UNIVERSITY OF RIJEKA

Doctoral School

Croatian Academy of Sciences and Arts, Deparment of Biomedical Sciences in Rijeka, Croatian Academy of Medical Sciences – Branch office Rijeka

The University of Rijeka Foundation

PhD SCIENTIFIC CONFERENCE

"Innovative Technologies in Biomedicine"

University Campus Rijeka, University Departments, Lecture Hall O-028, Radmile Matejčić, Rijeka

BOOK OF ABSTRACTS

Rijeka, September 19-20, 2019

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PROGRAMME

19th September 2019

8:00 – 9:00 Gathering, registration and coffee

9:00 – 9:20 Opening remarks:

- Madam Rector **Snježana Prijić-Samaržija,** PhD, Professor,
- **Alen Ružić,** PhD, Professor, Vice-rector for science and internationalization,
- **Daniel Rukavina,** PhD, Professor Emeritus, Head of Department of Biomedical Sciences of the Croatian Academy of Sciences and Arts,
- **Sandra Kraljević Pavelić,** PhD, Professor, Head of University of Rijeka Doctoral School

9:20 – 9:45 **David del Álamo Rodŕigez,** PhD, Head of the EMBO Fellowship Programme; "Introduction to the EMBO fellowships programmes"

9:45 – 10:15 Plenary lecture 1: **Marc Thorsten Hütt,** PhD, Jacobs University Bremen, Germany, Department of Life Sciences and Chemistry; "High-throughput data as patterns on biological networks"

10:15 – 10:45 Plenary lecture 2: **Michael Menden, PhD,** ICB Institute of Computational Biology, Neuherberg, Germany; "Computational Cancer Pharmacogenomics"

10:45 – 11:15 Plenary lecture 3: **Srećko Gajović,** PhD, University of Zagreb, School of Medicine, Croatian Institute for Brain Research, Zagreb; "In vivo molecular imaging in preclinical evaluation of medical interventions"

11:15 – 11:30 Discussion

11:30 – 12:00 Poster session

12:00 – 13:00 Lunch

13:00 – 14:30 PhD and post-docs oral presentations

20th September 2019

9:00 – 9:30 Plenary lecture 1: **Matthias Schwab,** PhD, Dr Margarete Fischer- Bosch Institute of Clinical Pharmacology, Sttutgart, Germany; „ADME Pharmacogenomics: Clinical relevance and future directions"

9:30 – 10:00 Plenary lecture 2: **Nandu Goswami,** PhD, Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation, Graz, Austria; "Molecular mechanisms of bedrest induced coagulation changes"

10:00– 10:30 Plenary lecture 3: **Riccardo Alessandro,** PhD, University of Palermo, Department of Biomedicine, Palermo, Italy; "The multifaced role of exosomes in cancer: from biology to therapy"

10:30 – 11:00 Poster session and coffee break

11:00 – 11:30 Plenary lecture 4: **Johan Wojta,** PhD, Medical University of Vienna, Vienna, Austria; "Monocyte subsets and macrophage polarization in cardiovascular disease„

11:30 – 12:00 Plenary lecture 5: **Nataša Pržulj,** PhD, Barcelona Supercomputing Center, Barcelona, Spain; "Computational challenges for data-driven medicine"

12:00 – 13:00 Poster session and lunch

13:00 – 14:30 PhD and post-docs presentations

Molecular mechanisms of bedrest induced coagulation changes

Nandu Goswami[1,2], PhD.

1. Head of Physiology Division, Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation, Medical University of Graz, Neue Stiftingtalstrasse 6, D-5, Graz, Austria
2. Director of Health Sciences Research, Alma Mater Europea, Maribor, Slovenia

