BIOCHEMISTRY AND MOLECULAR GENETICS IN MEDICINE

Scientific symposium with international participation on the occasion
of the 50th anniversary of the Institute of Biochemistry and Molecular Genetics (1972-2022)
and the 30th anniversary of the Medical Centre for Molecular Biology (1992-2022)
at the Faculty of Medicine, University of Ljubljana

6.–7. 7. 2023

Large lecture hall, Faculty of Medicine, Korytkova 2, Ljubljana

Univerza v Ljubljani
Medicinska fakulteta
Inštitut za biokemijo in molekularno genetiko

PROGRAMME OVERVIEW	06. 07. 2023	07. 07. 2023
MORNING SESSION	**OPENING CEREMONY** Opening of the symposia and welcome address	
	PLENARY SESSION 1 **Enzyme research from bench to bedside** *Chair: A. Bavec, Laboratory of enzyme research*	**PLENARY SESSION 5** **Functional genomics and systems medicine** *Chair: D. Rozman, Centre for functional genomics and biochips*
	PLENARY SESSION 2 **Pharmacogenetics and Personalized medicine** *Chair: V. Dolžan, Pharmacogenetics laboratory*	**PLENARY SESSION 6** **Molecular mechanisms and biomarkers in hormone-dependent diseases** *Chair: T. Lanišnik Rižner, Laboratory for molecular basis of hormone-dependent diseases and biomarkers*
	LUNCH BREAK	LUNCH BREAK
AFTERNOON SESSION	**PLENARY SESSION 3** **Extracellular vesicles - new source of biomarkers of disease** *Chair: M. Lenassi, Laboratory for extracellular vesicle research*	**PLENARY SESSION 7** **Molecular biology and clinical research on rare diseases of the skin** *Chair: M. Liović, Medical Centre for Molecular Biology*
	POSTER SESSION	POSTER SESSION
	PLENARY SESSION 4 **Translational medical biochemistry** *Chair: K. Trebušak Podkrajšek, Laboratory for translational medical biochemistry*	**PLENARY SESSION 8** **Molecular biology in clinical research of rare diseases** *Chair: N. Debeljak, Medical Centre for Molecular Biology* **PLENARY SESSION 9** **Molecular biology in preclinical research of cancer** *Chair: P. Hudler, Medical Centre for Molecular Biology*

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Distinguished colleagues, dear friends,

The international scientific symposium Biochemistry and molecular genetics in medicine that is taking place at the Faculty of Medicine of the University of Ljubljana from 6 to 7 July 2023 commemorates the 50th anniversary of the Institute of Biochemistry and Molecular Genetics (IBKMG; 1972-2022) and the 30th anniversary of the Medical Center for Molecular Biology (MCMB; 1992-2022).

In the decades of their existence, IBKMG and MCMB have significantly imprinted on the development of the field of biochemistry and molecular biology, both in fundamental research, as well as in translational research and the transfer of new technologies into clinical practice. Members of IBKMG and MCMB also successfully collaborate with many distinguished researchers and research institutions around the world, thus enabling the introduction of new research topics and methodology. Besides research, the key mission of the institute is also education and the knowledge transfer to younger generations of undergraduate students, especially medicine and dental medicine students, PhD students of Biomedicine and Biosciences doctoral programs and postdoctoral students.

The aim of this scientific symposium is to present the expertise and the most important research achievements of the laboratories and centers within IBKMG, as well as their scientific collaborations with researchers and institutions in Slovenia and abroad.

We hope that this scientific symposium may be of interest for researchers in the field of biomedicine and biosciences, medical doctors and other health care professionals, students of medicine, dental medicine and other fields of natural sciences, as well as patients and the general public who are interested in the field of biochemistry and molecular genetics.

Kindly welcome to celebrate our anniversary with us!

Prof. Vita Dolžan, MD, PhD, spec. lab. med. gen.
Head, Institute of Biochemistry and Molecular Genetics

Scientific Committee:

Vita Dolžan (head)
Aljoša Bavec
Nataša Debeljak
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Plenary session 1:

ENZYME RESEARCH FROM BENCH TO BEDSIDE

Chair: A. Bavec, Laboratory of enzyme research

The toxicity of organophosphorus compounds can be reduced by cholinesterase activity

Zrinka Kovarik

Institute for Medical Research and Occupational Health, Zagreb, Croatia

zkovarik@imi.hr

The main action mechanism of organophosphorus compounds (OP) is the inhibition of acetylcholinesterase (AChE) that causes the accumulation of the neurotransmitter acetylcholine and excessive stimulation of nicotinic and muscarinic receptors in the central and peripheral nervous system, leading to the paralysis of cholinergic synaptic transmission. Related enzyme, butyrylcholinesterase (BChE), is not physiologically essential, but it serves as a backup for AChE and protection of synaptic AChE from man-made and naturally occurring poisons. Both enzymes should be reactivated by strong nucleophiles such as oximes to avoid severe health effects after exposure to OP. However, both inhibition and reactivation are fine-tuning chemical processes that depend on the structure of all reactants. As the interactions of AChE and BChE with ligands and inhibitors are complex, a comprehensive approach involving *in silico*, *in vitro* and *in vivo* research that could lead to the development of new drugs and reactivators AChE and BChE will be presented.

Zrinka Kovarik is permanent research adviser at the IMROH and associate professor of biochemistry and medicinal chemistry at the University of Zagreb, Faculty of Science. She received Ph.D. at the University of Zagreb. For postdoctoral study she was trained at the UCSD, USA. She is PI of more than 10 research projects, supervisor of 9 PhD thesis, co-author of >100 paper with >1700 citations and h-index 30. Since 2023 she serves as Associate Editor of *Biofactors*, a IUBMB journal.

Structural and mechanistic enzymology of acetylcholinesterase at the crossroads between Ljubljana and Trieste

Alessandro Pesaresi, Doriano Lamba

Institute of Crystallography - CNR, Italy

alessandro.pesaresi@ic.cnr.it

When it comes to the study of enzymatic mechanisms, kinetic and structural approaches go along very well together: crystallography can provide atomic resolution snapshots of the enzymatic reaction path that are only static, while kinetic studies gather the dynamic features of the process. The combination of these two techniques enables a detailed and comprehensive understanding of the inner working of enzymes and of their regulation mechanisms.

The structural biology laboratory of the CNR-Institute of Crystallography, based in Trieste within the Italian Synchrotron Facility, and the group of the Institute of Biochemistry of the University of Ljubljana led by Prof. Jure Stojan, found in their common interest for cholinesterases a fruitful ground for collaboration. Since the first interactions, which date to twenty years ago, not only have several papers been written jointly, but a productive cultural and intellectual exchange has occurred between the two parties which resulted in valuable mutual scientific enrichment.

Here, three of the more recent works which combined structural and kinetic studies of cholinesterases will be briefly reported:
1. Reactivation of aged-like MSF-inhibited acetylcholinesterase by oximes.
2. Insight into acetylcholinesterase inhibition by anti-Alzheimer's drug galantamine.
3. Kinetic and structural study on the inhibition of cholinesterases by a series of new galantamine-peptide derivatives.

Alessandro Pesaresi graduated in "Biological Sciences" at the University of Bologna (2000) and went on to earn a PhD in "Structural and Functional Genomics" at SISSA/ISAS of Trieste (2005). Since 2011 he joined the Trieste outstation of the Institute of Crystallography of the Italian Research Council. His main scientific interests are in protein crystallography, structural enzymology and enzyme kinetics.

How does paraoxonase 1 function in cerebrospinal fluid? Some findings from a study of Alzheimer's dementia patients.

