of Molecular Biology, Giannina Gaslini Institute, Genova, Italy
(e-mail: paola.defilippi@unito.it)

The p140Cap adaptor protein is a scaffold molecule encoded by the SRCIN1 gene, which is physiologically expressed in several epithelial tissues and in the neurons. However, p140Cap is also strongly expressed in a significant subset of cancers including breast cancer and neuroblastoma. Notably, cancer patients with high p140Cap expression in their primary tumors have a lower probability of developing a distant event and ERBB2-positive breast cancer sufferers show better survival. In neuroblastoma patients, SRCIN1 mRNA levels represent an independent risk factor, which is inversely correlated to disease aggressiveness. Consistent with clinical data, SRCIN1 gain- or loss-of-function mouse models demonstrated that p140Cap may affect tumor growth and metastasis formation by controlling the signaling pathways involved in tumorigenesis and metastatic features. We will discuss data showing the relevance of SRCIN1/p140Cap in cancer patients, the impact of SRCIN1 status on p140Cap expression, the specific mechanisms through which p140Cap can limit cancer progression, the molecular functions regulated by p140Cap, along with the p140Cap interactome, to unveil its key role for patient stratification in clinics.

L11: Sirtuin 3 Exerts Its Tumour-Suppressive Role by Stabilizing p53 and Attenuating Response to Estrogen in MCF-7 Cells

Sandra Sobočanec[1], Marija Pinterić[1], Iva I. Podgorski[1], Marijana Popović Hadžija[1], Vedrana Filić Mileta[2], Mladen Paradžik[1], Bastien Lucien Jean Proust[1], Ana Dekanić[1], Ivan Ciganek[1], Denis Pleše[1], Dora Marčinko[1], Tihomir Balog[1]

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

MCF-7 is an estrogen (E2)-sensitive breast cancer cell line characterized by low expression of sirtuin 3 (Sirt3), a mitochondrial NAD + dependent deacetylase. Sirt3 regulates reactive oxygen species (ROS) production, cell metabolism, ATP synthesis, apoptosis and proliferation and thus plays an important role in tumorigenesis. We elucidated differences in the effects of E2 on proliferation, metabolism, and tumorigenic properties in estrogen receptor-α (ER-α) positive MCF-7 cell line stably transfected with overexpressed Sirt3 (MCF-7S3) or with absent expression of Sirt3 (MCF-7C). Cells were treated with E2 and ER inhibitor (ICI), and analyses of proliferation, metabolism, candidate protein interaction, mitochondrial function, and cell morphology were performed. Because of sensitivity of these cells to E2 availability, the E2 effect on the localization of Sirt3 and ER-α was also examined to determine a possible difference in the nuclear and cytoplasmic distribution of ER-α with respect to Sirt3. The results suggest that Sirt3 reduces the colony-forming ability and inhibits E2-induced proliferation of the MCF-7 breast cancer cells. This effect of Sirt3 is explained by the fact that the MCF-7S3 cell line has higher expression of the transcription factor p53, which initiates tumor-suppressive cell programs. In addition, Sirt3 enhances metabolic activity and increases the expression of mitochondrial oxidative phosphorylation complexes, switching cells to oxidative phosphorylation, a metabolic pathway less favourable for tumour cells. Moreover, Sirt3 decreases the interaction between ER-α and p53, leading to functional stabilization of p53 and activation of its tumor-suppressive activity, and finally reduces E2-induced cell proliferation. In the presence of Sirt3, E2 increases DNA damage and inhibits cell progression through the S phase of a cell cycle, reducing proliferation and decreasing the ability of the MCF-7 cell line to form new colonies. Overall, the results suggest an antagonistic effect of Sirt3 on E2-induced proliferation of MCF-7 breast cancer cells, suggesting that Sirt3 is a potential therapeutic target in E2-sensitive breast cancer. Moreover, restoration of p53 function is one of the most important therapeutic targets in the treatment of breast cancer, as many therapeutic agents lose efficacy over time. Therefore, reactivation of Sirt3 should be considered as a potential option for the treatment of E2-sensitive breast cancer.

L12: Development of αvβ6 Selective Precision Immunovirotherapies

Alan Parker

Division of Cancer and Genetics, Cardiff University School of Medicine, Cardiff, UK
(e-mail:parkeral@cardiff.ac.uk)

Our laboratory has a long-standing interest in defining interactions between adenoviruses and host cells and proteins and understanding how these interactions define viral spread and tropism. This information is not only valuable for understanding viral pathogenesis and developing antivirals, but also in engineering adenoviral platforms for therapeutic applications, for example as vaccines or oncolytic applications.

Using a rational engineering approach, we developed a therapeutic oncolytic adenoviral platform devoid of all native means of cellular infection, termed Ad5NULL. This was achieved by engineering each of the three major adenoviral capsid proteins, hexon, fiber and penton base, to prevent native means of cell entry, through heparan sulphate proteoglycans (HSPGs), Coxsackie and Adenovirus receptor (CAR) and αvβ3/5 integrins respectively. The resultant platform is basal and cannot be propagated without providing a surrogate means of cell entry. To this end, we incorporated a peptide, A20, which binds αvβ6 integrin with high affinity and selectivity, and we able to propagate the "precision virotherapy" Ad5NULL-A20 in αvβ6 expressing producer cell lines with high efficiency and purity. The resultant vectors do not infect healthy cells, absent in αvβ6 integrin expression *in vitro* or *in vivo*, and is able to effectively and precisely target αvβ6 integrin positive tumour cells *in vivo* following local, intraperitoneal and intravenous administration.

We are now generating a powerful suite of Ad5NULL-A20 based virotherapies expressing therapeutic, immunostimulatory transgenes, which home to tumours, inducing immunogenic cell death through viral replication, whilst secreting immune activating agents in the tumour microenvironment. We are stratifying a range of these agents, with a view to first in human clinical evaluation for the lead agent by the end of 2023, in partnership with Accession Therapeutics Ltd.

L13: The Role of NKG2D in MAFLD Pathogenesis

Sonja Marinović[1], Maja Lenartić[1], Karlo Mladenić[1], Marko Šestan[1], Inga Kavazović[1], Vedrana Jelenčić[1], Ante Benić[1], Mia Krapić[1], Tamara Turk Wensveen[2], Dora Fučkar Čupić[3], Lidija Bilić-Zulle[4], Adrian Hayday[5], Felix M. Wensveen[1], Bojan Polić[1]

[1]Department of Histology & Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [2]Department of Internal Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [3]Department of Pathology and Pathological Anatomy, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [4]Department of Laboratory Diagnostics, Clinical Hospital Centre Rijeka, Rijeka, Croatia; [5]Department of Immunobiology, King's College London, London, UK
(e-mail: bojan.polic@medri.uniri.hr)

Metabolic (dysfunction) associated fatty liver disease (MAFLD) is the most common chronic liver disease worldwide. It is a hepatic manifestation of metabolic syndrome, which includes insulin resistance, hyperglycemia, dyslipidemia, hypertension and abdominal obesity. MAFLD is a spectrum of progressive liver disease from relatively benign steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular carcinoma (HCC). The key event in progression of MAFLD is activation of the immune system and development of inflammation. However, mediators and immune cells involved in early immune sensing of metabolically stressed liver cells are largely unknown.

To investigate the mechanism laying behind the transition from steatosis to NASH we developed a steatosis-steatohepatitis dietary (SSD) mouse model that mimics a western lifestyle. After 16 weeks of SSD feeding, we noticed several clinical hallmarks of liver damage such as hepatomegaly and elevated ALT levels when compared to chow-fed mice. Moreover, histological quantification revealed formation of micro- and macro-vesicular steatosis at early time points upon SSD onset and development of inflammatory foci, hepatocyte degeneration and hepatic stellate cells (HSC) activation at later stages. This model closely resembles MAFLD progression in humans. *Ex vivo* stimulation of liver leukocytes revealed IL-17A as the most prominent cytokine that is increased early upon the start of SSD. We identified hepatic γδ T cells as a dominant source of IL-17A. TCRδ-/- mice showed reduced NASH and confirmed the importance of γδ T cells in the disease progression. Furthermore, we found that metabolically stressed hepatocytes express high levels of NKG2D ligands on their surface, which suggested the importance of NKG2D signaling axis in sensing of cellular stress. Indeed, employment of NKG2D-deficient mice showed striking reduction of liver inflammation and fibrosis. Altogether, activation of T cells via NKG2D plays a crucial first step in the progression of the disease and provides a potential new target for MAFLD treatment.

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ST1: Unique Hallmarks of Targeted Therapy Resistant Melanoma Cell Lines

Ignacija Vlašić[1], Martina Radić[1], Maja Jazvinšćak Jembrek[1,2], Anđela Horvat[1], Ana Tadijan[1], Maja Sabol[3], Marko Dužević[1], Maja Herak Bosnar[1], Neda Slade[1]

[1]Laboratory for Protein Dynamics, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia; [2]School of Medicine, Catholic University of Croatia, Zagreb, Croatia; [3]Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: ignacija.vlasic@irb.hr)

Melanoma is skin cancer developed from melanocytes and is considered to be one of the most aggressive tumor types due to high metastatic potential. One of the reasons why melanoma is prone to metastasis is a process known as a phenotype switching which is similar to epithelial–mesenchymal transition (EMT) since it includes EMT-related genes and signaling pathways. In addition, this process is shown to be implicated in resistance to targeted therapy. Depending on the BRAF mutation status, first line treatment options for melanoma can include targeted therapy or immunotherapy for BRAF-mutated melanoma, or immunotherapy for wild-type BRAF melanoma. Around 60% of melanoma carry BRAF mutation, of which almost 90% harbor BRAF V600E mutation that causes continuous activation of the MAPK signaling pathway. BRAF V600E mutated kinase can be targeted by BRAF inhibitor (BRAFi), vemurafenib, which is a first targeted therapy licensed for treatment of advanced melanoma. Although initial response in the clinic was very powerful, the disease relapses within several months of therapy introduction due to occurrence of acquired resistance to BRAFi therapy. Reactivation of the MAPK pathway occurs in the majority of BRAFi-resistant tumors, while the second most frequently activated signaling pathway is PI3K/AKT. So to investigate the underlying molecular mechanisms of BRAFi targeted therapy resistance in more detail, we generated and characterized two melanoma cell lines with acquired resistance to vemurafenib. The resistant cell lines exhibited specific features of slow-cycling cells possibly as a consequence of vemurafenib-driven phenotype switching. These characteristics included morphological and molecular features of EMT-like cells, enhanced resistance to chemotherapy, changed levels of cell-cycle regulators and thus reduced proliferation. Additional features included decreased migration ability but tendency toward collective migration, and reactivation of MAPK or activation of PI3K/AKT signaling pathways, depending on the cell line. Reduced levels of NME1 and NME2 metastasis suppressor proteins were observed in primary vemurafenib-resistant cell line, which could originate from vemurafenib-acquired resistance and is one of the reasons for increased PI3K/AKT signaling. Further studies are required to reveal the vemurafenib-dependent suppressors of NME proteins, their association with PI3K/AKT signaling, and their impact on vemurafenib-resistant melanoma cell characteristics.