Orthostatic challenge could lead to dizziness upon standing up, especially if adequate brain perfusion is not able to be maintained by the cardiovascular system. This condition occurs quite frequently in older persons and returning astronauts. Spaceflight environment of microgravity, for example, influences several physiological systems, including cardiovascular system, cerebral autoregulation, and musculoskeletal function; some of these factors alone, and in combination, could contribute to post spaceflight orthostatic intolerance. This presentation provides an overview of these microgravity induced physiological effects (deconditioning) and then discusses important similarities and connections to the aging process. Bedrest immobilization often occurs due to aging-associated illness and/or falls-related injuries. During hospitalization, older persons are confined to long periods of bedrest, which can result in substantial physical deconditioning. As bedrest is routinely used by space agencies to model and research the effects of spaceflight de-conditioning due to microgravity, bedrest experimental protocols can increase insight and knowledge regarding both the de-conditioning impact of bed confinement in older persons and the deconditioning occurring in astronauts during spaceflight. This information can be applied

synergistically to design and improve appropriate deconditioning countermeasures for bed-confined older persons and astronauts. In particular, for older persons, such countermeasures can help break the negative spiral of bed confinement leading to deconditioning, dizziness upon standing up, and consequently, falls, resulting in recurring hospitalization and increased frailty.

Keywords: Spaceflight, aging, falls, orthostatic intolerance, immobilization, bedrest.

The multifaceted role of exosomes in cancer: from biology to therapy

Riccardo Alessandro, PhD.

Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Italy

Cell-Cell communication occurs not only via the canonical pathways (cytokines, neurotransmitters, direct contact, ECM-mediated interactions or hormones) but also releasing extracellular vesicles that can reach different regions of the organism acting as a new “signalling organelles”. Among extracellular vesicles, exosomes are considered efficient players to modulate target cells phenotype through the delivery of several molecules such as mRNAs, microRNAs, long non-coding RNAs, DNA, lipids, metabolites and proteins. Exosomes are cup-shaped vesicles of 30-150 nm, released by all cell types, that originate when a multivesicular body (MVB) fuses with the plasma membrane, releasing intraluminal vesicles in the extracellular space. In recent years the scientific community has focused its attention on altered intercellular communication between malignant cells and host non-malignant cells and a number of studies have evidenced the role played by cancer-cell-derived EV in promoting cancer progression and metastasis. Exosomes have been demonstrated to affect cancer invasion, angiogenesis, immunoescape and drug-resistance both in solid and haematological cancers. Recent progress in the biology of EVs also suggests that they might serve as optimal delivery systems of therapeutics. Exploitation of their molecular

composition and physical properties, together with improvement in bio-techniques to modify their content are critical issues to target them to specific cells/tissues/organs thus attaining the most from this incredible “cell treasure”.

Introduction to the EMBO fellowships programmes

David del Álamo Rodŕigez, PhD, MBA.
Programme Head, Fellowships, EMBO - Excellence in Life Sciences, Heidelberg, Germany

EMBO is an organization of more than 1800 leading researchers that promotes excellence in the life sciences in Europe and beyond. The major goals of the organization are to support talented researchers at all stages of their careers, stimulate the exchange of scientific information, and help build a research environment where scientists can achieve their best work. EMBO fulfils these goals by means of its programmes and activities: Fellowships, Young Investigators, Courses and Workshops, Science Policy, Global Activities and Scientific Publications.

High-throughput data as patterns on biological networks

Marc-Thorsten Hütt, PhD.

Jacobs University Bremen, Bremen, Germany

At the core of systems thinking in Biology is the concept of networks: A wide range of empirical observations – about interacting genes, interacting proteins, biochemical reactions in a cell – all can be summarized in the mathematical language of nodes and links. The challenge of Systems Biology is to relate the architecture of such networks to their biological function. Hence, network-based analyses of high-throughput (or 'omics') data are a cornerstone of Systems Biology and, more recently, Systems Medicine. The statistical task is to quantify the clustering of biological signals (e.g., significant expression changes) in a network (e.g., a metabolic network or a protein-interaction network).

Network coherences – topological indices evaluating the connectivity of subnetworks spanned by the 'omics' signal [1,2] – have been highly successful in analyzing basic principles of biological regulation [2,3], as well as in identifying patient subgroups in disease cohorts [4,5]. Underlying this comparison of data with network architectures is the idea of considering the data as self-

organized patterns on graphs.

In this talk I will briefly review this field, starting from investigations of network architectures and then moving to dynamics on networks and, finally, to the concept of 'omics' data as patterns on graphs and its application to data in biology and medicine.

References:

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Computational Cancer Pharmacogenomics

Michael Menden, PhD.

Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), ICB Institute of Computational Biology, Neuherberg, Germany

Pharmacogenomics high-throughput screens (HTS) successfully enable the identification of drug response biomarkers in cancer. In the last 5 years, multiple HTSs were published, e.g. the Genomics of Drug Sensitivity in Cancer (GDSC) project, the Cancer Cell Line Encyclopaedia (CCLE) and the Cancer Target Discovery and Development (CTD2) portal. Those screens raised high expectations towards patient stratification and advances in personalised cancer treatment; however, they did not always measure up to those expectations. This is partially due to cancer cell lines being simplified models, which do not capture the full complexity of tumours, as well as the lack of matched normal and overwhelming large genetic space of putative biomarkers. Therefore, smart computational approaches considering the limitations of HTSs and leveraging knowledge of cancer biology are necessary to successfully identify biomarkers of drug response, and thereby ultimately deliver personalised treatments. In this talk, I will present innovative computational methods to tackle the bottlenecks of drug HTS.

In vivo molecular imaging in preclinical evaluation of medical interventions

Srećko Gajović, PhD.

University of Zagreb, School of Medicine,
Croatian Institute for Brain Research, Zagreb, Croatia

Small laboratory animals serve as an essential tool to evaluate the future medical interventions in preclinical setting, before translation to clinical trials. The clinical relevance of the mouse models of the human diseases, and the ability to predict the successful candidates in the subsequent human-based trials are under constant pressure for improvements. This is mportant in particular for brain diseases, where neuroprotective or neurorestorative treatments are still elusive. Therefore, we created a platform to longitudinally monitor the molecular events in the mouse brain, which would indicate critical elements for design of medical interventions or in validation of their effects. The platform is based on the multimodal in vivo imaging of the experimental animals. This includes magnetic resonance imaging (MRI) with 7T preclinical scanner (Bruker) to visualize the morphology of the ischemic lesion, and bioluminescence imaging (BLI) by optical imager (IVIS Spectrum, Perkin Elmer) to get insight in gene activity in the living mouse brain using luciferase reporter. The ischemic brain lesion was achieved by middle cerebral aretery occlusion (MCAO) for 60 minutes, followed by filament removal and reperfusion. The affected animals were monitored during 28 days by multiple imaging sessions, functional evaluation by neurological scoring and subsequent brain analysis at the end of the experiment. The molecular activity monitored by BLI included neuroinflammaton by Tlr2 gene, neurorepair by Gap43 gene and

apoptosis by innovative approach developed in our laboratory using caged luciferin, DEVD-luciferin (VivoGlo, Promega). The analysis of Tlr2-deficient mice indicated that modified neuroinflammation could enhance the neurorepair, but it was as well accompanied by increase in apoptosis, in particular in the chronic phase of the stroke. The in vivo imaging allowed to follow animals through the time, and evaluate both morphology and molecular aspects of the lesioned brain. The modified inflammation in our model had controversial effects by enhancing neurorepair but as well the apoptosis.

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Monocyte subsets and macrophage polarization in cardiovascular disease

Johan Wojta [1,2,3], PhD.

[1] Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria;
[2] Core Facilities, Medical University of Vienna, Vienna, Austria;
[3] Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria

Human monocytes can be divided into functionally distinct subsets, based on expression of CD14 and CD16, into classical monocytes (CD14++CD16-), intermediate monocytes (CD14++CD16+) and non-classical monocytes (CD14+CD16++).1 The CD16+ subsets are associated with pathologies characterized by a chronic inflammatory state including coronary artery disease.2 We demonstrated recently that in humans with mild inflammation, the CD16+ subsets express the highest levels of inflammatory cytokines.3 Macrophages also exhibit distinct functional heterogeneity and plasticity.4 Exposure to interferon-γ and lipopolysaccharide primes macrophages towards a proinflammatory phenotype, whereas macrophages exposed to interleukin-4 (IL-4) and IL-13 are linked to tissue repair processes.4 Recently, we showed that proinflammatory human macrophages exhibit significantly higher matrix degradation activity

compared to reparative macrophages and that this reduced ability to degrade matrix by reparative macrophages is due to increased expression of plasminogen activator inhibitor-1.5 Moreover, we showed that Pro-inflammatory CD14++CD16+ human monocytes and Ly6Chigh mouse monocytes expressed the highest levels of a specific receptor for the serine protease plasminogen and bound significantly more plasminogen compared to the other respective subsets and, that in an in vivo peritonitis model significantly less Ly6Chigh monocyte recruitment was observed in Plg-RKT-/- compared with Plg-RKT+/+ mice.6 The pathophysiological roles of these subsets of monocytes and macrophages in cardiovascular disease will be discussed.