Boštjan Petrič, Vita Dolžan, Marko Goličnik, Aljoša Bavec

Institute of Biochemistry and Molecular Genetics,
Faculty of Medicine, University of Ljubljana, Slovenia

Paraoxonase 1 (PON1) is an enzyme that is produced in the liver and found mainly in blood plasma as part of high-density lipoprotein (HDL) vesicles. It is known to have an antioxidative role, yet its biological substrates remain uncertain. For measuring PON1 activity *in vitro*, several artificial substrates are used, each one of them corresponding to one of PON1's three main enzymatic activities: arylesterase, lactonase, and aryldialkylphosphatase.

We assembled a group of 161 patients with suspected Alzheimer's dementia (AD), of whom 32 turned out to have mild cognitive impairment (a precursor of AD), 56 had AD and 73 had other or unspecified dementias. For each patient, we determined the genotype for 4 SNPs (rs662, rs854560, rs705379 and rs705381). For each patient's blood plasma, we measured the enzymatic parameters K_m and V_{max} for dihydrocoumarin (a lactone) and phenylacetate (an arylester) and the rate of hydrolysis for paraoxon (an aryldialkylphosphate). For each patient's cerebrospinal fluid (CSF), we measured K_m and V_{max} for phenylacetate, and for patients with AD or MCI confirmed, we also determined PON1 concentration with ELISA. We then compared the results with the patients' diagnosis and with their MMSE cognitive test score.

We found no correlation between any measure of PON1 activity or concentration or *PON1* genotype and cognitive status. The correlations between blood plasma activities for different substrates were strong, indicating that the substrates are mostly interchangeable; we also found strong correlation between specific genotypes and blood plasma K_m and/or V_{max}, which were mostly already described in literature. However, we found no significant correlation between measured parameters in blood plasma and the equivalent parameters in CSF, as well as between genotype and any parameters in CSF. Our results indicate that a seeming correlation between PON1 in blood plasma and in CSF is only present when we don't account for blood contamination of CSF. Since genotype or PON1 in blood do not seem to influence PON1 in CSF, it remains to be discovered what the main influence on PON1 in CSF is.

Plenary session 2:

PHARMACOGENETICS AND PERSONALIZED MEDICINE

Chair: V. Dolžan, Pharmacogenetics laboratory

The missing heritability in pharmacogenomics

Magnus Ingelman-Sundberg

Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

magnus.ingelman-sundberg@ki.se

The heritability of interindividual differences in drug metabolism and transport is higher than what can be explained by known genetic variants in genes encoding enzymes for drug absorption, distribution, metabolism, and excretion (ADME). Twin studies have shown that pharmacokinetic parameters for the CYP2D6 substrate metoprolol and the CYP2C9 substrate torsemide are highly heritable. However, only 30-40% of the inherited variability can be explained by known genetic polymorphisms, indicating that an important amount of heritability remains unexplained. The missing pharmacogenomic information could be due to various factors, including rare genetic variants, incomplete next-generation sequencing, the occurrence of functionally different haplotypes of alleles, the altered specificity of enzyme variants, and the global inheritance of genetic variants indirectly affecting the level of enzyme expression. Rare genetic variants in ADME genes are significant for genetic polymorphism in about 50% of genes encoding important enzymes and transporters. Analysis of the data from the UK Biobank revealed that among eight ADME genes, 6.1% of subjects carried at least one deleterious variant. Rare variants alone can explain 6-10% of the missing heritability in ADME gene pharmacogenomics.

The prediction of genetically influenced drug pharmacokinetics relies mainly on the distribution of established genetic variants, but a CYP allele could be in linkage disequilibrium with genetic variants in the vicinity of the gene locus, causing alterations in gene expression. Substrate specificity for the influence of ADME variant alleles is also important to consider. Additionally, non-ADME polymorphic genes, such as NFIB, which controls CYP2D6 expression, can influence drug

metabolism, and it is anticipated that more examples will be presented in the future, explaining a substantial amount of missing heritability.

The lecture will provide an overview of these aspects as well as looking forward to the future development of pharmacogenomics for improved use in clinical care.

Magnus Ingelman-Sundberg, PhD; BSc.Med is Professor of Molecular Toxicology and research group leader in Pharmacogenetics at the Department of Physiology and Pharmacology, Karolinska Institutet. He has more than 500 original papers and a h-factor of 95 (ISI/Clarivate) or 124 (Google Scholar). Assigned "Highly Cited Researcher" for 2014, 2015, 2016, 2017, 2021 and 2022 by Thomson & Reuters/Clarivate. He was a member of The Nobel Assembly at Karolinska Institutet 2008-2018 and a member of Editorial Advisory Boards of e.g. Trends in Pharmacological Sciences (Edit Board), Pharmacogenetics and Genomics, Pharmacogenomics, Drug Metabolism Reviews, Drug Metabolism and Disposition, Human Genomics. He has received numerous Awards, most recently the 2018 BCPT Nordic Prize in Basic and Clinical Pharmacology and Toxicology and The RT Williams award and an honorary doctorship at SydDansk University. His research focuses on genetics, polymorphism, regulation, function and toxicology of the hepatic ADME system with aims at understanding interindividual differences in drug response. Furthermore he develops novel hepatic in vitro systems for studying liver function, liver diseases and validation of hepatic drug targets.

Safety in polymedication and pharmacogenetics based precision dosing: the clinical perspective of drug drug gene interactions

Julia C. Stingl

Institute of Clinical Pharmacology,
University Hospital of RWTH Aachen, Germany

jstingl@ukaachen.de

The role of pharmacogenetic diagnostics has transformed in recent decades, initially playing a more exploratory role by explaining differences in metabolism and excretion of drugs, then investigating differences in individual response to drugs and drug safety, and now evolving into companion diagnostics for personalization of drug therapies.

With the knowledge of our pharmacogenome, new methods for genetic diagnostics also emerged, and large genomic analyses now became convenient, easy to handle, and relatively inexpensive.

Extensive studies on pharmacogenetics-guided therapy have been conducted both in Europe and other countries, demonstrating that personalization of drug therapy based on pharmacogenetic signatures can help improve drug therapy.

Different types of pharmacogenetic information are provided in drug descriptions that scale information to tailor drug therapy. Depending on the impact on individual drug prescribing, this information may be informative, actionable, or mandatory. Pharmacogenetic profiles that impact drug indications would most likely result in mandatory pharmacogenetic diagnostics prior to initiation of therapy. If pharmacokinetic parameters such as drug clearance are affected by pharmacogenetics, it is usually information that can be taken into account when adjusting the dose or selecting an alternative drug.

Pharmacogenetics is now in the application phase with the use of pharmacogenetic testing for drug therapy indication, individualized dosing, and adverse event prevention. The recently published implementation study of pharmacogenetics-assisted dosing showed that safety can be increased by about 30% when pharmacogenetics-guided therapy is used for those drugs for which actionable information is available. Interestingly, the study found that the effect of pharmacogenetically controlled therapy was apparent regardless of a patient's genotype. Post-hoc analysis of the "actionable" genotypes in this patient cohort revealed that the effect may be more pronounced in the extreme genotypes but not completely absent in the more common "normal metabolizer" genotypes.

However, drugs that are metabolized through a major pharmacogenetic pathway may also be susceptible to drug-drug interactions. We analyzed drug-drug interactions together with pharmacogenetic profiles in a cohort study of adverse drug events leading to emergency department visits (ADRED study with n=2939 cases). In this analysis, adverse drug events were associated not only with specific pharmacogenetic risk genotypes but generally with the number of medications a patient is taking. In polymedication situations, drug interactions may cause a decrease in enzyme activity leading to phenocopy of the pharmacogenetically predicted metabolizer group.

For personalized drug therapy in the future, it will be important to integrate all drug information and pharmacogenetic parameters that influence individual drug metabolism. In a recently launched European project (SafePolyMed), both pharmacogenetic information and drug interactions will be integrated using Data Science methods. In the future, individual modeling of the most appropriate dose and therapy choice will be possible, leading to personalized drug therapy with optimized benefit risk for the individual patient.