ST2: The miR-200c/TUBB3 Regulatory Axis is Part of the Cellular Stress Response to Carboplatin in Drug-Resistant Ovarian Cancer Cell Lines

Margareta Pernar Kovač[1], Vanja Tadić[1], Juran Kralj[1], Darija Stupin Polančec[2], Sanja Dabelić[3], Maja T. Tomicic[4], Anamaria Brozovic[1]

[1]Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia; [2]Selvita d.o.o., Zagreb, Croatia; [3]University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia; [4]Institute for Toxicology, University Medical Center Mainz, Mainz, Germany
(e-mail: mpernar@irb.hr)

Acquired drug resistance remains a major problem for successful chemotherapy, contributing to poor long-term prognosis of ovarian cancer (OC) patients. The combination of platinum drugs and taxanes is commonly used to treat patients with OC, due to their different modes of action. Despite the positive initial response to chemotherapy, up to 80% of OC patients will eventually relapse, and become platinum/taxane unresponsive. Moreover, cross-resistance occurs in almost 30% of OC cases. We established two OC cell line models, i.e., MES-OV CBP and SK-OV-3 CBP characterized by clinically relevant acquired resistance to carboplatin (CBP), a mesenchymal-like phenotype, CBP-induced class III β-tubulin (TUBB3) overexpression, and different responsiveness to paclitaxel (TAX) treatment. We showed previously that despite increased TUBB3 protein expression in both CBP resistant variants only MES-OV CBP cells are sensitized to CBP upon TUBB3 silencing. The lack of compensation phenomena with other β-tubulin isotypes noticed in SK-OV-3 CBP, but absent in MES-OV CBP cell line, underlined that pan TUBB should be considered together with TUBB3 for the prediction of treatment efficacy. We noticed further that the presence of compensation phenomena correlated with the expression pattern of miRNA-200 family members, known regulators of TUBB3 expression. We focused on the miR-200c that is predominantly investigated in the context of drug resistance and epithelial-mesenchymal transition. We showed that in MES-OV CBP cells, long-term exposure to CBP induced stable miR-200c downregulation via alterations in epigenetic regulation, and consequently led to the upregulation of TUBB3. Transient transfection of MES-OV CBP cells with mimic miR-200c sensitized them to CBP, while transduction of MES-OV cells with lentiviral particles carrying miR-200c inhibitor rendered them less sensitive to CBP and TAX as well. Most importantly, decreased constitutive expression of miR-200c in MES-OV cells was accompanied by elevated expression of TUBB3 and TUBB. This finding shows for the first time that the miR-200c/TUBB3 axis is part of the cellular stress response to CBP.

ST3: Antimelanoma Potential of New Telmisartan Analogues Without AT1 Receptor Activity

Ana Damjanović[1], Miodrag Vuković[1], Dragana Vukadinović[2], Vladimir Dobričić[2], Jelena Grahovac[1]

[1]Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
(e-mail: miodragvuk97@gmail.com)

Melanoma is one of the most aggressive malignancies, where the prognosis for metastatic patients remains extremely poor. Our group has shown that the antihypertensive drug telmisartan has antimelanoma potential[1]. Given that the antihypertensive effect is not favorable in cancer patients, the aim of this study was to design and test novel telmisartan derivatives without the angiotensin receptor 1 (AT1R) binding activity. New derivatives were designed by modification of the carboxylic group, in order to alter telmisartan geometry and its AT1R binding properties. Eight derivatives, from which the lack of AT1R antagonistic activity could be expected based on molecular docking, were synthetized and selected for *in vitro* testing. After the cytotoxicity test on human melanoma cell lines A375 and 518a2, three derivatives that were twice more potent than telmisartan itself were selected for further analysis. The new derivatives induced mitochondrial fragmentation, generation of the mitochondrial reactive oxygen species, and decrease of mitochondrial membrane potential in melanoma cells, the mechanism we previously shown for induction of apoptosis by telmisartan in melanoma cells. As the new derivatives showed more potent effect on melanoma cells than telmisartan these results lay a ground for further preclinical testing in melanoma.

[1]Grahovac *et al*. (2019) Cancer Biol Med, 16(2):247-263.

ST4: PTCH1 as a Novel B-arrestin Target Suggests a Regulatory Role in Non-canonical Hedgehog Signalling

Hattie Ollerton, Mohammad Arastoo, Natalia A. Riobo-Del Galdo

School of Molecular and Cellular Biology, University of Leeds, Leeds, UK
(e-mail: bs16ho@leeds.ac.uk)

The tumour suppressor protein Patched1 (PTCH1) is the receptor of the Hedgehog (Hh) protein family and regulates canonical and non-canonical signalling. Somatic mutations in PTCH1 are common in basal cell carcinoma and medulloblastoma while heterozygous germline mutations underlie Gorlin syndrome.

In the absence of Hh, PTCH1 inhibits Smoothened (SMO), a G protein-coupled receptor (GPCR) which drives Gli transcriptional activity. β-arrestins (βarrs) are small soluble proteins reported to interact with SMO, promote ciliary trafficking and exert a positive effect on Gli activation. Here we report that PTCH1 interacts with βarr1 and βarr2 despite not being a GPCR. The goals of this study are to identify the PTCH1-βarr binding site and determine the functional consequence of the interaction.

Co-immunoprecipitation (co-IP) showed that the C-terminal domain (CTD) of PTCH1 contains the βarr binding site. We hypothesised that βarrs are recruited to PTCH1 via a similar mechanism to GPCRs, by binding a known phosphorylation 'code' in the C-tail. Indeed, co-IP of βarr1-GFP or endogenous βarr1/2 together with variants of PTCH1, showed that mutations and deletions in the SEYSSQT motif of the PTCH1 CTD impairs βarr binding. Specifically, S1223A and/or T1229A mutations prevented interaction, and phosphomimetic mutation of both residues enhanced binding to βarrs. Both residues are predicted to be CDK1 substrates; suggesting the strength of interaction may vary during the cell cycle.

The specific binding of βarrs to PTCH1 suggests a regulatory function. We tested whether repression of SMO/Gli by PTCH1 necessitates its interaction with βarr1/2. Transfection of WT and mutant PTCH1 together with a Gli-luciferase reporter in Ptch1-/- MEFs showed that all mutants retain SMO inhibitory capacity and respond to Shh. Moreover, silencing of βarr1/2 increased Gli-luciferase and Gli1 expression, suggesting that βarrs are not required for SMO activation as previously reported. Having ruled out a role of PTCH1-βarr interaction in canonical Hh signalling, we investigated PTCH1-dependent ERK activation in response to Shh. βarr1/2 siRNA increased ERK phosphorylation and retarded PTCH1 degradation in response to Shh.

Our study reveals that βarr1/2 are negative regulators of PTCH1-dependent non-canonical signalling through binding to a phosphorylated motif in the CTD. Future studies are needed to determine its role in Hh-dependent cancers.

ST5: Liquid Biopsy for Detection of Resistance Mutation T790M in EGFR Gene from Lung Cancer Patients

Gordana Bubanovic[1], Marija Milavic[1], Diana Hrg[1], Ivona Kaloper Lovrekovic[1], Ivana Birek[1], Sven Seiwerth[1,2], Lovorka Batelja Vuletic[2], Silvana Smojver Jezek[3], Marko Jakopovic[4]

[1]Laboratory for Molecular Pathology, Department of Pathology and Cytology, University Hospital Centre Zagreb and Department of Pathology, University of Zagreb School of Medicine, Zagreb, Croatia; [2]Department of Pathology and Cytology, Department of Pathology, University Hospital Centre Zagreb and Department of Pathology, University of Zagreb School of Medicine, Zagreb, Croatia; [3]Department of Pathology and Cytology, Division of Pulmonary Cytology Jordanovac, University Hospital Centre Zagreb, Zagreb, Croatia; [4]Department of Respiratory Diseases Jordanovac, University Hospital Centre Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia
(e-mail: gordana.bubanovic@kbc-zagreb.hr)

Targeted treatment options have rapidly emerged for non-small cell lung cancer (NSCLC) within the past decade. Testing for molecular aberrations is mainly done by biopsy but in some cases a biopsy is not possible and liquid biopsy remains the only option. Once the mutation of epidermal growth factor receptor (EGFR) is identified in tissue NSCLC patients, liquid biopsy can be useful to monitor the treatment, since the mutation load decreases when the patient responds to therapy and increases again at progression. Retesting with tissue and/or liquid biopsy detect resistance mechanisms for the therapy. The resistance mechanisms to epidermal growth factor receptor - tyrosine kinase inhibitors (EGFR-TKIs) are heterogeneous. Secondary T790M mutation was the most frequently acquired resistance mechanism to early generation EGFR-TKIs and osimertinib was the standard second line therapy.

The cobas EGFR Mutation Test v2 (Roche Diagnostics) has been approved for the qualitative detection in plasma of EGFR, to select patients with advanced NSCLC for targeted therapy. An advantage of this real-time PCR test is that it is standardized, and it can detect up to 42 EGFR mutations simultaneously. For the cobas EGFR Mutation Test v2, cfDNA was isolated from 2-3 mL plasma using the cobas DNA Sample Preparation Kit (Roche Diagnostics) according to the manufacturer's instructions. The PCR reactions were run on the cobas z 480 analyzer with the cobas 4800 software that reports automatically results as semiquantitative index (SQI) when an EGFR mutation is detected in ctDNA.

Plasma cfDNA, hystological and citological DNA in 6264 samples from patients with stages III to IV NSCLC were analyzed for EGFR mutations from 2016 to September 2022. Data will be presented.

We show that EGFR resistance mutation T790M, in cfDNA can be helpful in those patients with already detected EGFR mutations in tissue. cfDNA analysis helps to evaluate mutational status, especially if the tissue biopsy is not recent or unavailable. The availability of starting plasma sample before targeted therapy and retesting samples allows the monitoring of the efficacy of therapy and the detection of resistance mutations.

ST6: H4K20me3 Upregulated by Reactive Oxygen Species is Associated with Tumor Progression and Poor Prognosis in Patients with Hepatocellular Carcinoma

Suchittra Phoyen[1], Anapat Sanpavat[2], Chakriwong Ma-on[3], Ulrike Stein[4,5], Nattiya Hirankarn[6], Pisit Tangkijvanich[1,7], Depicha Jindatip[8], Chanchai Boonla[1]

[1]Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [2]Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [3]Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [4]Translational Oncology of Solid Tumors, Experimental and Clinical Research Center, Charité Universitätsmedizin Berlin and Max-Delbrück-Center for Molecular Medicine, Berlin, Germany, [5]German Cancer Consortium, Heidelberg, Germany; [6]Center of Excellence in Immunology and Immune-mediated Diseases, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [7]Center of Excellence in Hepatitis and Liver Cancer, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [8]Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
(e-mail: chanchai.b@chula.ac.th)

Epigenetic alteration by oxidative stress is vitally involved in carcinogenesis and cancer progression. Previously, we demonstrated that oxidative stress was increased in hepatocellular carcinoma (HCC) patients and associated with aggressiveness. Herein, we investigated whether histone methylation, specifically H4K20me3, was altered in human HCC and if this alteration was induced by reactive oxygen species (ROS) and contributed to HCC progression. H4K20me3 was overexpressed in human HCC tissues (n = 100) compared with the adjacent noncancerous liver tissues. H3K9me3 and H3K4me3 expression in HCC tissues were also upregulated. Cox regression analysis revealed that the elevated H4K20me3 expression was associated with tumor recurrence and short survival in HCC patients. Experimentally, H2O2 (ROS representative) provoked oxidative stress and augmented H4K20me3 expression in HepG2 and Huh7 cells. Transcript expression of histone methyltransferase Suv420h2 (for H4K20me3), Suv39h1 (for H3K9me3), and Smyd3 (for H3K4me3) were upregulated by H2O2 in HCC cell lines. The epithelial-mesenchymal transition (EMT) was induced by H2O2 in HCC cells, indicated by decreased E-cadherin but increased α-SMA and MMP-9 mRNA expression. Migration, invasion, and colony formation in HCC cells were markedly increased following the H2O2 exposure. Conclusion, we firstly demonstrated that H4K20me3 was overexpressed in human HCC tissues, and this H4K20me3 overexpression was independently associated with poor prognosis. ROS upregulated H4K20me3 expression, induced EMT, and promoted tumor progression in human HCC cell lines. Decrease H4K20me3 formation by antioxidant or histone methyltransferase inhibitor might be a new therapeutic approach to slow the HCC progression.