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ADME Pharmacogenomics: Clinical relevance and future directions

Matthias Schwab, PhD., M.D.

Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany

Professor and Chair of Clinical Pharmacology, University Tuebingen director of Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart and Department of Clinical of Pharmacology, University Hospital Tuebingen, Germany. He participates in and/or coordinates a number of national/international research networs and is member of several committees. His scientific interests focus on pharmacogenomics in cancer therapy particularly related to ADME genes under consideration of the application of novelomics technologies such as genomics, proteomics and metabolomics. His special interest lies in the implementation of research findings into clinical practice. Beyond that he is particularly interested in the application of new techonogies, such as pharmacological genome research. His theme on the Doctoral Scientific Conference was “ADME Pharmacogenomics: Clinical relevance and future directions”.

Computational challenges for data-driven medicine

Nataša Pržulj, PhD.

Barcelona Supercomputing Center, Barcelona, Spain

Dealing with complex "omic" data is computationally intractable. Hence, we must develop methods for extracting new biomedical knowledge from them. Our new computational methods uncover the patterns in molecular networks and in the multi-scale network organization indicative of biological function, translating the information hidden in the network topology into biomedical knowledge. Also, we introduce a versatile data fusion (integration) framework to address key challenges in precision medicine: better patient stratification, prediction of driver genes in cancer, and re-purposing of approved drugs to particular patients and patient groups. Our new methods stem from novel network science approaches coupled with machine learning, such as graph-regularized non-negative matrix tri-factorization. We utilize our new methodologies for performing other related tasks, including uncovering new cancer mechanisms and disease re-classification from modern, heterogeneous molecular level data, inferring new Gene Ontology relationships, and aligning multiple molecular networks.

Scientific achievements

Oral presentation sections were incorporated within the conference program (13 doctoral presentations were given in the form of ppt presentations and posters). The oral presentations were evaluated by a panel composed of invited plenary speakers. The three best presentations were awarded as best presentations:

- Petra Grbčić – "*Sphingolipid metabolism is dysregulated in BRAF V600E mutant colon cancer cells resistant to vemurafenib*",

The present study investigates the involvement of sphingolipid metabolism in the development of resistance to clinically relevant doses of vemurafenib in colon cancer cell line RKO harbouring BRAF V600E mutation. Developed chemoresistance was confirmed by the MTT assay showing higher IC50 values for resistant cells (29.76 μM) in comparison to parental cells (3 μM). Besides distinct morphological features, resistant cells showed higher clonogenic and migratory potential and upregulation of PI3K/Akt and RAF/MEK/ERK signalling in comparison with parental cells. Further Western blot analyses showed differential expression of several enzymes regulating the metabolism of simple sphingolipids including acid ceramidase (ASAH1), ceramide synthase 2 and 6 (Lass2 and 6), neutral sphingomyelinases 1 and 2 (NSmase1 and 2) and sphingosine kinases 1 and 2 (SphK1 and 2) between resistant and sensitive RKO cells. Pharmacological inhibition of selected sphingolipid metabolic enzymes was further evaluated either alone or in combination with vemurafenib to investigate the potential of targeting sphingolipid metabolism as a novel strategy to overcome vemurafenib resistance in colon cancer.