Stingl Julia, University professor in clinical pharmacology, is director of the Institute of Clinical Pharmacology at the University hospital of RWTH University Aachen, Germany. Before her move to the University RWTH Aachen, Dr. Stingl worked for seven years in drug regulation and research as vice president at the German drug regulatory authority, BfArM. Her research mostly focuses on Personalized Medicine and individual pharmacogenetic diagnostics. She pioneered the systematic development of personalized dose adjustments based upon differences in drug clearances caused

by pharmacogenetic polymorphisms promoting the way of pharmacogenetics from bench to bedside. She explored individual variability in molecular or genetic influences on drug response and also worked on characterization of the physiological role of genetic polymorphisms in cytochrome P450 enzymes such as the brain expressed CYP2D6. She integrated new methods into pharmacogenetic research such as brain imaging techniques for visualization of individual drug effects and pharmacogenetic modulation. She is involved in several European projects on pharmacogenetics and personalized medicine (UPGx, and SafePolyMed), and is currently coordinator of the EraPerMed project Artipro on Artificial Intelligence methods for the prediction of response to antidepressant drugs involving multimodal biomarkers in several European countries and Israel. She has authored more than 270 publications in peer-reviewed scientific journals, has been cited more than 10,000 times with an average citation of 30 per article and an H-index of 54 (ISI web of science, July 2022).

Biomarkers of neurodegeneration: towards personalized management of Alzheimer's and Parkinson's disease

Sara Redenšek Trampuž[1], David Vogrinc[1], Maja Trošt[2], Milica Gregorič Kramberger[2], Katja Goričar[1], Vita Dolžan[1]

[1]Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Neurology, University Medical Centre Ljubljana, Ljubljana, Slovenia

sara.redensek@mf.uni-lj.si

Alzheimer's disease (AD), which usually begins with mild cognitive impairment (MCI), and Parkinson's disease (PD), mostly presenting with motor signs and symptoms, are the most common neurodegenerative brain diseases, especially in the elderly population. Molecular pathogenesis begins years before the onset of the clinical symptoms, therefore molecular biomarkers are needed to support earlier diagnosis and prediction of the course of the disease.

Our data indicates that genetic variability in oxidative stress and neuroinflammatory pathways, as well as some well-known AD risk loci from localization and signaling pathways are associated with cerebrospinal fluid (CSF) biomarker levels and cognitive test results in patients with MCI and AD. Furthermore, expression of candidate miRNA is being assessed in blood plasma and CSF as well. Although motor deficits are the prevailing clinical symptoms in PD, these patients also experience various non-motor symptoms. Some of them are caused by dopaminergic medication. Dementia is a common non-motor symptom in advanced PD. We aimed to predict dementia

with different types of potential biomarkers, such as telomere length and single nucleotide polymorphisms. Overall, the course of the disease as well as the response to dopaminergic treatment are difficult to predict. Our group identified several different genetic polymorphisms in dopaminergic and serotonergic pathways and also pathways of neuroinflammation and oxidative stress associated with the development of motor and non-motor adverse events of dopaminergic treatment. Furthermore, we developed clinical-pharmacogenetic models for prediction of time to occurrence of motor adverse events as well as clinical-pharmacogenetic models to predict the occurrence of psychiatric adverse events in PD patients. We have also shown that two miRNAs (hsa-miR-34a and hsa-miR-132) related to AD and PD are shared with neurodegeneration as a COVID-19 sequela and may thus have a valuable potential for prediction of neurodegeneration also in COVID-19 patients. Biomarkers predicting neurodegeneration-related phenotypes would present a tremendous asset in diagnostic procedures of such complex diseases, thus further biomarker research is of utmost importance.

Sara Redenšek Trampuž is an assistant professor of Biochemistry and molecular biology at the Faculty of Medicine, University of Ljubljana. She investigates different types of diagnostic and prognostic biomarkers and biomarkers of treatment response of neurodegenerative brain diseases, especially Parkinson's disease, as well as cancer, particularly breast cancer. She aims to develop clinical-pharmacogenetic models combining different types of molecular biomarkers and clinical characteristics of patients to predict specific phenotypes in order to facilitate personalized management of the disease.

Plenary session 3:

EXTRACELLULAR VESICLES - NEW SOURCE OF BIOMARKERS OF DISEASE

Chair: M. Lenassi, Laboratory for extracellular vesicle research

Stem cell-derived extracellular vesicles as therapeutic and diagnostic tools in kidney diseases

Benedetta Bussolati

Dept. of Molecular Biotechnology and Health Sciences, University of Torino, Italy

Benedetta.bussolati@unito.it

Increasing evidence has now established a central role of EVs in renal physiology and pathology. EVs mediate cross-talk between glomerular and tubular cells, and among different tubular segments, and subsequently, the molecular profile of EVs collected in the urine might be exploited for diagnostic and prognostic purposes. We recently identified an EV-based signature, reflecting repairing/regenerative features of the graft, that correlated with eGFR, creatinine and proteinuria, and predicted patient outcome after kidney transplant. On the other hand, several reports provide convincing evidence of the regenerative potential of EVs released by stem cells and, in particular, mesenchymal stromal cells (MSCs) in different kidney injury models. In addition, other stem cell-bioproducts, such as mitochondria, are of increasing interest for renal regeneration. Strategies to further increase EV activity are in study, engineering their cargo while exploiting their biocompatibility. In particular, Klotho, a renal hormone involved in the control of ageing and tissue fibrosis, is present in urinary EVs and mediates kidney protection.

Benedetta Bussolati obtained her medical degree at the University of Torino, Italy and her PhD on Physiopathology of the Renal Insufficiency at the University of Parma. She currently holds the position of Full Professor of Laboratory Medicine at the University of Torino and director of a Research group at the Molecular Biotechnology Centre. She acquired a strong background on renal pathophysiology, on angiogenesis and on the mechanisms of renal damage and progression.

In addition, she has extensive experience in studies of stem cell biology and regenerative medicine that include characterization of various stem cell types and their potential use for tissue regeneration. Actual interests focus on the role of stem cell-derived extracellular vesicles (EVs) in regenerative medicine as therapeutic tool, to induce cell reprogramming and modulate angiogenesis and renal repair. She is deeply involved in the EV community, being President of the Italian Society of Extracellular Vesicles (EVIta), Treasurer of the ISEV and Member of the Task forces on Urinary EVs and on Regulatory Affairs and Clinical Use of EV-based Therapeutics.

Molecular biomarkers in kidney transplantation

Miha Arnol [1, 2]

[1]Department of Nephrology, Division of Internal Medicine, University Medical Center Ljubljana, Ljubljana, Slovenia

[2]Department of Internal Medicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

miha.arnol@kclj.si

Despite progress in transplant medicine, long-term outcomes after kidney transplantation continue to fall short of ideal, with approximately 50% of kidneys from deceased donors still functioning 10 years after transplantation. Allograft rejection remains the major risk factor for transplant failure and is becoming a major cause of end-stage kidney disease. Post-transplant kidney allograft monitoring includes the surveillance of serum creatinine, glomerular filtration rate, and proteinuria, but these biomarkers are nonspecific and have low sensitivity, thereby missing subclinical allograft changes. Histopathological characterization of kidney biopsies remains the standard for diagnosis of kidney allograft injury, but the procedure is invasive, complications may occur; while sampling errors may jeopardize their diagnostic usefulness. New non-invasive biomarkers are thus needed that will allow frequent monitoring and earlier detection of kidney allograft injury.