ST7: Reciprocal Regulation of p21 and Chk1 Controls the Cyclin D1-RB Pathway to Mediate Senescence Onset After G2 Arrest

Anđela Horvat[1*], Gérald Lossaint[2*], Véronique Gire[3], Katarina Bačević[2], Karim Mrouj[2], Fabienne Charrier-Savournin[3], Virginie Georget[3,4], Daniel Fisher[2], Vjekoslav Dulić[2]

[1]Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia; [2]Institute of Molecular Genetics of Montpellier, University of Montpellier, CNRS, Montpellier, France; [3]CRBM, University of Montpellier, CNRS, Montpellier, France; [4]Montpellier Ressources Imagerie, BioCampus, University of Montpellier, CNRS, INSERM, Montpellier, France (e-mail: arunje@irb.hr)

Cellular senescence is a permanent withdrawal from the cell cycle which can be induced by various stimuli, including dysfunctional telomeres and DNA damage. Senescence is generally recognized as a tumor suppressive mechanism, because it prevents malignant transformation and proliferation of cancer cells. However, there is increasing evidence of its involvement in several physiological and pathological processes that can contribute to the development of age-related disorders and cancer. Although it is usually considered that senescence is preceded by G1 cell cycle arrest, it can also be triggered after DNA damage induced G2 arrest thus preventing genomic instability. Tumor suppressors p53 and retinoblastoma protein (RB) are involved in the senescence onset, but the precise mechanisms of their regulation during the transition from the temporary to permanent cell cycle arrest in G2 are still unknown. The aim of this research was to investigate the complex interplay of kinases that regulate switch from DNA damage-induced G2 arrest to permanent cell cycle exit preceding senescence. We found that in non-transformed cells, cyclin-dependent kinase (CDK) inhibitor p21 inactivates cyclin D1-CDK2/4 complexes thus blocking RB phosphorylation and leading to G2 exit which precedes the appearance of senescence markers. This event was associated with a mitotic bypass, downregulation of the checkpoint kinase Chk1 (but not Chk2) and reduction in the number of DNA damage foci. In contrast, in p53/RB-proficient cancer cells we found sustained Chk1 activity and delayed p21 induction after DNA damage in G2, which coincided with altered mitotic bypass, delayed G2 exit and more frequent endoreplication. After Chk1 depletion by siRNA, we observed increased p21 binding to cyclin D1- and cyclin E1-CDK complexes and downregulation of CDK6 leading to promotion of senescence. On the contrary, downregulation of Chk2 enabled RB phosphorylation and delayed G2 exit, indicating the role of this kinase in senescence onset. Our results strongly suggest that a balance between p21 and Chk1 regulates cyclin D1-CDK complex activity during G2 arrest thus modulating RB activity and senescence onset. We showed that Chk1 impairs, whereas p21 and Chk2 induce senescence onset in G2. Therefore, pharmacological inhibition of Chk1 in combination with DNA damaging agents might be investigated as a potential novel approach in cancer treatment that could contribute to improved patient outcome.

ST8: Unique and Overlapping Transcriptional Targets of GLI1, GLI2 and GLI3 in Melanoma Cell Lines

Matea Kurtović[1], Nikolina Piteša[1], Nenad Bartoniček[2], Petar Ozretić[1], Vesna Musani[1], Tina Petrić[1], Josipa Čonkaš[1], Maja Sabol[1]

[1]Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bosković Institute, Zagreb, Croatia
[2]Garvan Institute, Darlinghurst, Australia
(e-mail: matea.kurtovic@irb.hr)

Despite significant progress in therapy, melanoma still has a rising incidence worldwide, and novel treatment strategies are needed. Understanding the interplay of different molecular pathways leading to development and progression of melanoma is important for developing new therapeutic strategies. Recently, researchers have recognized the involvement of the Hedgehog-GLI (HH-GLI) signaling pathway in melanoma and its consistent crosstalk with the MAPK pathway. In order to elucidate the role of GLI proteins in melanoma and to find new target genes that could be considered for combination therapy, we performed RNA-sequencing on human melanoma cell lines with overexpressed GLI1, GLI2 or GLI3 and ChIP-sequencing on endogenous GLI1, GLI2 and GLI3 proteins in same cell lines.
Chromatin immunoprecipitation was performed on three cell lines with the strongest basal expression of GLI proteins (CHL-1, A375, and MEL224). For the purpose of RNA-sequencing, the same melanoma cell lines were transfected with GLI1, GLI2 or GLI3 plasmids. Non-transfected cell lines were used as controls. In order to select GLI target genes for further validation, a list of differentially expressed genes (DEGs) was filtered according to FDR values and logFc. After performing KEGG pathway analysis, genes were selected for further investigation according to their role in diseases, role in different signaling pathways and their importance in melanoma. 21 genes were chosen for validation by qPCR. The validation was performed on seven melanoma cell lines. Our results confirmed GLI1 and PTCH1 as known targets of the HH-GLI pathway. By ChIP-seq, we have identified 2183 genes that contained GLI TFs binding sites in their promoters: 527 for GLI1 (24%), 1103 for GLI2 (50%), and 553 for GLI3 (25%). Also, we found that the majority of the genes are overlapping targets of GLI1 and GLI2 which is to be expected, as GLI1 is a known transcriptional target of GLI2. GLI3 regulates a small number of separate gene targets compared to GLI1 and GLI2.
Out of 21 selected targets, we validated 15 as novel targets of GLI proteins, considering their expression in melanoma cell lines and possession of GLI binding motifs. To our knowledge, this is the first comprehensive study of transcriptional targets of all three GLI proteins in melanoma. Our findings provide new potential targets to consider while designing melanoma-targeted therapy, especially in the case of recurrent disease due to therapy resistance.

ST9: Tracking Molecular Actors of Progression in Intracranial Meningioma

Anja Bukovac[1,2], Anja Kafka[1,2], Božana Blažević[1,3], Matea Katić[1,3], Lovro Marjanović[1,4], Katarina Dragičević[5], Antonia Jakovčević[6], Danko Müller[7], Kristijan Marčić[1], Nives Pećina-Šlaus[1,2]

[1]Laboratory of Neurooncology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia; [2]Department of Biology, School of Medicine, University of Zagreb, Zagreb, Croatia; [3]Faculty of Science, University of Zagreb, Zagreb, Croatia; [4]Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia; [5]Biotech Research & Innovation Centre, University of Copenhagen, Copenhagen, Denmark; [6]Department of Pathology, University Hospital Center Zagreb, Zagreb, Croatia; [7]Clinical Department of Pathology and Cytology, Dubrava University Hospital, Zagreb, Croatia
(e-mail: nina@mef.hr)

Meningioma progression and invasion is usually attributed to higher tumor grades (II and III) which are less likely to be found in the population but are more difficult to treat. However, new findings suggest that grade I or benign meningioma can also harbor invasive molecular characteristics. Epithelial to mesenchymal transition (EMT) is a leading process governing tumor invasion that has also been implicated in progression of intracranial meningioma. The most prominent feature of EMT is a cadherin switch responsible for the loss of cellular epithelial properties (loss of E-cadherin) and gain of mesenchymal ones (gain of N-cadherin). We studied this feature in meningiomas of different grades along with EMT transcriptional activity and the activation of closely related Wnt signaling pathway. The results of our analysis showed that CDH2 (N-cadherin) was very frequently mutated with 70% of samples harboring MSI and/or loss of heterozygosity while CDH1 (E-cadherin) was lost in 12.5% of samples. Furthermore, microsatellite instability (MSI) was the major genetic aberration in both E- and N-cadherin, found in 19,4% and 40% of samples, respectively. Investigation on protein levels showed that N-cadherin expression was a bit higher than E-cadherin which was lost in 30% of samples. The presence of both proteins suggests partial EMT, which is considered more effective for invasion than when E-cadherin is completely lost. The immunohistochemistry results revealed strong expression of EMT transcription factors: TWIST1, SNAIL and SLUG, where SNAIL and SLUG expression was significantly correlated to higher grades (p=0,001). Key regulators of Wnt signaling – β-catenin and DVL1, were also activated and associated with meningioma progression. The higher nuclear expression of DVL1 was significantly associated with higher meningioma grades (p=0.030) and accompanied with higher levels of active β-catenin (p=0.029). Mutational hotspot of CTNNB1 (β-catenin) exon 3 was sequenced showing 22,2% of samples with mutations, which may result in faulty protein product as predicted by Porter 5.0: Prediction of protein secondary structure. Our results showed cadherin switch and strong EMT transcriptional activity, as well as the activation of Wnt signaling pathway, which can all contribute to meningioma progression.

ST10: Focal and Reticular Adhesions Composition in Melanoma Cell Lines MDA-MB-435S and RPMI-7951

Anja Rac[1], Marija Tomić[1], Nikolina Stojanović[1], Ana Tadijan[2], Junzhe Zha[3], Mahak Fatima[3], Jonathan D. Humphries[3], Martin J. Humphries[3], Andreja Ambriović-Ristov[1]

[1]Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia; [2]Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia; [3]Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, UK
(e-mail: arac@irb.hr)

Cell adhesion mediated by integrins, cell surface heterodimers composed of α and β subunits, integrate signalling between the extracellular matrix (ECM) and cells and control many aspects of normal and tumour cells behaviour. Molecular mechanisms related to cell adhesion have been extensively studied, especially in cancer in which mutations and/or changes in expression of these proteins have been observed contributing to its proliferation, migration, and invasion. Through binding to the ECM and clustering, integrins form multimolecular adhesion complexes (IACs) which are connected to, and can regulate the cell cytoskeleton and are termed nascent adhesions, focal adhesions (FAs), fibrillar adhesions and hemidesmosomes. A new class of IACs, named reticular adhesions (RAs) were firstly described as clathrin lattices composed of hexagonal clathrin structures, enriched in dynamin and AP2 (adaptor protein complex 2), both involved in clathrin-mediated endocytosis. They are formed by integrin αVβ5, lack association with actin and are devoid of vinculin which is marker of FAs. While FAs are completely depleted during mitosis, RAs are maintained to enable effective mitosis and also transmit spatial memory from pre-mitotic to post-mitotic daughter cells. We have previously analysed IACs of two melanoma cell lines MDA-MB-435S and RPMI-7951, grown in long term culture, using biochemical isolation and mass spectrometry (MS)-based proteomics, and demonstrated that both cell lines use preferentially integrin αVβ5 for adhesion forming FAs. Immunofluorescent analysis has shown that integrin αVβ5, in both cell lines, was localized in vinculin positive FAs and vinculin negative, ring-like or reticular structures thus resembling RAs. To determine their composition, we exposed MDA-MB-435S and RPMI-7951 cells grown in long term culture to inhibitor of actin polymerisation Cytochalasine D to disrupt FAs, thus enabling us to isolate only RAs. The composition of RAs was then analysed by (MS)-based proteomics, Western blot and immunofluorescence. Our results show the absence of FA components such as Talin1, filamins and alpha actinins and enrichment of proteins such as AP-2, disabled homolog 2 (DAB2) and Numb. Interestingly, in RAs isolates we observed the presence of Talin2. The comprehensive analysis of FA and RA compositions in two cell lines, sharing the same integrin αVβ5, will enable us to analyse their regulatory interplay/relationships.