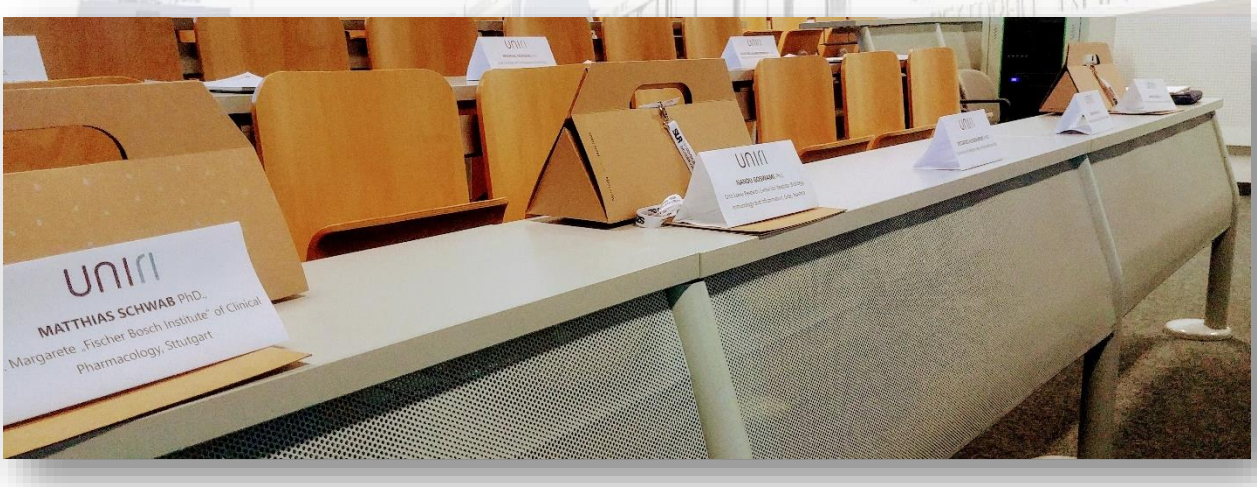
- Iva Vukelić "*Chlorogenic acid ameliorates experimental colitis in mice by suppressing signaling pathways involved in inflammatory response and apoptosis*"

Oleanolic acid (OA) is a natural triterpenoid that possesses beneficial health effects such as antioxidant, anti-inflammatory, and anti-apoptotic activities. We investigated the therapeutic effect of OA (10 and 40 mg/kg) on cisplatin (CP)-induced (13 mg/kg) nephrotoxicity. Treatment with OA 40 mg/kg once daily for 2 days, 48 h after CP-intoxication, ameliorated the increased serum markers and histological features of kidney injury. Also, CP administration increased renal expression of HO-1 and 4-HNE as well as the expression of TNF-α and NF-κB, which was reduced by OA, indicating the inhibition of oxidative stress and inflammation. Increased expression of caspase-3/9 with a concomitant increase in PARP cleavage, which suggested CP-induced apoptosis in the kidneys, was inhibited by OA. Treatment with OA also ameliorated LC3B-II and Atg5 expression induced by CP, indicating the anti-autophagic activity of OA in the kidneys. Interestingly, OA increased CP cytotoxicity in HeLa cancer cells by inducing autophagic cell death. The

chemosensitization of HeLa cells to CP suggests an additional beneficial effect of OA in cervical cancer patients due to reduced CP dosage requirements.

- Jelena Železnjak - "*MCMV-altered MHC I molecules in NK cell immunoevasion and Ly49 receptor evolution*".

Cytomegaloviruses (CMVs) efficiently downregulate MHC I molecules to avoid recognition by cytotoxic T cells. However, the absence of MHC I on the cell surface makes infected cells susceptible to NK cell killing via missing-self recognition. To prevent this, mouse CMV (MCMV) uses viral m04 protein to escort some MHC I molecules to the cell surface where they engage inhibitory Ly49 receptors and inhibit NK cell response. Here we show a new MCMV protein, MATp1, that is crucial for mentioned MHC I surface rescue by enabling proper m04/MHC I complex formation. Such MATp1/m04 altered-self MHC I molecules egress to the cell surface and bind to inhibitory Ly49 receptors stronger then MHC I alone. Consequently, NK cell activation is impaired which leads to inability of NK cells to adequately control the virus despite dramatically reduced surface levels of MHC I. Moreover, we show that these MATp1/m04 altered-self MHC I molecules are also specifically recognized by several activating Ly49 receptors indicating that MATp1 has prompted the evolution of virus-specific Ly49 receptors capable of recognizing CMV altered-self MHC I molecules.



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