Recent developments in molecular techniques have advanced the identification of new biomarkers, including molecular microscope diagnostic system (MMDx), donor-derived cell-free DNA (dd-cfDNA), extracellular vesicles (EVs) and EV-bound DNA (evDNA), which have the potential to assist clinicians in therapeutic decision making and predict transplant outcome. MMDx uses genome-wide microarrays for transcriptomic profiling of allograft biopsy, and interprets the results using predefined machine learning-based algorithms and comparison to a reference set of previously characterized biopsies. Thereby MMDx circumvents the limitations of conventional

histologic assessment, but it still requires kidney biopsy. Transplant recipient's plasma levels > 1% of dd-cfDNA are indicative of kidney allograft rejection, as supported by the pivotal DART study. Rejection namely entails injury in the allograft, leading to increased release of dd-cfDNA into the bloodstream. Recently, we developed a digital droplet PCR-based approach to quantify the dd-cfDNA fraction in plasma of kidney transplant recipients by measuring 6 single nucleotide polymorphisms. EVs in urine are potentially a powerful tool for liquid biopsy and a noninvasive biomarker for kidney transplant rejection. Urinary EVs contain proteins and nucleic acids from the parent cells and reflect the biological function of the parent cells in the kidney, including immune cells. Urinary evDNA represents up to 29.2 % cfDNA, but is less fragmented. ddDNA is detectable in uEV samples of kidney allograft recipients, but its quantity is highly variable. Several evDNA characteristics correlated with clinical and histological parameters, supporting further studies.

These new tools will hopefully translate into a personalized therapy that would reduce over-immunosuppression in patients at low risk of rejection and allow early intervention in patients at high risk of rejection. Such precision medicine could reduce patient morbidity and increase long-term allograft survival.

Prof. **Miha Arnol,** MD, PhD, is one of the leading experts in nephrology and kidney transplant medicine in Slovenia. He graduated from the Faculty of Medicine at the University of Ljubljana in 2000. After receiving his master's degree in 2002, he continued his clinical training as an internal medicine resident and then as a nephrology fellow. In 2007, the last year of his clinical training, he focused on transplant medicine and spent a year as a Visiting Fellow at Oregon Health & Science University, Portland, USA. In 2008, he completed his fellowship and earned a PhD in biomedical sciences. Today, he is the Head of the Department of Nephrology and Medical Director of the Centre for Kidney Transplantation at the University Medical Centre Ljubljana. His research interests include cardiovascular risk reduction in kidney patients, optimization of immunosuppression in transplant recipients, and noninvasive clinical and molecular biomarkers of kidney allograft injury.

Variability of blood-derived extracellular vesicles in healthy humans

Marija Holcar[1], Ivica Marić[2,3], Tobias Tertel[4], Katja Goričar[1], Nina Mavec[1], Bernd Giebel[4] and Metka Lenassi[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Immunohaematology, Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

[3]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[4]Institute for Transfusion Medicine, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

marija.holcar@mf.uni-lj.si

Extracellular vesicles (EVs) are phospholipid bilayer-enclosed particles, released from all cells into body fluids, reflecting their cell of origin. Blood is the most frequently used biofluid for EV biomarker research, yet little is known about plasma EVs (pEVs) in healthy humans, limiting the biomarker data interpretation. Here, we analysed pEVs in healthy adults and the influence of clinical, demographic, and lifestyle parameters on interindividual variability.

Blood and clinical (height, weight, blood type, menopause), demographic (sex, age), and lifestyle (exercise, smoking) data were collected from 208 healthy blood donors (ethically approved, informed consent). A complete blood count (CBC) was performed in the blood, and CRP and insulin were analysed from blood sera. Imaging flow cytometry was used to phenotype EVs in plasma. pEVs were enriched from plasma (ultracentrifugation on a 20% sucrose cushion, sUC), then analyzed for concentration (NTA) and surface proteins (MACSPlex). The variability in pEV characteristics was assessed, and correlation with individuals' parameters was analyzed.

Subjects, evenly distributed by sex and age, were healthy with normal CBC, insulin, and CRP levels. The average concentration of EV types in plasma was 2.3*107/mL, but there was high interindividual variability within the same EV type and between the median of different EVs. After enrichment of EVs from plasma with sUC (median 5.7*109 particles/mL plasma), mostly blood-cell and endothelial markers were detected on tetraspanin+ EVs, with the highest signal for platelet-derived EVs. The concentration of pEVs correlated with sex, smoking, menopausal status and blood type of study subjects. Smoking correlated with the increase in enriched tetraspanin+ EVs of platelet and endothelial origin and with a decrease in EVs of leukocyte origin.

There is a high interindividual variability in concentrations of plasma EVs in healthy adults, which also correlates with some patients' characteristics.

Marija Holcar is a postdoctoral researcher at the Faculty of Medicine, University of Ljubljana. She is involved in various studies of extracellular vesicles as new biomarkers of disease. Currently, she is investigating the impact of interpersonal differences in healthy subjects on their blood EV characteristics. Findings may greatly aid biomarker discovery.

Predicting surgical resectability of pancreatic cancer based on plasma extracellular vesicle characteristics

David Badovinac[1,3], Katja Goričar[2], Hana Zavrtanik[1], Teja Lavrin[2], Vita Dolžan[2], Metka Lenassi[2], Aleš Tomažič[1,3]

[1]Department of Abdominal Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia

[2]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[3]Department of Surgery, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

david.badovinac@kclj.si

Pancreatic ductal adenocarcinoma (PDAC) is among cancers with worst prognosis. In resectable disease, upfront resection is indicated, while in borderline cases neoadjuvant therapy is initiated. However, even some patients with resectable PDAC could benefit from systemic therapy before surgery due to biology of their disease. Better preoperative characterisation of these patients would aid in their treatment optimisation and liquid biopsy with extracellular vesicles (EV) is promising in this regard.

83 patients undergoing surgery of presumably resectable PDAC were enrolled in our prospective study. Patient data and plasma samples were collected pre- and intraoperatively, then again one, six and 12 months postoperatively. Small plasma EV concentration and size were determined by nanoparticle-tracking analysis.

Significantly higher small plasma EV concentrations before surgery were found in patients undergoing resection compared to those without PDAC resection. Furthermore, small plasma

EV concentration before surgery was significantly higher in patients that underwent radical (R0) resection than in patients with micro- or macroscopic tumour remnant (R1 or R2 resection). Median follow-up was 25.7 months and OS 11.3 months. Association of OS with certain cutoff values of small EV characteristics were determined based on patients' resection status.

Plasma EV concentration before surgery could predict PDAC resectability and it correlated with radicality of tumour resection. Similar studies are needed to further evaluate the clinical value of liquid biopsy EV characteristics, however, such findings could help optimise patient treatment approach and aid in evaluation of the resected specimen.

Plenary session 4:

TRANSLATIONAL MEDICAL BIOCHEMISTRY

Chair: K. Trebušak Podkrajšek, Laboratory for translational medical biochemistry

Fabry nephropathy in focus: diagnostics and management

Bojan Vujkovac

Centre for Fabry Disease, General Hospital Slovenj Gradec, Slovenj Gradec, Slovenia

bojan.vujkovac@sb-sg.si

Fabry nephropathy is a very common complication of Fabry disease, a rare genetic disorder caused by mutations in the GLA gene leading to deficient activity of the enzyme alpha-galactosidase A. This deficiency results in the accumulation of globotriaosylceramide (Gb3) and related glycosphingolipids in various organs, including the kidneys. Fabry nephropathy is characterized by progressive renal dysfunction and is a significant cause of end-stage renal disease (ESRD) in affected individuals.

The diagnostic process involves a thorough clinical evaluation, family history assessment, and laboratory investigations. Enzyme activity assays and genetic testing play a crucial role in confirming the diagnosis and identifying disease-causing mutations. Additionally, kidney biopsy may be performed to assess the extent of renal involvement and guide treatment decisions.