ST11: Sex and Age-related Changes in Kidney and Liver Expression of Inducible Metallothioneins in Correlation with Essential and Toxic Trace Elements Accumulation: Influence of Antioxidants

Marija Ljubojević[1], Ivana Vrhovac Madunić[1], Dean Karaica[1], Vedran Micek[4], Marko Gerić[5], Goran Gajski[5], Dubravka Rašić[3], Maja Peraica[3], Tatjana Orct[2], Jasna Jurasović[2], Ivana Novak Jovanović[3], Lucia Nanić[6], Ivica Rubelj[6], Ivan Sabolić[1], Davorka Breljak[1]

[1]Molecular Toxicology Unit, [2]Analytical Toxicology and Mineral Metabolism Unit, [3]Toxicology Unit, [4]Laboratory Animals Unit, [5]Mutagenesis Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia; [6]Laboratory for Molecular and Cellular Biology, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: mljubo@imi.hr)

Mechanisms of aging, and cancer as one of main associated pathology are only on verge of understanding. Even in healthy aging gradually loss of function in excretion organs kidneys and liver appears. Previously, we did not find expected elevated tissue concentrations of reactive oxidative species (ROS), that are assumed to be present in old humans and experimental animals and that they can be reversed by antioxidants (AO) treatment. The new hypothesis was that the expression of ROS inducible, potent antioxidant and metal scavenging proteins, metallothioneins Mt1 and Mt2 (MTs), among others, increases in old age to maintain ROS levels and that they can be further modulated with AO. To that aim we studied male and female Wistar rats regularly aged together with these treated with melatonin and resveratrol. Starting from 3 months of age, for the next 9 or 21 months treated rats were drinking AO in water (~1 mg/kg b.w./day), whereas the control animals were drinking water or AO vehicle (0.01% ethanol). Trace elements (TE) were analyzed by ICP-MS and the expression of MTs mRNA and proteins in kidneys and liver were determined by end-point RT-PCR and immunochemical methods, respectively. AO did not change TE accumulation and expression of MTs in both sexes, but known iron accumulation in aged animals was followed by copper and toxic metal cadmium with known female domination. After 1 year, aging increased MTs expression in both sexes that did only slightly further increased after 2 years. We conclude that Fenton reactive metals accumulated in aged animals may influence MTs mRNA and protein expression to help retain physiological ROS level in old rats. (Croatian Science Foundation project IP-2013-11-1481).

ST12: TLN2 and KANK2 are Potential Targets for Enhanced Sensitivity to Paclitaxel Treatment in MDA-MB-435S Cells

Marija Tomić[1], Nikolina Stojanović[1], Anja Rac[1], Kaatje Coopmans[1], Jonathan D. Humphries[2], Martin J. Humphries[2], Andreja Ambriović-Ristov[1]

[1]Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia; [2]Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom (e-mail: mtomic@irb.hr)

Despite development of targeted agents and immune-based therapies, a fraction of patients diagnosed with melanoma are still treated with conventional antitumor drugs such as microtubule (MT) poison paclitaxel (PTX). Since chemotherapy is usually not as effective for melanoma as it is for some other tumours, there is an urge to characterise novel therapeutic targets that can improve effectiveness of classic chemotherapies. One of the potential targets within the cell are proteins of integrin adhesion complexes (IACs) formed through binding and clustering of integrins to the extracellular matrix. IAC composition analysis in MDA-MB-435S melanoma cell line using mass spectrometry (MS)-based proteomics revealed integrin αVβ5 as the predominant integrin used for adhesion in long term cell culture. Concomitantly, (MS)-based proteomics upon integrin αV knockdown revealed components of integrin αVβ5 focal adhesions (FAs), i.e., talin (TLN) 1 and 2, and KANK 1 and 2. Since the integrin αV knockdown increased sensitivity to PTX we aimed to analyse the underlying mechanism. The immunofluorescence analysis of TLN1, TLN2, KANK1 and KANK2 demonstrated that KANK1 does not localise near integrin αVβ5 FAs and its expression does not change upon integrin αV knockdown. Conversely, we found KANK2 localised near αVβ5 FAs and integrin αV knockdown decreased its expression. Moreover, KANK2 knockdown mimicked the effect of αV depletion i. e. increased sensitivity to PTX and inhibited cell migration. TLN1 knockdown resulted in complete breakdown of integrin αVβ5 FAs, decreased proliferation and expression of KANK2 as well as changed cell morphology, actin and MT appearance. On the other hand, TLN2 knockdown did not affect the number of αVβ5 FAs, cell proliferation, KANK2 expression or localisation, but it increased sensitivity to PTX, decreased migration and altered the MT appearance. The velocity of MT growth, using transfection of fluorescently labelled end-binding protein 3, demonstrated increased velocity upon TLN1, TLN2 or KANK2 knockdown. Finally, the western blot analysis of isolated IACs indicated that TLN2 knockdown reduced KANK2 levels within IACs. Together, these results indicate that TLN2-KANK2 interaction controls MT dynamics and sensitivity of MDA-MB-435S cells to PTX. Therefore, our study identifies TLN2 and KANK2 as additional targets within αVβ5 FAs whose knockdown might be used to increase efficacy of PTX treatment and at the same time inhibit migration.

ST13: Neoadjuvant Oncolytic Virotherapy of a Localized Breast Cancer Recurrence: A Case Study

Dubravko Forčić[1,6], Karmen Mršić[2], Melita Perić-Balja[3], Tihana Kurtović[1,6], Snježana Ramić[3], Tajana Silovski[4], Ivo Pedišić[5], Ivan Milas[3], Beata Halassy[1,6]

[1]Centre for Research and Knowledge transfer in Biotechnology, University of Zagreb, Zagreb, Croatia; [2]Clinical Department of Diagnostic and Interventional Radiology, University Hospital Centre "Sestre Milosrdnice", Zagreb, Croatia; [3]University Hospital for Tumours, University Hospital Centre "Sestre Milosrdnice", Zagreb, Croatia; [4]Department of Oncology, University Hospital Centre Zagreb, Zagreb, Croatia; [5]Radiochirurgia Zagreb, Sveta Nedelja, Croatia; [6]Center of Excellence for Virus Immunology and Vaccines, Zagreb, Croatia
(e-mail: bhalassy@unizg.hr)

Intratumoral oncolytic virotherapy may have promise as a means to debulk and downstage inoperable tumours in preparation for successful surgery. Here we describe the pioneering case of a 50-year-old virologist with locally recurrent muscle-invasive breast cancer who was able to proceed to tumour resection after receiving multiple intratumoral injections of viruses, first an Edmonston Zagreb measles vaccine strainMeV) then vesicular stomatitis virus Indiana strain (VSV). The intratumoral virus therapy was well tolerated and led to transient pseudoprogression followed by partial tumour remission. Frequent imaging studies and regular clinical observations documenting size, consistency and mobility of the injected tumour demonstrate that both MeV and VSV contributed to the overall favourable response. Two months after the start of virus injections, the shrunken tumour was no longer invading the skin or underlying muscle and was surgically excised. The excised tumour showed strong lymphocytic infiltration, with increase in both CD20-positive B cells and CD8-positive T cells, as well as macrophages. PD-L1 expression was detected in contrast to the PD-L1 negative phenotype before the treatment. The patient completed one-year trastuzumab adjuvant therapy, remains well and recurrence free 22 months post-surgery. Although an isolated case, the study provides a strong rationale for the formal testing of oncolytic virotherapy also in patients with early stage cancer.

ST14: The Contribution of TLR4 And MYD88 to Bladder Cancer Development

Dora Knezović[1], Blanka Roje[1], Katarina Vilović[2], Lucija Franković[1], Jelena Korać Prlić[1], Janoš Terzić[1]

[1]Laboratory for Cancer Research, University of Split School of Medicine, Split, Croatia
[2]Department of Pathology, University Hospital Split, Split, Croatia
(e-mail: dknezovi@mefst.hr)

Bladder cancer (BC) is the seventh most common type of cancer and the most common neoplasm of the urinary tract, causing significant impairment of life quality and mortality. The importance of microbiota in the development of BC is becoming more evident. It is emphasized by the fact that the gold-standard therapy for non-muscle invasive BC is the intravesical instillation of the bacterium Bacillus Calmette-Guerin. Furthermore, the urinary microbiota of BC patients differs from healthy controls. Thus, it is crucial to test the roles of the microbial receptor TLR4 and signaling molecule MyD88 as key molecules reflecting cellular interaction with bacteria. We challenged TLR4-/- and MyD88-/- mice and respective controls with the 0.05% N-butyl-N-(4-hydroxylbutyl)-nitrosamine (BBN) in drinking water during two (acute) or twelve weeks (chronic protocol). Urinary bladder specimens were pathologically analyzed and a sample of stool was collected for 16S sequencing. To our surprise, we found no difference in the prevalence of bladder pathologies including degenerative changes, stages of bladder tumor, and the inflammatory score between WT controls, and TLR4 and MyD88 knock-out mice. The differences were neither observed in the acute nor the chronic experiment. This could be a consequence of microbiota similarity between tested mice groups, however, microbiota composition analysis is underway. In conclusion, we found no evidence suggesting a role for the microbiota-inflammation pathway in bladder cancer development.

ST15: TFEB Drives Chemo-Immuno-Resistance in Lung Cancer

Muhlis Akman[1], Camilla Ariano[1,2], Gabriella Doronzo[1,2], Federico Bussolino[1,2], Chiara Riganti[1]

[1]Department of Oncology, University of Turin, Turin, Italy.
[2]Candiolo Cancer Institute-FPO, IRCCS, Candiolo, Italy
(e-mail: muhlis.akman@unito.it)

Transcription factor EB (TFEB) is a leucine zipper protein and a major regulator of lysosomal biogenesis and autophagy. These two events confer chemoresistance in solid tumors, by sequestrating chemotherapeutic drugs, and also modulate the immune-recognition. In this study, we investigated if TFEB affects the response to chemotherapy and to Vγ9δ2 T-lymphocytes in non-small cell lung cancer (NSCLC).