Management of Fabry nephropathy focuses on slowing disease progression, alleviating symptoms, and preventing complications. Enzyme replacement therapy (ERT) is the primary treatment approach, which involves intravenous administration of recombinant alpha-galactosidase A. ERT has demonstrated efficacy only if started early, before irreversible changes develop. In recent years, alternative treatment options, such as chaperone therapy and gene therapy, have shown promise in potentially providing long-term benefits. Regular monitoring of renal function, proteinuria, and cardiac parameters is essential to assess disease progression and guide treatment decisions. Multidisciplinary care of different is recommended to provide comprehensive management for individuals with Fabry disease.

In conclusion, early diagnosis and intervention are crucial in treating Fabry nephropathy, therefore ongoing research efforts are focusing to identify new biomarkers of early kidney involvement

and patients at risk for disease progression. In addition, further studies are needed to explore the potential of emerging therapies in achieving better outcomes for affected individuals.

Bojan Vujkovac completed his undergraduate studies at the University of Ljubljana, Slovenia, and his residency at the University Clinical Center in Ljubljana. He works at the General Hospital Slovenj Gradec, where he founded and directs a national centre for Fabry disease. His main scientific interests include coagulation disturbances in patients receiving haemodialysis; Fabry disease diagnosis, treatment and management; and chronic kidney disease, epidemiology and progression factors. He is currently principal investigator in numerous studies and has authored over 150 original and review articles.

Biomarkers in Fabry nephropathy

Albina Nowak

University Hospital Zurich, Zurich, Switzerland

albina.nowak@usz.ch

Fabry disease (FD) is a rare X-linked lysosomal storage disease with a deficiency of α-galactosidase A leading to progressive sphingolipid accumulation in different organs, among them heart and kidney. Deposition of undegraded glycosphingolipid substrate occur in all kidney cells and structures including podocytes, mesangial, tubular, interstitial and vascular endothelial cells. The aim of this overview is to provide a summary of nephropathy biomarkers in Fabry patients that are used for the diagnosis and disease monitoring. Fabry-unspecific routine biomarkers such as glomerular filtration rate and the quantity of proteinuria and albuminuria are associated with late signs of kidney damage. Thus, there is a need to develop new biomarkers associated with early stages of kidney damage that would enable early diagnosis, in order to timely initiate the disease-specific treatment to avoid irreversible kidney damage. Moreover, appropriate biomarkers for therapy-monitoring are still an unmet clinical need.

Albina Nowak, MD, PhD is senior clinician, researcher and lecturer at the University Hospital Zurich, Department of Endocrinology.
Her main interest is understanding the disease mechanisms underlying inborn errors of metabolism and particularly Fabry disease. She has >10 years of experience in treating patients

with Fabry disease. She is nephrologist, specialist of general internal and pharmaceutical medicine. Her undergraduate training residency was at the University Hospitals Bern, Basel and Zurich Switzerland as well as at the Royal Sussex Hospital Brighton, UK. Her clinical and scientific research interests also include vitamin D, iron and mineral metabolism. She is currently a Principal Investigator of clinical trials and has authored numerous original research publications and review articles. She is reviewer of several international scientific journals.

Genetic and epigenetic biomarkers for the development and progression of Fabry nephropathy

Tina Levstek[1,2], Bojan Vujkovac[3], Katarina Trebušak Podkrajšek[1,2]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

[3]Centre for Fabry Disease, General Hospital Slovenj Gradec, Slovenj Gradec, Slovenia

tina.levstek@mf.uni-lj.si

Fabry nephropathy contributes significantly to the morbidity and mortality associated with Fabry disease, as patients with Fabry nephropathy are also at higher risk for dysfunction in other organ systems. Current biomarkers are associated with late signs of kidney damage. Therefore, there is a need to identify non-invasive biomarkers associated with early signs of kidney damage that allow early diagnosis, which is crucial for effective treatment and prevention of irreversible and life-threatening complications. We aimed to investigate genetic and epigenetic biomarkers that may be associated with the development and progression of Fabry nephropathy.

In the first part of the study, we examined the expression of microRNAs (miRNAs) isolated from urinary extracellular vesicles. First, we determined the expression of 87 miRNAs and then confirmed the expression of selected miRNAs on chronological samples. miR-21-5p and miR-222-3p were upregulated in patients with stable renal function and in patients with progressive nephropathy compared with control subjects. In addition, miR-10b-5p, miR-30a-5p, and miR-204-5p were downregulated in patients with progressive nephropathy compared with control subjects, but only the expression of miR-204-5p decreased significantly in chronological samples.

Functional enrichment analysis revealed that dysregulated miRNAs are associated with various pathophysiological pathways in the kidney.

In the second part of the study, we investigated the dynamics of leucocyte telomere length (LTL) over a 10-year period and its association with late complications in Fabry patients. LTL was shorter in Fabry patients compared with control subjects, but the difference was not statistically significant. Further analysis showed that male Fabry patients had significantly shorter LTL compared with control subjects, whereas no significant difference in LTL was found in females. In chronological samples, LTL shortened significantly over time. On the other hand, the presence of late complications did not significantly alter LTL shortening.

Identification of new biomarkers and related pathways may provide an opportunity for early detection of patients at risk and consequently reduce or even prevent the development of Fabry nephropathy.

Tina Levstek is a PhD student in biomedicine at the Faculty of Medicine. She obtained her master's degree in laboratory biomedicine at the Faculty of Pharmacy, University of Ljubljana. She then completed an internship at the Institute of Oncology in Ljubljana and passed the professional examination of the Ministry of Health. She is currently employed as a young researcher at the Institute of Biochemistry and Molecular Genetics at the Faculty of Medicine. Her PhD thesis is focused on the identification of genetic, epigenetic, and biochemical biomarkers for nephropathy in patients with Fabry disease.

Plenary session 5:

FUNCTIONAL GENOMICS AND SYSTEMS MEDICINE

Chair: D. Rozman, Centre for functional genomics and Biochips

(Nano)Technologies empowering understanding, prognosis and treatment of liver diseases

Ruchi Bansal

Translational Liver Research, Department of Medical Cell BioPhysics, Technical Medical Centre, Faculty of Science and Technology, University of Twente, Enschede, The Netherlands

r.bansal@utwente.nl

Non-alcoholic steatohepatitis (NASH) associated with obesity and diabetes is a growing burden worldwide affecting more than 25% world population and is the most-common indication for liver Transplantation. NASH in a complex disease involving several cell types and mechanisms which have been unraveled in the past decade, revealing novel diagnostic and therapeutic targets. Despite tremendous efforts, NASH remains a challenging disease to diagnose (lack of early and predictive biomarkers) and treat with many drugs failed in phase 3 clinical trial. In my team, we tackle NASH using the state-of-the-technologies. We have developed technologies-modalities-nanotherapeutics enabling understanding of liver diseases, biomarkers identification, and treatment to advance liver research and to impact patient management. We have developed single cell phenomics platform with the aim to unravel communication networks and to identify the key therapeutic cellular and molecular targets. Using click-chemistry and magnetic beads, we have developed extracellular vesicles (EVs) capture technology to enrich EVs and study their content to identify diagnostic and prognostic biomarkers. We have expanded our research into (liver) cancer where we isolate and characterize circulating tumor cells and fibroblasts derived from liquid biopsy and correlate these with disease prognosis. Using in silico modeling approaches, we have engineered therapeutic peptides that showed promising therapeutic effects in vitro and in vivo. We have also worked with several nanocarrier systems including targeting peptides, polymersomes, super-paramagnetic ironoxide nanoparticles, polylactic-co-glycolic acid (PLGA) nanoparticles, liposomes, and lipid nanoparticles to deliver biologicals including therapeutic

proteins, peptides, bioactive lipids and/or microRNAs selectively to macrophages and/or liver myofibroblasts to inhibit liver inflammation and fibrosis respectively. In future, (nano)technologies will enable unraveling the disease mechanisms, personalized diagnosis, prognosis, and treatment.

Dr. **Ruchi Bansal** is an Associate Professor at the University of Twente, The Netherlands. She is a group leader of Translational Liver Research and supervises 2 Postdocs, 4 PhDs, and several students. She has received several awards, honors, grants and have published several publications and is recognized as the University of Twente featured scientist.

In search for biomarkers of neuropsychiatric disorders: new perspectives on early diagnosis and personalized therapy

Dubravka Švob Štrac[1], Alja Videtič Paska[2], Matea Nikolac Perković[1], Katarina Kouter[4], Nela Pivac[1], Suzana Uzun[4]

[1]Laboratory for Molecular Neuropsychiatry, Division of Molecular Medicine, Ruder Boskovic Institute, Zagreb, Croatia

[2]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[3]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[4]Department for Biological Psychiatry and Psychogeriatrics, University Psychiatric Hospital Vrapce, Zagreb, Croatia

dsvob@irb.hr

Neuropsychiatric disorders are frequent and heterogeneous multifactorial mental disorders that carry a high social impact, enormous public health costs, and various comorbidities. Since their complex neurobiological basis is still not clear, novel integrative approaches, based on the systems biology and focused on better understanding of biological underpinnings might offer new specific biomarkers. During long-term collaboration between Faculty of Medicine, University

of Ljubljana and Rudjer Boskovic Institute in Zagreb, we aimed to identify potential biomarkers, which might be useful for an early diagnosis and tailored, personalized approach to therapy of various neuropsychiatric disorders, such as suicidal behavior, Alzheimer's disease, and depression. This lecture will give an overview of our several previous and current joint collaborative projects, focusing on genetic (target gene polymorphisms) and epigenetic (DNA methylation, miRNA) alternations involved in the regulation of gene expression and resulting protein concentration. Moreover, altered metabolites as the final products of genetically controlled biochemical pathways, which are highly influenced by both external and internal changes, might represent potential metabolomic biomarkers that could facilitate early diagnosis and identification of novel treatment strategies. Specifically, our research aims to offer easily obtainable and non-invasive biomarkers, such as those obtained from human peripheral blood mononuclear cells, plasma and circulating extracellular vesicles. Such biomarkers are of the utmost importance for the populations of both Slovenia and Croatia, but also for neuropsychiatric patients in EU and all around the world. Additionally, well-established international collaboration between our research groups allows acquisition of new knowledge from different research models and methodological approaches, as well as further training and scientific development, especially for young scientists.

Assoc. prof. **Dubravka Svob Strac,** PhD, works as a Head of the Laboratory for Mol. Neuropsychiatry, Department of Mol. Medicine, Ruder Boskovic Institute, Zagreb. She received her MSc and PhD degrees in Mol. Biology at the Faculty of Science, University of Zagreb, and MSc degree in Project Management at Baltazar University, Zapresic. In addition to scientific work (90 papers, 22 book chapters, 1967 citations; h-index 24, 30 projects), she teaches at the Universities of Zagreb, Rijeka and Osijek (mentor of 18 MSc and 5 PhD students). She received several stipends and awards, including awards from Ruder Boskovic Institute, Department of Biotechnology Rijeka, and Croatian National Award.

Slovenian Reference Genome Project

Tadej Battelino[1,2], Jernej Kovač[2,3]

[1]Clinical Department of Endocrinology, Diabetes and Metabolic Disorders, University Children's Hospital, UMC Ljubljana, Ljubljana, Slovenia

[2]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[3]Clinical Institute of Special Laboratory Diagnostics, Ljubljana, Slovenia

tadej.battelino@mf.uni-lj.si

Embracing the era of genomics, the Slovenian Reference Genome Project (SRGP) seeks to decode the genetic blueprint of Slovenia's population, contributing to the broader 1+ Million Genomes (1+MG) and Genome of Europe initiatives. The SRGP aims to sequence at least 1000 genomes from healthy Slovenian volunteers, using an innovative combination of short and long reads sequencing to probe into the unexplored "dark and hard-to-resolve genome regions". By understanding the genomic diversity of a healthy population, the project aspires to identify rare diseases faster and with greater precision, potentially illuminating new diagnostic markers and genetic causes of diseases. The project's implications extend beyond the scientific realm, offering the promise of drastically reducing the journey towards a correct diagnosis for rare diseases, reducing false positives and relieving the burden on the healthcare system. Key Slovenian partners, such as the University Medical Centre Ljubljana (UMCL), the Faculty of Medicine at the University of Ljubljana (ULMF), and the Faculty of Electrical Engineering and Computer Science at the University of Maribor (UMFERI) collaborate to integrate the SRGP into the broader EU Genomic Data Infrastructure (GDI) project. As a beacon of ambition and innovation, the SRGP signals a new dawn in genetic exploration, with Slovenia at the forefront of this monumental stride in human genomics. The preliminary analysis of the first ~10% of the genomes will be discussed, highlighting the challenges encountered and the solutions implemented.

Tadej Battelino, a respected pediatric endocrinologist, researcher, and professor, holds key roles at UMC Ljubljana and the University of Ljubljana. He's a PI on numerous diabetes and endocrinology research projects, with 300+ international papers. His work has earned him awards such as the ISPAD Achievement Award (2020). Active in various professional associations, he's internationally recognized authority in pediatric endocrinology.

Jernej Kovač, a dedicated biochemist and geneticist, excels in research on rare diseases and pediatric genetics. His expertise spans genomics, epigenetics, and NGS technology. A published author with >50 international papers, Jernej actively contributes to national and European initiatives. His work drives advancements in personalized medicine, rare disease diagnostics and the development of a comprehensive national genomic database.

Biobanking in Slovenia

Vladka Čurin Šerbec[1], Maja Černilec[1], Natalija Lampreht[1], Valerija Kovač[1], Melita Gracar[1], Marjana Šprohar[1], Jugoslav Njenjić, Dražen Franić[1], Slavica Stanišić[1,] Tadeja Režen[2], Katarina Nahtigal[2]

[1]Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

[2]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

vladka.curin@ztm.si

Biobanks are repositories of human biological material that responsibly receive, store and distribute biological samples and relevant clinical data for research, study purposes and for clinical use. They are a complex system of processes through which unique and valuable samples are managed. The results of our recent research which has been done in the scope of the Interreg project C3B, where the Blood Transfusion Centre of Slovenia and the University of Ljubljana, Faculty of Medicine were partners, will be presented. The goals of the project were the following: 1) to review the situation in the field of biobanks in Slovenia - to identify the institutions that are already collecting or intend to collect human biological material or already have a human biological material bank (market analyses), 2) to check the interest in biological samples of healthy donors for research, education and clinical studies and to adapt to customers, 3) to transfer good practices from the Blood Transfusion Centre of Slovenia and the biobank in Venice to all project partners and to interested institutions in both countries, 4) to arrange and organize the collection, storage and use of samples of blood from healthy donors - a pilot model, 5) to prepare the prerequisites for the establishment and operation of a national biobank of the blood samples of healthy controls - based on a pilot model, our experience and good practices in blood banking and according to the "Strategy of the Blood Transfusion Centre of Slovenia", 6) stakeholder education and promotion of the biobanks. The project C3B was chosen twice as one of the best projects in the programme - in the field of management and in the field of health.

Professor **Vladka Čurin Šerbec** led several national and international projects. Her research area covers primarily antibodies and their use in research, diagnostics and therapy. She was granted international patents, for which non-exclusive licenses were sold and has documented technology transfer. She was a part of the teams that established a graduate study of Biochemistry and postgraduate study of Biomedicine at the University of Ljubljana and served as a mentor to many PhD and graduate students who were awarded for their research work. She received the Zois Award for inventions and technological achievements.