We silenced TFEB in H441 and H2228 cells. Changes in ABC transporters involved in chemo-immunoresistance (ABCA1, ABCB1, ABCC1) and metabolic associated pathways were measured by RT-PCR, immunoblotting, and radiolabelling. Co-cultures between NSCLC cells and γδ T-lymphocytes were set-up to measure their expansion and cell killing. By reducing the pERK1/2-SREBP2 axis that modulates genes of cholesterol homeostasis, TFEB silencing decreased expression and activity of the cholesterol/IPP transporter ABCA1, the efflux of IPP, and the NSCLC killing by γδ T-lymphocytes. shTFEB cells had increased expression of ABCB1 and ABCC1 and significantly higher IC50 to cisplatin. shTFEB NSCLC xenografts implanted in Hu-CD34+ NSG mice, were resistant to cisplatin, but were resensitized by zoledronic acid, which re-activates γδ T-lymphocytes killing and down-regulates ABCB1/ABCC1.

We propose TFEB as a novel driver of chemo-immuno-resistance in NSCLC, since its silencing reduced IPP efflux and γδ T-lymphocyte killing (by decreasing ABCA1), and cisplatin cytotoxicity (by increasing ABCB1 and ABCC1). Zoledronic acid can be repurposed as a chemo-immuno-sensitizer agent in TFEB-expressing NSCLC.

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POSTER PRESENTATIONS

P1: MOLECULAR DIAGNOSTICS OF THE L265P MUTATION IN THE MYD88 GENE

Ivona Arić, Branimir Gizdić, Katarina Marija Tupek, Ivanka Sviben, Zdravko Mitrović, Ozren Jakšić, Vlatko Pejša, Rajko Kušec

Department of Hematology, Dubrava University Hospital, School of Medicine, University of Zagreb, Zagreb, Croatia
(e-mail: ivonaaric5@gmail.com)

The L265P mutation in the MYD88 gene is associated with the development and progression of Waldenström's macroglobulinemia. According to the national consensus of modern diagnostics in hematology in the Republic of Croatia, it was agreed to develop a test for the L265P mutation in the MYD88 gene at the Dubrava Clinical Hospital in the Laboratory for Molecular Diagnostics and Genetics. A Sanger sequencing method was developed which is preceded by the DNA isolation from bone marrow, peripheral blood and tumor tissue biopsies followed by PCR amplification of the desired DNA fragment. By comparing the results of HRM analysis with the Sanger sequencing method the L265P mutation in the MYD88 gene was found in 21% of patients in the first series, which included 14 patients with a histological diagnosis of lymphoplasmacytoid lymphoma or Waldenstroem's macroglobulinemia. Three patients were positive for this mutation. Sanger sequencing was selected as the primary method in the diagnosis of the L265P mutation in the MYD88 gene due to its greater sensitivity and specificity.

P2: Analysis of Alternative LDLRAD4 Gene Promoters and Transcripts in Colorectal Cancer

Luka Velimirov, Tamara Babic, Sandra Dragicevic, Aleksandra Nikolic

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia
(e-mail: tamara@imgge.bg.ac.rs, sandra.d@imgge.bg.ac.rs)

Gene LDLRAD4 plays a role in cell proliferation, apoptosis, immunosuppression and cancer progression. Transcription of LDLRAD4 is regulated by several alternative promoters, two of which were indicated by *in silico* analyses to be differentially active in rectal cancer. Promoter A encodes for a truncated protein-coding transcript and is down-regulated in rectal cancer. Promoter B encodes for a non-coding transcript up-regulated in rectal cancer identified as lnc-RNMT-2:5. The aim of this study was to characterize the two alternative promoters *in silico* in order to explain their differential activity and to investigate the profile of LDLRAD4 transcripts in colon cell lines. Nucleotide sequences used in the analyses were downloaded from the Ensemble genome database (reference GRCh37). Three bioinformatics tools were used for core promoter element prediction: GPMiner, YAPP and CNNPromoter. Four bioinformatics tools were used for transcription factor binding site prediction: PROMO, TFBIND, CiiiDER and Tfsitescan. Only the predictions made by two or more tools were considered. Primer extension followed by fragment analysis was used to characterize LDLRAD4 transcripts present in colon cell lines. The promoter element predictions showed that the promoter A is typical, while promoter B has most typical elements and lacks GC boxes. The transcription binding site predictions indicate that three different transcription factors bind only to the promoter A (NF-kB, EGR1 and IRF-7), while four different transcription factors bind only to the promoter B (HNF1, POU2F1, POU2F2 and PTF1). The predicted transcription factors are mostly involved in regulation of cell differentiation and proliferation. The primer extension experiment performed with primer specific for exon 2-exon 3 junction produced multiple signals of relatively low intensity, indicating the presence of multiple LDLRAD4 transcripts in colon cell lines. The results obtained by *in silico* analysis may explain promoter B activation in rectal cancer. However, based on the results of primer extension, neither of the LDLRAD4 transcripts is dominant in colon cell lines. Considering that promoter B generates long non-coding RNA that can exert its function even at low expression level, it can serve as potential colorectal cancer biomarker and its potential role in carcinogenesis should be investigated.
Acknowledgments: This research was supported by the Science Fund of the Republic of Serbia, PROMIS, #6052315, SENSOGENE.

P3: Membrane Androgen Receptor OXER1 Indicates a Potential Role in Metastasis of Head and Neck Tumors

Josipa Čonkaš[1], Janja Josić[1], Nikolina Piteša[1], Matea Kurtović[1], Tina Petrić[1], Vesna Musani[1], Maja Sabol[1], Ozren Vugrinec[2], Dinko Leović[2], Petar Ozretić[1]

[1]Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
[2]Department of Otorhinolaryngology and Head and Neck Surgery, Zagreb University Hospital Centre, Zagreb, Croatia
(e-mail: Josipa.Conkas@irb.hr)

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer by incidence, with almost 900 000 new cases in 2020 and a frequency of 5% in men and 2% in women. The high prevalence of HNSCC is associated with alcohol and tobacco consumption, infections with oncogenic strains of HPV and long-term exposure to environmental pollutants. It develops from the mucosal epithelium of the oral cavity, larynx, pharynx, and salivary glands, most often in males over 50 years of age. The three main treatments for HNSCC are surgery followed by chemotherapy and/or radiation therapy. Due to the higher incidence of HNSCC in men and the association between sex hormone receptors and pathogenesis of HNSCC shown in some recent studies, the focus of our research was to investigate the potential role of androgen and androgen receptors in the metastasis of head and neck tumors. The expression of nuclear (AR) and membrane (CACNA1C, OXER1 and SLC39A9) androgen receptors was determined using the qPCR method in 74 tissue samples of primary HNSCC tumors, 26 metastatic lymph nodes and 26 healthy controls. Furthermore, as a cell model was used Detroit 562, a metastatic cell line of pharyngeal carcinoma, purchased from the ATCC (CCL-138™, Manassas, VA, USA). Treatment with dihydrotestosterone (DHT) was used for androgen receptors activation, while with simvastatin (SIM) for androgen depletion. Effects of dihydrotestosterone and simvastatin treatments on relative gene expression and migratory ability, using wound healing assay and colony forming assay, of untreated and treated cells was determined. Our preliminary results have shown statistically increased OXER1 gene expression in metastases compared to primary tumors, as well as decreased migration and colony forming potential of Detroit 562 cells after treatment with simvastatin. Therefore, our results indicate a potential role of membrane androgen receptor OXER1 in metastasis of head and neck tumors, while the therapeutic potential of statins still needs to be investigated.

P4: Screening of the Antiproliferative Activity of Novel Cu (II) Complex: Comparison of 2D and 3D Cell Culture Models *in vitro*

Nikolina Filipović[1], Berislav Marković[1], Hrvoje Bekina[2], Katarina Mišković Špoljarić[3]

[1]*Department of Chemistry, University of Osijek, Osijek, Croatia*
[2]*Croatia Institute for Public Health, Osijek, Croatia*
[3]*Faculty of Medicine, University of Osijek, Osijek, Croatia*
(e-mail: nfilipovic@kemija.unios.hr)

The first ideas about the antitumor effect of copper complexes appeared in the 1960s. The biological activity of these complexes is highly dependent on the nature of the ligand. The focus of our research is the new Cu (II) complex with chromone-2-carboxylic acid. The copper (II) complex was synthesized from $Cu(NO_3)_2$ x $3H_2O$ and chromone-2-carboxylic acid as ligand. After synthesis, using classical solution chemistry in the stoichiometric ratio (1:1, metal: ligand), the compound was dissolved in DMSO (10^{-3} mol/dm^3) and subjected to cytotoxic appraisal. Evaluated copper (II) complex suppresses cell growth of Hep-G2 cells at highest concentration (10^{-5} M) by 64.8 % followed by NCI-H358, MRC-5 and HT-29 (45.6 %; 45.3 %; 43.4 %). The inhibitory effect of copper (II) complex on KATO III, MDA-MB 231 and Caco-2 cell lines is less than 25 % indicating low antiproliferative efficacy. The activity of the pure ligand was also tested using the MTT test, and the percentage of survival of all cell lines was more than 95 %. The biological activity of the Cu (II) coordination complex with chromone-2-carboxylic acid was also tested in 3D cell culture (Caco-2; HT-29; MRC-5). The results after a single treatment on the Caco-2 cell line showed that the complex has an inhibitory effect of 55 % at a concentration of 10^{-6} mol/dm^3 and a 65 % inhibitory effect at a concentration of 10^{-5} mol/dm^3. The action of the complex on the same cell line during repeated treatment showed a lower inhibitory effect: about 20 % at a concentration of 10^{-6} mol/dm^3, and about 30 % at a concentration of 10^{-5} mol/dm^3. During a single treatment, the tested complex inhibits the growth of the HT-29 cell line by about 50 % at a concentration of 10-6 mol/dm3 while it shows extremely high activity on that cell line at a concentration of 10^{-5} mol/dm^3 (cca. 80 %). The action of the complex on the same cell line during repeated treatment again showed a lower inhibitory effect: about 30 % at a concentration of 10^{-6} mol/dm^3, and about 60 % at a concentration of 10^{-5} mol/dm^3. The tested complex did not show a significant cytotoxic effect on the MRC-5 cell line in any type of treatment, at any concentration. The results clearly indicate that the novel copper (II) complex may represent a promising structural starting point for the development of a new generation of potential antitumor agents.