Plenary session 6:

MOLECULAR MECHANISMS AND BIOMARKERS IN HORMONE-DEPENDENT DISEASES

Chair: T. Lanišnik Rižner, Laboratory for molecular basis of hormone-dependent diseases and biomarkers

Metabolomics studies of mechanisms and biomarkers of complex human diseases

Jerzy Adamski,[1, 2, 3, 4]

[1]Institute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg, Germany

[2]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

[3]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[4]Metaron Diagnostics i.G., Neuherberg, Germany

info.adamski@gmx.org

Human diseases like obesity with manifested cardiovascular disease (CVD) or endometriosis lack in-depth understanding on mechanistic background of disease progression. They further lack reliable biomarkers supporting early diagnostics or therapies. Metabolomics is very instrumental in research on these aims.

In a mechanistic study we recently discovered that leptin sensitivity is regulated by 17beta-estradiol (Cell Metabolism, 2023). In the mouse model we have demonstrated that the 17beta-estradiol acts in hypothalamus by accruing Pomc neurons action through Cited-ERalpha-Stat3 pathway. Loss (knockout) of Cited1 exacerbates diet induced obesity in female mice. The Pomc neurons integrate endocrine inputs from gonadal and adipose axes via Cited1, thereby contributing to the sexual dimorphism in diet-induced obesity. The neuroendocrine mechanism represents a mode by which melanocortin neurons integrate energy stores with reproductive signals for metabolic adaptation in diet-induced obesity. This observation contributes to the understanding why after menopause women are no longer protected by 17beta-estradiol from developing metabolic diseases in comparison to man.

Metabolomics has been applied in another study to identify biomarkers of endometriosis. After non-invasive analyses of plasma metabolites (lipids, acylcarnitines, amino acids, biogenic amines) of multicenter cohort of 500 patients we were able to discover unique signatures

specific of different types of endometriosis. The advantage of metabolomics was demonstrated by the fact that the specific signatures are independent of menstrual cycle, BMI or nutrition. Novel algorithms (patent pending) involving metabolite ratios have been found superior in discriminating endometriosis progression. The signatures are further revealing better AUC for diagnostics in comparison to other invasive methods employed now or to use of reference values for diagnostic decision-making process.

Jerzy Adamski is a medicinal scientists and worldwide renowned expert in endocrinology and metabolomics. He has been working in the field of endocrine-related cancers and frequent human diseases like diabetes for 20 years. He published 463 papers (e.g. Nature Genetics, 2010; Nature 2011 and 2020; Nature Reviews in Nephrology, 2017; Cell, 2018, 2021 and 2023, Nature Biotechnology 2023) holds several patents and has H-index of 78. He acts as an Editor-in-Chief for J. Steroid Biochemistry and Molecular Biology (IF 5.03). He developed prototypes for early diagnosis of endometriosis, diabetes type 2, kidney dysfunction and cardiovascular complications. Recently he edited and co-authored a book "Metabolomics for biomedical research" (ISBN: 978-0-12-812784-1) published in 2020. He recently co-founded a biotech company Metaron Diagnostics where he acts as CSO. At Metaron Diagnostics he implements validated assays for diagnostics of complex human diseases by metabolomics. His further interests lie in networking and team building for the efficient problem solutions in biomedical discovery and applications in personalized medicine.

Biosynthesis, metabolism and bioactivity of 11-oxygenated androgens

Karl Storbeck

Department of Biochemistry, Stellenbosch University, Stellenbosch, South Africa

storbeck@sun.ac.za

Until recently, the prevailing paradigm in endocrinology was that testosterone and 5α-dihydrotestosterone (DHT) are the only potent androgens relevant to human physiology. However, the discovery of adrenal-derived 11-oxygenated androgens, notably 11-ketotestosterone (11KT), has challenged this established view, leading to a reassessment of the androgen pool. In the last decade, 11-oxygenated androgens have been implicated in several disease states, including polycystic ovarian syndrome, congenital adrenal hyperplasia, and castration-resistant prostate cancer. This presentation will provide an overview of the biosynthesis, peripheral metabolism and bioactivity of 11-oxygenated androgens. Using a combination of *in vitro* assays and *in silico* modelling as well, as *ex vivo* and *in vivo* data, we show that 11-oxygenated androgen biosynthesis and peripheral metabolism

is different to that of classic androgens and involves enzymes traditionally only considered in regulating glucocorticoid activity. The clinical implications of the overlap between 11-oxygenated androgen and glucocorticoids will be presented. Other differences between 11-oxygenated and classic androgens, including their conversion to estrogens, will also be highlighted.

Karl Storbeck (PhD) is an Associate Professor at the Department of Biochemistry, Stellenbosch University and an Honorary Senior Research Fellow at the Institute of Metabolism and Systems research, University of Birmingham, UK. He was awarded his PhD in Biochemistry by Stellenbosch University in 2008 and was subsequently appointed to faculty in 2012. His research interests are focused on investigating the biosynthesis and metabolism of 11-oxygenated androgens and understanding the role of these androgens in health and disease.

11-Oxyandrogens in endometrial and ovarian cancers

Marija Gjorgoska[1], Lea Sturm[1], Angela E. Taylor[2], Spela Smrkolj[3,4], Tea Lanisnik Rizner[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia

[2]Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, United Kingdom

[3]Department of Gynaecology and Obstetrics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[4]Division of Gynaecology and Obstetrics, University Medical Centre, Ljubljana, Slovenia

marija.gjorgoska@mf.uni-lj.si, tea.lanisnik-rizner@mf.uni-lj.si

11-oxyandrogen hormones are a unique set of androgen metabolites that have not yet been studied in the context of endometrial (EC) and ovarian cancers (OC). In this study, we aimed to determine the systemic levels of 11-oxyandrogens in a cohort of EC patients and tumor-free women and investigate their associations with EC risk and clinical parameters. Additionally, we examined a panel of EC and OC cell lines and steroid precursors to determine if endometrial and ovarian tumors could potentially contribute to the 11-oxyandrogen pool.

A cohort of 70 tumor-free women and 62 EC patients was enrolled for multi-steroid profiling using liquid chromatography tandem mass spectrometry (LC-MS/MS). In vitro experiments were

conducted using EC and high-grade serous OC (HGSOC) cell lines, along with corresponding control lines, to examine androgen metabolism by LC-MS/MS.

EC patients exhibited elevated systemic levels of 11β-hydroxy-androgens compared to controls. Increasing levels of the latter were associated with higher EC risk, while higher 11-keto-androstenedione levels demonstrated a protective effect against myometrial invasion, a negative prognostic factor in EC. Altered 11β-OH-androgen levels were not attributed to intra-tumoral formation from classic androgen precursors. Furthermore, both EC and HGSOC cell lines exhibited a notable metabolic profile enriched with androgen receptor-activating 11-oxyandrogens, derived from metabolic processing of 11-oxyandrogen precursors.

Our findings highlight the dysregulation of 11-oxyandrogen levels in EC patients compared to tumor-free women. Moreover, EC and HGSOC cell lines exhibit a unique metabolic profile characterized by the production of bioactive 11-oxyandrogens. Ongoing profiling of 11-oxyandrogen levels in HGSOC patients holds promise for further understanding their role in OC.

Study supported by J3-2535 grant to T.L.R. from the Slovenian Research Agency and a Practical Skills Grant to M.G. from the Society of Endocrinology.

Marija Gjorgoska, PhD candidate in Biomedicine, field Biochemistry and Molecular Genetics from University of Ljubljana, Ljubljana, Slovenia.