P5: Ascorbate in Absence of FGF-2 Interferes with Energetic Pathways of Sarcoma Stem Cells

Maja Ledinski, Katarina Caput Mihalić, Marijana Šimić Jovičić, Zara Škibola, Lorena Zubaj, Karla Ostojić, Inga Urlić

Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Croatia (e-mail: maja.ledinski@biol.pmf.hr)

Positive outcomes of antitumor therapy can be prevented by resistance mechanisms provided by a small population of cells, cancer stem cells (CSC). Their properties enable them to evade chemotherapy and radiotherapy and sequentially lead to tumor recurrence and even metastasis. Therefore, their eradication is essential for successful recovery. Although vitamin C has a controversial history in cancer treatment, many pre-clinical studies are now focused on the mechanisms of its effect. We would like to investigate the effect of vitamin C on CSCs derived from sarcoma biopsies. Isolated CSC were characterized by dye exclusion assay and analysis of stemness markers on qPCR. CSC showed strong efflux of dye Hoechst 33342. Also, levels of SOX2 and Oct-4 were higher when compared to parental cell line. CSCs are grown in the presence of fibroblast growth factor 2 (FGF-2) as it is a key regulator of their stemness properties. However, FGF-2 potentially has the ability to modulate the answer of CSCs to the treatment. To test their sensitivity, CSC were treated with a range of concentrations of vitamin C in two forms – ascorbic acid (AA) and dehydroascorbic acid (DHA). Both treatments were applied in media with/without FGF-2 for 72 h and viability was assessed by MTT. CSC showed sensitivity to the treatment in 1-5 mM range and absence of FGF-2 contributed to their enhanced sensitivity. Upon choosing the concentration suitable for longer treatment, CSC were treated with 1 mM AA and DHA in media with/without FGF-2, during 7 days. RNA and proteins were isolated for downstream expression analyses. qPCR analysis showed that both treatment with AA and DHA in absence of FGF-2 affect expression of GAPDH and PGC-1α. The absence of GAPDH was confirmed on Western blot. To conclude, depriving CSC of FGF2- in some cases makes CSC more susceptible to treatment with ascorbate. Also, treating CSC with ascorbate in the presence of FGF-2 can enhance their metabolic properties for energy generation. Therefore, our results show that ascorbate in absence of FGF-2 interferes with energetic pathways and that could affect the viability of CSC.

P6: Primaquine Derivatives Reverse Multidrug Resistance of Tumor Cells by Modulation of ABCG2 Transporter Activity

Marija Mioč[1], Ágnes Telbisz[2], Maja Beus[3], Zrinka Rajić[3], Branka Zorc[3], Balázs Sarkadi[2], Marijeta Kralj[1]

[1]*Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*
[2]*ELKH Research Centre for Natural Sciences, Budapest, Hungary*
[3]*Faculty of pharmacy and biochemistry, University of Zagreb, Croatia*
(e-mail: marijacosic1990@gmail.com)

Multidrug resistance (MDR) is a well-known characteristic of many cancers and a major cause of chemotherapy treatment failure. Although several cellular mechanisms are responsible for this phenomenon, the overexpression of ATP-binding cassette (ABC) transporters named ABCB1 (MDR1/P-glycoprotein) and ABCG2 (BCRP) by cancer cells seems to be the most important. The ability to modulate their expression or block their function is of great clinical importance to achieve more effective antitumor therapy and to improve the availability and absorption of other drugs that are substrates of these transporters. To date, many pharmacological agents have been discovered that modulate the activity of the ABC transporters, but none of them has been in clinical use due to toxicity, drug interactions, or insufficient clinical efficacy. One of them is antimalarial drug primaquine, for which it has been shown to have adjuvant or direct antitumor effect and is able to inhibit the activity of the ABCB1 transporter and reverse tumor cell resistance to chemotherapeutic agents that are substrates of this transporter. Modifications of primaquine structure are often made with the aim of developing primaquine derivatives with improved antimalarial activity and lower toxicity, but also as new drugs with different biological activity.

In this study, we investigated the effect of two groups of primaquine derivatives - primaquine and halogenaniline fumardiamide and bis-urea on ABCG2 transporter activity and expression. We demonstrated that a few compounds have potent inhibitory effects towards the ABCG2 transporter and are able to sensitize cancer cells to the conventional chemotherapeutic agent mitoxantrone, the substrate of the ABCG2. We have also shown that these compounds are substrates of the ABCB1 transporter, making them selective inhibitors of ABCG2. These data are promising and indicate that primaquine derivatives could be used in cancer therapy due to their ability to reverse MDR. This work also contributes to the development of new compounds with even better inhibitory properties.

P7: Strict Regulation of the KEAP1-NRF2 Pathway and its Interaction with the Aquaporin 3 Expression in Breast Cancer Cell Lines

Monika Mlinarić, Ana Čipak Gašparović

Laboratory for Oxidative Stress, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: Monika.Mlinaric@irb.hr)

Aquaporins (AQP) are membrane proteins that facilitate the transport of water and other small uncharged solutes across the plasma membrane, which makes them important in the regulation of cellular water homeostasis, proliferation, and migration. Several AQPs can transport hydrogen peroxide and are therefore named peroxiporins, who by facilitating hydrogen peroxide in and potentially out of the cell influence cell's oxidative status. In the state of oxidative stress, characterized by an imbalance between oxidants and antioxidants, AQPs have a significant role and could contribute to oxidative damage in the cell. To prevent oxidative damage and associated undesirable effects, cells have a strong antioxidant system. As a major regulator of cellular resistance to oxidative stress, Nrf2 (nuclear factor erythroid 2–related factor 2) induces the expression of many antioxidant genes and therefore regulates cellular response to oxidative stress. Since it has an important role in fine-tuning of ROS, Nrf2 regulation is crucial. Keap1 (Kelch Like ECH Associated Protein 1) acts as a sensor for oxidative stress and is responsible for Nrf2 ubiquitination and degradation in physiological conditions, and its activation induced by oxidants. Once activated Nrf2 translocates to the nucleus where it exerts its effect. The Nrf2-Keap1 axis, as well as AQPs, are often dysregulated in tumors, who use them for their protection against constantly present oxidative stress. Our aim was to study the Nrf2 regulation in breast cancer cell lines, and to see if it somehow affects the AQP expression. According to the literature, AQP3 is often overexpressed in breast cancer cells compared to normal breast cells, so we put our efforts into finding its potential interaction with Nrf2. Our results indicate a strong regulation of Nrf2 activation independent of its expression. Cell line-dependent changes in AQP expression were also observed, but additional research is needed to determine whether there is an interaction between these proteins.

P8: Molecular-Morphological Diversity of Atypical Spitz Tumor

Ivan Novak[1], Bernardica Jurić[2], Majda Vučić[2,3], Cvjetko Lež[4,5], Božo Krušlin[2,6], Monika Ulamec[2,6,7]

[1]School of Medicine, University of Zagreb, Zagreb, Croatia; [2]Ljudevit Jurak Clinical Department of Pathology and Cytology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia; [3]Department of Pathology, School of Dental Medicine, University of Zagreb, Zagreb, Croatia; [4]General Hospital and Croatian Veterans Hospital Zabok; Zabok, Croatia; [5]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Croatia; [6]Department of Pathology, School of Medicine, University of Zagreb, Zagreb, Croatia; [7]Scientific Group for Research on Epigenetic Biomarkers, University of Zagreb School of Medicine, Zagreb, Croatia
(e-mail: novak.ivan264@gmail.com)

Spitz neoplasms comprise a spectrum of melanocytic tumors which include benign Spitz nevi, malignant Spitz melanomas and atypical Spitz tumors (AST) for which precise distinction between nevi and melanomas is very challenging. Specific driver mutations are the most important distinguishing factor of Spitz lesions, and some of the most common are tyrosine kinase fusions of ALK, ROS1, NTRK1, NTRK3, MET and RET, as well as serine-threonine kinase fusions of MAP3K8 and BRAF. Current studies are focused on correlations between pathohistological features and molecular changes in order to increase the power to distinguish between benign and malignant Spitz neoplasms. In our research, we analyzed the clinical and pathohistological features, and the immunohistochemical profile of 24 Spitz nevi and 24 ASTs. Pathohistological analysis included cell type, pagetoid extension, lymphocytic infiltration, Kamino bodies, cellular pleomorphism, mitotic activity and pigmentation. Semiquantitative immunohistochemical analysis was performed after staining the preparations with classical markers p16, Melan-A, HMB45 and Ki67, and unconventional antibodies ALK, ROS1 and Pan-TRK. A greater number of malignant pathohistological features were found in ASTs (intensity of pagetoid extension showed most significant difference, P=0.02) except for the absence of Kamino bodies and deeply located dermal mitoses. Clinical features of malignancy (diameter and depht of lesions) were also more pronounced in ASTs, although without statistical significance. Among the classical immunohistochemical markers, the most intense difference was found in the Ki67 profile, which points to a higher proliferative nature of ASTs (P=0.01). Moreover, ASTs showed expression of ALK in 8% of lesions, ROS1 in 17% and Trk in up to 33%, while Spitz nevi showed expression of each protein in 4% of lesions. In all cases expression of these proteins was mutually exclusive. It was observed that the expression of ROS1 protein correlates with a notable pagetoid extension, and domination of epithelioid type of cells might be a potential distinguishing feature of Spitz tumors which express ALK, ROS1 or Trk proteins. Further research using cytogenetic or molecular techniques such as next generation sequencing is necessary to test concordance between expression of the proteins and gene fusions.

P9: Impaired Integrin αVβ5-Adhesion Affects Mitochondrial Biogenesis And Shifts Metabolism Toward Catabolic Processes in the Triple-Negative Breast Cancer Cell Line MDA-MB-231

Mladen Paradžik[1], Nikolina Stojanović[2], Jonathan D. Humphries[3,4], Iva I. Podgorski[5], Marija Pinterić[5], Lucija Dončević[6], Zrinka Vitković[2], Sandra Sobočanec[5], Martin J. Humphries[3], Andreja Ambriović-Ristov[2]

[1]Laboratory of Experimental Therapy, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia; [2]Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia; [3]Wellcome Centre for Cell-Matrix Research, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK; [4]Department of Life Sciences, Manchester Metropolitan University, Manchester, UK; [5]Laboratory for cell metabolism and aging, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia; [6]Laboratory for Bioanalytics, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb Croatia(e-mail: mladen.paradzik@irb.hr)

Integrins are heterodimeric receptors that bind cells to the extracellular matrix (ECM). Upon integrin clustering, multimolecular integrin adhesion complexes (IACs) are formed, facilitating the linkage between integrins and the cytoskeleton. The αV-family of integrins contributes to tumorigenesis, metastasis, and response to therapy. To understand the role of αV heterodimers in triple negative breast cancer cell line MDA-MB-231, we isolated cell clones transfected with control plasmid (MDA-MB-231sh) or plasmid expressing shRNA specific for αV (MDA-MB-231shαV). IACs following crosslinking from both cell clones, grown in long-term culture, were isolated and their molecular composition analysed using mass spectrometry (MS)–based proteomics, enabling us to detect IACs as well as ECM proteins secreted by cells. We detected 223 proteins, of which 26 proteins were significantly altered in MDA-MB-231shαV cells. The most prominent integrin subunits identified in this analysis were αV and β5, suggesting that these cells mainly use integrin αVβ5 for adhesion. Using gene ontology enrichment analysis, we showed that the majority of downregulated proteins in MDA-MB-231shαV cells were associated with focal adhesions, whereas all upregulated proteins were part of the extracellular space, suggesting that cells compensate for the absence of αV-integrin heterodimers by enriching their ECM. Knockdown of the αV subunit reduced the ability to form colonies and inhibited cell proliferation. To further investigate the downstream effect of αV knockdown, metabolic parameters were measured. Impaired adenylate energy charge, higher AMP/ATP ratio, and increased mitochondrial function were observed in MDA-MB-231shαV cells. We hypothesize that knockdown of αV, in addition to reducing adhesion, reduces proliferation by shifting metabolism to catabolic pathways, and upregulating mitochondrial biogenesis via 5' AMP-Activated Protein Kinase (AMPK), through Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha (PGC1α)-Sirtuin 3 (Sirt3) axis. These data indicate that αV integrin heterodimers regulate metabolism, providing new avenues for the identification of novel drug targets.

P10: Cytotoxic Effects of *Lavandula angustifolia* Mill. and *Laurus nobilis* L. Essential Oils on Human Cervical Adenocarcinoma Cells

Ivana Pašić[1], Ivana Srbljak Ćuk[1], Nina Petrović[1,2], Ivana Matić[1], Hurija Džudžević-Čančar[3], Alema Dedić[3], Amra Alispahić[3], Emina Boškailo[4], Tatjana Stanojković[1]

[1]Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia; [2]Laboratory for Radiobiology and Molecular Genetics, "VINČA" Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Serbia; [3]Department of Chemistry in Pharmacy, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina; [4]Department of Pharmacy, Faculty of Health Studies, University of Modern Sciences-CKM, Mostar, Bosnia and Herzegovina
(e-mail: ivannapasic@gmail.com, dragoninspiration@yahoo.com)

Lavandula angustifolia Mill. (lavender) is an aromatic and medicinal herb whose flower essential oils (EO) are widely used for the treatment of gastrointestinal, nervous, and rheumatic disorders, and in the perfume industry. Laurus nobilis L. (laurel bay) is an evergreen tree whose EOs have antimicrobial and anti-inflammatory effects. Lavender and bay were collected from Sarajevo and Mostar in Bosnia and Herzegovina. The extraction was performed by hydrodistillation in Clevenger-type apparatus. Phytochemical composition was analyzed by gas chromatography coupled with mass spectrometry. Cytotoxic activities of lavender EO and bay leaf, fruit and seed EOs were investigated against human cervical adenocarcinoma HeLa cells and non-transformed human lung fibroblasts MRC-5 by MTT cell survival assay. Cell cycle phase distribution was examined by flow cytometry. In bay EOs the most abundant component was 1,8-cineole, followed by linalool, bicyclic monoterpenes sabinene, α-pinene, and β-pinene. Components identified in the fruit and seed, but not in the leaf were (E)-β-ocymene, camphene, β-elemene, bornyl acetate and trans-caryophyllene. The major component of lavender extract was linalool accompanied by linalyl acetate, lavandulyl acetate, camphor, 1,8-cneole, borneol, α-terpineol, and terpinene-4-ol. The four tested EOs showed concentration-dependent cytotoxic effects on HeLa and MRC-5 cells. Among examined EOs, lavender EO exerted the strongest cytotoxic activity on HeLa cells with IC50 value of 0.11 μL/mL. Bay seed and fruit EOs exerted stronger cytotoxicity on HeLa cells than bay leaf EO (IC50 values: 0.17, 0.21, and 3.35 μL/mL, respectively). When compared with sensitivity of HeLa cells, normal MRC-5 cells showed similar sensitivity to the cytotoxic activity of the four tested EOs. Lavender EO applied at IC50 concentration, during 24 h caused remarkable increase in the percentage of HeLa cells within the subG1 cell cycle phase, in comparison with control cells (64.69% vs 2.47%). Pretreatment with caspase-3, caspase-8 or caspase-9 inhibitor before 24 h treatment with lavender EO did not cause changes in the percentage of cells in the subG1 phase in comparison with HeLa cells exposed only to lavender oil. Our results showed that lavender and bay EOs exerted potent cytotoxic activity against HeLa cells. Additional investigations are necessary to explore cytotoxic effects of these EOs against various cancer cell lines and mechanisms underlying anticancer effects.

P11: 3D Culture of Prostate Cancer as a Model to Study Hedgehog-GLI Signaling Pathway

Tina Petrić, Matea Kurtović, Nikolina Piteša, Josipa Čonkaš, Vesna Musani, Petar Ozretić, Maja Sabol

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

Prostate cancer is the second most frequent cancer diagnosed in men worldwide. Albeit radical prostatectomy and radiation have been proven as quite effective strategies to fight the tumor while still localized, management of advanced stages is limited due to the development of biological mechanisms of resistance to medication. One of the relevant pathways in prostate cancer progression is the Hedgehog-GLI (HH-GLI) signaling pathway which is crucial for normal embryonic development, stem cell maintenance and tissue homeostasis in adult organisms but its aberrant activation in adult cells has been linked with the development of various tumors, including prostate cancer. The aim of this research was to better understand the differences in HH-GLI signaling pathway in co-culture of prostate cancer and stromal cells in two-dimensional (adherent) and three-dimensional (spheroid) models. We used adenocarcinoma prostate cell line LNCaP and cancer-associated prostate fibroblasts WPMY-1. For the two-dimensional models, cells were co-cultured in different ratios (5:1, 2:1, 1:1, 1:2 and 1:5). For the generation of spheroid models, the hanging drop system was employed and cells were co-cultured in the same ratios as 2D models. The response to HH-GLI signaling pathway inhibition was investigated using four different treatments and observing these cell lines with MTT assay. Differences in expression of key genes involved in HH-GLI signaling pathway were also analyzed between cancer and stromal cell lines, both grown in 2- and 3-dimensional system by Real-time PCR. Results demonstrated that cancer and stromal cells are forming spheroids of different shapes and sizes. HH-GLI signaling pathway inhibition showed that the cell death is delayed in the cancer-fibroblast co-culture compared to monocultures. From the analysis of the expression of key genes involved in HH-GLI signaling pathway it is visible that stromal cells are changing their characteristics to the higher extent compared to the cancer cell line. Results obtained so far are pointing out the importance of interaction between the cancer cells and cancer associated fibroblast in the prostate cancer progression. It is known that tumor-stroma interaction is crucial for Hedgehog-GLI signaling in the prostate, but here we demonstrate the resistance of co-culture compared to monocultures on an *in vitro* model.

P12: Signaling Switch from Hedgehog-GLI to MAPK Potentially Drives Primary Cilia Loss in NRAS Mutated GANT61-Resistant Melanoma Cell Line

Nikolina Piteša[1], Nenad Bartoniček[2], Matea Kurtović[1], Tina Petrić[1], Josipa Čonkaš[1], Vesna Musani[1], Petar Ozretić[1], Maja Sabol[1]

[1]Laboratory for Hereditary Cancer, Department of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
[2]Genome Informatics Laboratory, Garvan Institute of Medical Research, Sydney, Australia
(e-mail: nikolina.rincic@irb.hr)

Hedgehog-GLI signaling pathway is extremely important for normal embryonal development and often deregulated in cancer. Its activity is highly dependent on the primary cilia and can be non-canonically modified through the interaction with other signaling pathways, like MAPK signaling. BRAF and NRAS can activate GLI proteins directly regardless of the upstream membrane events, but the exact order in which this occurs, mediators of these interactions and final outcomes are still not fully understood. These interactions may be crucial in establishment and maintenance of drug resistance, a known issue for metastatic melanoma treatment. To investigate in more depth the interaction between HH-GLI and MAPK in resistance, we established two melanoma cell lines MEL224 (NRAS Q61R) and CHL-1 (NRAS WT) resistant to GANT61, a specific GLI protein inhibitor. Cell lines were treated 8-12 months with increased GANT61 concentrations and afterwards validated with the MTT test. To characterize the established cell lines, we examined if the response to other HH-GLI and MAPK inhibitors has changed after resistance development, colony formation and migration capacity. HH-GLI, MAPK protein signaling components and autophagy markers were examined by western blot. In our previous study (Kurtović et al. 2022), using a combined ChIP-seq and RNA-seq approach we identified novel GLI transcription targets involved in MAPK signaling. Therefore, by using qPCR we examined if any of these potential targets were changed in the resistant cell lines. Our results suggest that a signaling switch from HH-GLI to MAPK signaling has occurred in the resistant NRAS mutated cell line MEL224. Both cell lines exhibit a higher colony formation and migration capacity, but they differ in HH-GLI and MAPK signaling activity. A newly identified GLI2 transcription target and potential MAPK substrate, RAB34 essential for ciliogenesis, was downregulated in MEL224 resistant cell lines. For that reason, we checked primary cilia formation using immunofluorescence and detected primary cilia loss in resistant cell line. Kuonen et al. have previously reported cilia loss after HH-GLI and MAPK signaling switch in basocellular carcinomas resistant to SMO inhibitors. We believe that primary cilia represent a potential link between HH-GLI and MAPK signaling in GANT61 resistant NRAS mutated melanoma. Therefore, our future studies will focus on ciliogenesis regulation via MAPK signaling and its function in drug resistance.

P13: Prostate Cancer and Environmental Exposure to Toxic Metal(loid)s: Impact on Prostate Specific Antigen (PSA)

Alica Pizent[1], Blanka Tariba Lovaković[1,2], Tanja Živković Semren[1,3], Marija Gamulin[4,5]

[1]Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia; [2]Toxicology Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia; [3]PMI R&D, Philip Morris Products S.A., Neuchâtel, Switzerland; [4]University of Zagreb, School of Medicine, Zagreb, Croatia; [5]Department of Oncology, University Hospital Centre, Zagreb, Croatia
(e-mail: apizent@imi.hr)

Prostate cancer is among the most commonly diagnosed malignancies in men and its incidence increases with age. In addition to family history, race, and ethnicity, various external risk factors may contribute to its increased risk. Arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) are pervasive environmental contaminants and several experimental and epidemiological studies linked exposure to these metal(loid)s with an increased risk of prostate cancer. However, research on the impact of low-level exposure is scanty and largely inconclusive. Prostate specific antigen (PSA), combined with a digital rectal examination of prostate, has been routinely used for early detection of prostate cancer in spite of its insufficient specificity for clinically important cancers. Moreover, several studies in presumably healthy men indicated that various external factors can affect PSA levels, including metal(loid) exposure. Therefore, we aimed to evaluate the role of environmental exposure to As, Cd, Pb and Hg in prostate cancer risk and their impact on serum PSA. Blood As, Cd, Pb, and Hg and serum PSA were measured in 62 prostate cancer patients and 30 control men with no occupational exposure to metals. Prostate cancer patients had significantly higher serum PSA and blood Hg than control subjects. The results of Spearman's correlation and a simple linear regression analysis in all 92 subjects showed a positive association between blood Hg and PSA. However, after control for the impact of the remaining elements in the models of multiple regression, this association was lost. Although we did not confirm a linear association between measured metal(loid)s and PSA in conditions of environmental exposure, further investigation in larger cohorts, taking into consideration possible synergistic or non-linear mixture effects, are warranted.

P14: NME6 Negatively Impacts Mitochondrial Respiration in Tumor Cell Lines

Bastien Proust[1], Martina Radić[1], Nikolina Škrobot Vidaček[2], Malgorzata Tokarska-Schlattner[3], Uwe Schlattner[3], Maja Herak Bosnar[1]

[1]Laboratory for Protein Dynamics, Ruđer Bošković Institute, Zagreb, Croatia
[2]Laboratory for Molecular Genetics, Ruđer Bošković Institute, Zagreb, Croatia
[3]Laboratory of Fundamental and Applied Bioenergetics, University Grenoble Alpes, Grenoble, France
(e-mail: bproust@irb.hr)

The nucleoside diphosphate kinases (NDPK/NME/Nm23) family raised considerable interest after the discovery of metastasis suppressor role of NME1 in the early 2000. The enzymes catalyze the transfer of gamma phosphate from nucleoside triphosphates to nucleoside diphosphates (NDPK activity), thus maintaining locally the availability of nucleotides for cellular processes. Decades of investigation separated the family into two groups: Group I proteins (NME1-NME4) are highly similar and all display NDPK activity, while Group II proteins (NME5-NME9) are less homologous and are deficient of NDPK activity. Three members, NME3, NME4 and NME6 were shown to localize with mitochondria. The poorly studied NME6 was found to be overexpressed in colon and gastric carcinoma, as well as in colorectal cancer tissues. We recently published an extensive description of the human NME6 protein revealing, among other things, that NME6 is enzymatically inactive but interacts with RCC1L in the mitochondrial matrix. RCC1L, a component of the mitochondrial-RNA maturation module (pseudouridylation module), is strongly associated with mitoribosome biogenesis and was found essential for oxidative phosphorylation, the final metabolic pathway of mitochondrial respiration. We showed that NME6 is ubiquitously expressed in a large panel of cancer cell lines with different tissues of origin, as well as in non-cancer cell lines using Western blot. We confirmed the mitochondrial localization of the protein by immunofluorescence and live cells imaging, and further refined it to the mitochondrial matrix localization using mitochondrial subfractionation. We described, using oxygraphy, its negative impact on mitochondrial respiration upon overexpression, directly linked with a decreased abundance of respiratory chain complexes at the protein level. Finally, we propose a joint role of NME6 and RCC1L within the mitochondrial matrix, affecting the abundance of respiratory chain complexes and eventually affecting the mitochondrial respiration.

P15: IL6R Polymorphism rs2228145 and GP130 Polymorphism rs3729960 do not Contribute to Microsatellite Instability in Sporadic Colorectal Cancer

Anamarija Salar, Tamara Čačev, Sanja Kapitanović

Laboratory for Personalized Medicine, Division of Molecular Medicine, Ruđer Bosković Institute, Zagreb, Croatia (e-mail: asalar@irb.hr)

Chronic inflammation over time contributes to the accumulation of genetic and epigenetic alterations leading to malignant transformation. Elevated microsatellite alterations at selected tetranucleotide repeats (EMAST) is a biomarker of a specific kind of microsatellite instability caused by hMSH3 dysfunction due to chronic inflammation. Interleukin 6 (IL6) represents one of the mediators of chronic inflammation whose high serum levels contribute to the formation and progression of tumors. Signal transmission via IL6 can be classical and trans, the latter being associated with pro-inflammatory and pro-tumorigenic effects. Since IL6 receptor is not present on colon cells they can only undergo trans-signaling. In addition, soluble form of IL6 coreceptor gp130 acts as a selective antagonist of the IL6 trans-signaling pathway. Thus the response of colon cells to IL-6 is regulated by the amount of IL6, the presence of the soluble form of IL6R, and the presence of the soluble and membrane form of gp130 coreceptor. The aim of this study was to examine the distribution of two functional polymorphisms one in the IL6 receptor (rs2228145; 48892 A>C) and another in the gp130 coreceptor (rs3729960; 148 G>C) in tumors classified according to the different types of microsatellite instability.

In this study, we have examined the IL-6R rs2228145 and gp130 rs3729960 polymorphisms in 190 sporadic colorectal tumors. The PCR-RFLP followed by either agarose gel or polyacrylamide electrophoresis was used for SNP genotyping and the data were examined according to the presence or absence of a specific type of microsatellite instability.

Homozygous carriers of functional variants of rs2228145 and rs3729960 which have previously been associated with regulatory influence on IL6R and gp130 were not associated with either MSI or EMAST type of microsatellite instability.

We can conclude that since neither rs2228145 variant allele C nor rs3729960 variant allele C were more common in tumors with either MSI or EMAST type of microsatellite instability they are not contributing to these two types of genome instability.

P16: Prediction of Cell-of-Origin of Cancers Using Gene Mutation Profiles

Paula Štancl, Rosa Karlić

Bioinformatics Group, Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia
(e-mail: pstancl@bioinfo.hr)

Cancers of unknown primary origin (CUP) account for 3-5% of all cancers and still present a challenge for treatment in clinical practice. We previously developed a method that successfully identifies the cell-of-origin (COO) of a cancer by modeling the relationship between chromatin features of the COO fand the cancer's mutational landscape, obtained from whole-genome sequencing (WGS) data. Although several high-performing WGS-based methods to predict the COO were developed recently, methods of similar accuracy based on whole-exome sequencing (WXS) data are still missing. Since the cost and computational time needed for analysis is much lower for WXS compared to WGS data, the development of such a method might be very useful for diagnosis and treatment of cancer patients. Our study aimed to develop a statistical model that utilizes mutational profiles in genes obtained by either WXS or WGS, and chromatin state across those genes, to predict the COO. We analyzed a publicly available melanoma cohort from the International Cancer Genome Consortium, consisting of 183 patients with mean 224 single-base substitutions per gene. ChIP-seq data for six histone modifications (H3K27ac, H3K27me3, H3K36me3, H3K4me1, H3K4me3, H3K9me3) and input were downloaded from the ENCODE project. Read counts were normalized using FPKM over all genes on the hg19 human genome. We used wavelet transformation to determine the optimal scale for analysis of diverse data types and trained a multiple linear regression model with 10-fold cross validation to compute the amount of variance of aggregated mutations across genes explained by the epigenome of each COO. The model with the highest variance explained indicates the COO for a specific cancer type. Our WXS-based model correctly identified melanocytes as the COO of melanoma. The variance explained for the best-performing model was ~33% and ~52% for all and only protein-coding genes, respectively, when using non-normalized epigenetic features. Wavelet transformation caused a significant increase of prediction accuracy, with explained variance of around ~60% for both all and only protein-coding genes. Residual and over-representation analyses detected a specific group of protein-coding genes involved in melanin metabolic processes and pigmentation as informative for predicting COO. The results show that we are able to use the cell's epigenome and cancer's gene mutation profile to predict the cell-of-origin.

P17: Diversed Expression of Short p53 Family Isoforms May Affect Melanoma Aggressiveness

Ana Tadijan[1], Francesca Precazzini[2,3], Nikolina Hanžić[1], Martina Radić[1], Nicolò Gavioli[2], Ignacija Vlašić[1], Petar Ozretić[4], Lia Pinto[2], Lidija Škreblin[1], Giulia Barban[2], Yari Ciribilli[2], Neda Slade[1]

[1]Laboratory for Protein Dynamics, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
[2]Laboratory of Molecular Cancer Genetics, Department of Cellular, Computational, and Integrative Biology (CIBIO), University of Trento, Povo, Italy
[3]Laboratory of RNA Biology and Biotechnology, Department of Cellular, Computational, and Integrative Biology (CIBIO), University of Trento, Povo, Italy
[4]Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: Ana.Tadijan@gmail.com)

Metastatic melanoma is the most aggressive form of skin cancer. Despite currently available therapy targeting BRAF and MEK kinases, as well as immunotherapy, the treatment of melanoma remains a challenge due to resistance to therapy. Thus, it is of utmost importance to investigate the molecular pathways crucial for melanoma development and therapy resistance. The *TP53* gene, the guardian of the genome, is altered in more than 50% of human cancers but is rarely mutated in melanoma. Shorter p53 family isoforms, whose significance has just recently become evident, can act as modifiers of the p53-dependent responses including its tumor suppressive function. We have analyzed the gene and protein expression of p53 and p73 isoforms in a panel of human melanoma cell lines with different TP53 and BRAF status, in normal conditions and after the treatment with common DNA-damaging agents or targeted therapy. We generated stable clones of H1299 p53 null cells expressing the less characterized short isoforms Δ160p53α, Δ160p53β, and Δ160p53γ. Furthermore, we developed two human melanoma cell lines resistant to the BRAF inhibitor vemurafenib and examined the change in the expression of the p53 family isoforms after acquisition of resistance. Our results show that human melanoma cell lines express a wide array of p53 and p73 isoforms. We demonstrated for the first time that Δ160p53α, and to a lesser extent Δ160p53β, can be recruited on chromatin, and that Δ160p53γ can localize in perinuclear foci. Importantly, H1299 cells stably expressing Δ160p53 isoforms demonstrated higher proliferation and *in vitro* migration. Finally, melanoma cells resistant to vemurafenib exhibited an altered expression of p53 and p73 isoforms, specifically increased expression of potentially prooncogenic Δ40p53β and a decreased level of tumor-suppressive TAp73β. Therefore, we propose that p53 family isoforms play a role in the aggressiveness of melanoma cells and could be a potential marker and target for melanoma therapy.

P18: TLR3 Stimulation Induces the Expression of Damage Associated Molecular Patterns in Head and Neck Cancer Cell Line

Tea Vasiljević, Emilija Zapletal, Tanja Matijević Glavan

Laboratory for Personalized Medicine, Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia (e-mail: tvasilj@irb.hr)

Toll-like receptor 3 (TLR3) is a member of the Toll-like receptor family that belongs to pattern recognition receptors which recognize motifs derived from pathogens. It is located on the endosome and is stimulated by viral dsRNA or its synthetic analogs poly(I:C) and poly(A:U). It can also be stimulated by endogenous ligands, called damage associated molecular patterns (DAMPs). TLR3 participates in innate immunity but also plays a role in tumor cells. TLR3 stimulation induces apoptosis but may also stimulate tumor progression through increased cell proliferation, migration and metabolic re-programming. Here, we have tried to determine whether TLR3 plays a role in cancer stem cells (CSC). Pharyngeal cancer cell line Detroit 562 was used as the experimental model. The cells were treated with poly(I:C) or poly(A:U). Cells were grown in normal conditions to obtain adherent cell cultures or specific conditions to enrich cancer stem cells (CSC) (tumorospheres). Proteins were isolated from both adherent cells and tumorospheres, and detected by western blot.

We observed a significantly higher expression of endogenous ligands in tumorospheres and adherent cells treated with synthetic analogues of dsRNA compared with untreated controls. Among the endogenous ligands observed, S100A9 had the highest upregulation after both treatments with poly(I:C) and poly(A:U) in CSC and in adherent cells compared to control. S100A9 is overexpressed in many cancers, associated with poor prognosis, and may promote immune evasion. HMGB1 was induced through poly(A:U) treatment in CSC and with poly(I:C) in adherent cells, whereas RAGE as a receptor for HMGB1 had equally robustly expressed bands for both treatments in CSC and adherent cells compared with the control. HSP70 can suppress apoptosis, interfere with tumor immunity, promote angiogenesis and support metastasis. In our experiments, it has shown highest upregulation with poly(I:C) treatment in CSC compared to the control. TLR4 was not significantly changed in all of the samples.

We have determined that the TLR3 stimulation induces the expression of DAMPs. This demonstrates that no external signal is necessary, but only endogenous ligands, to induce TLR3 and cancer progression. Moreover, we have shown that endogenous ligands released from cancer stem cells can also contribute to head and neck cancer progression.

AUTHOR INDEX

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