Models including angiogenic factors as candidate biomarkers of endometrial cancer

Luka Roškar[1,2], Marko Kokol [3,4], Irena Roškar[5], Maja Pušić[5], Teja Klančič[5], Tamara Knific[5], Renata Pavlič[5], Špela Smrkolj[1,6], Tea Lanišnik Rižner[5]

[1]Department of Gynaecology and Obstetrics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia;

[2]Division of Gynaecology and Obstetrics, General Hospital Murska Sobota, Murska Sobota, Slovenia;

[3]Faculty of Electrical Engineering and Computer Science, University of Maribor, Maribor, Slovenia;

[4]Semantika Research, Semantika d.o.o., Maribor, Slovenia;

[5]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia;

[6]Division of Gynaecology and Obstetrics, University Medical Centre, Ljubljana, Slovenia.

e-mail: luka.roskar@outlook.com

Endometrial cancer (EC) is a prevalent gynaecological cancer whose growth and spread are facilitated by angiogenic switch in the early stages of cancerogenesis. This occurs through the release of pro-angiogenic and suppression of anti-angiogenic factors (AFs). Screening AFs as biomarkers in patients' plasma and the tumour tissue might generate new knowledge on cancerogenesis and enable more precise diagnosis and a tailored treatment. Our investigation of angiogenesis in EC was divided into three phases. The first case-control study included 76 postmenopausal women, and 37 AFs were measured as potential biomarkers for EC. The plasma levels of sTie-2 and G-CSF were significantly lower whereas the plasma levels of leptin were significantly higher in EC patients compared to control patients. In the validation study, we included 202 patients, 91 EC patients and 111 patients with benign diseases. We measured plasma concentrations of six selected AF – leptin, IL-8, sTie-2, follistatin, neuropilin-1, and G-CSF. Through machine-learning approach we created a robust diagnostic models based on AFs. Multivariate model, utilizing a combination of all six AFs, BMI and age reached a ROC AUC of 0.89 on both the training and test dataset, indicating the capability for predicting the risk of EC even on unseen data. Using the qPCR method on a cohort of 36 EC patients we tested the expression of 15 angiogenesis-associated genes, preselected from TCGA library, in tumour (T) compared to tumour-adjacent tissue (TA). Our findings suggest angiogenesis in EC is primarily driven by reduced anti-AF expression, with altered regulation in tumour-adjacent tissue of EC patients with less favourable prognosis. If validated in multicentre studies plasma concentrations of AFs could represent a supplementary tool for early detection and prognostic characterization of EC, which could advise on the extent of treatment.

This research was funded by ARRS grant J3-2535 to T.L.R. and UMCL TP 202110160 to Š.S.

Luka Roškar, MD, is a gynecologist and obstetrician who works at the General Hospital in Murska Sobota, Slovenia. He is a physician focused on the laparoscopic and hysteroscopic surgery, and a researcher with a focus on gynecological cancers and the development of new diagnostic and treatment methods.

Plenary session 7:

MOLECULAR BIOLOGY AND CLINICAL RESEARCH ON RARE DISEASES OF THE SKIN

Chair: M. Liović, Medical Centre for Molecular Biology

Skin fragility in epidermolysis bullosa and novel therapy approaches development

Mirjana Liović

Medical Center for Molecular Biology, Institute for Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

mirjana.liovic@mf.uni-lj.si

Within the heterogeneous group of rare inherited skin blistering diseases called epidermolysis bullosa (EB), there are four disease types: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler syndrome (KINDS). Altogether 18 genes are causative of the different EB phenotypes. While EBS is mostly due to mutations in constituents of the IF cytoskeleton, keratins 5 and 14, JEB is linked to mutations of laminin (extracellular matrix (ECM) protein) or structural components of hemidesmosomes (HD) i.e. integrin subunits (α6 or β4) or collagen XVII (180-kDa bullous pemphigoid antigen, BP180), while DEB is due to ECM protein collagen VII mutations. KINDS is a rare type of EB caused by mutations of kindlin 1, which is part of focal adhesions (FA). All these proteins form an interconnected network that provides physical integrity to skin by extending through the epidermis, basal lamina and dermis. Hereditary EB disorders are incurable and together they affect around 500.000 people worldwide. In the last decade lentiviral and retroviral constructs to supplement the missing gene or to repair the mutation *in vitro* were tested, as well as cell therapy through mesenchymal stromal cells, allogeneic fibroblasts, bone marrow transplantation, and a combination of *ex vivo* gene repair of patient-derived keratinocytes followed by epidermal sheet transplantation. The focal point of my research are keratin cytoskeletal proteins in health and disease, and it spans molecular and cell biology, biochemistry and biophysical studies on the keratin cytoskeleton, its role in the development of hereditary skin fragility disorders, development of new therapy delivery systems (archaesomes, GPMVs), induced pluripotent stem cells disease models and *in vitro* 3D skin models. EB is a systemic disease but localized therapies

that may enhance wound healing and decrease fragility in body locations subject to recurrent trauma could significantly improve patients' quality of life. In my talk I will present past and present research in my lab, our recent work on EB iPSC lines and the search for compounds suitable for pharmacological interventions.

Mirjana Liović is Senior Scientific Associate and Principal Investigator at the Medical Center for Molecular Biology (MCMB), IBKMG, Faculty of Medicine, University of Ljubljana. She is currently the Acting Director of MCMB. With broad scientific knowledge and research experience on the international level, she worked at the University of Wales Hospital, Cardiff, UK; New York University, Dept. of Dermatology in New York, USA; School of Life Sciences, University of Dundee, Scotland, UK and King's College London, UK. Her main research interests are the cytoskeleton and extracellular matrix function in health and disease.

DEBRA Slovenia

Polona Zakošek

Debra Slovenia, Trzin, Slovenia

info@debra-slovenia.si

DEBRA Slovenia, an association of people related to epidermolysis bullosa (EB), brings together sufferers, their family members and medical staff. For many years, he has been trying to offer patients with a severe hereditary skin defect as much quality life as possible, as this disease is currently incurable. As a humanitarian association of patients, DEBRA Slovenia has the status of an association that works in the public interest and is closely related to similar organizations around the world.

Epidermolysis bullosa is a group of genetic, mainly hereditary skin diseases. There are twenty different forms of the disease, which are roughly divided into three large groups, namely simplex, junctional and dystrophic EB. The cause of the disease is the lack of certain keratins and collagen, which are necessary to maintain normal intercellular contacts in the epidermis of the skin and mucous membranes. The result of the disease is daily blisters and sores on the body and mucous membranes, which cause ulcers of varying depth and constant pain.

In the dystrophic form of EB, unlike the other groups, wound healing proceeds significantly more slowly and with scars. Scars that affect the fingers of the hands cause the fingers to fuse into a

skin bag, scars in the mouth make it difficult to open the mouth and feed. The fingernails and toenails soon fall off, the hair is thinned, and the teeth are mostly carious. Swallowing hard food is not possible, as blisters also appear in the mouth and in the esophagus, which therefore narrows over the years. The result of all these problems is anemia, a delay in growth and development, and disability due to stiff joints and fused fingers. Over the years, these patients are most at risk of skin cancer, which appears at the sites of chronic wounds.

When dealing with such a difficult chronic disease, patients absolutely need all-round support, not only from family and society, but from all medical staff. They need special treatment adapted to their disease by a large number of specialists in various fields, as the affected skin is only one of the many concurrent problems where the patient needs support.

Polona Zakošek has been the president of DEBRA Slovenia since its establishment. She faced EB in 1993 with the birth of her son. She has been a member of the EB without Borders group for many years, and was also a member of the board of directors of DEBRA International for one term.

Stem cells in treatment of rare diseases of skin, from disease modelling to therapy

Nikola Kolundžić[1],Preeti Khurana[1], Jakob Jeriha[1], Mirjana Liović[2], Duško Ilić[1]

[1]Department of Women and Children's Health, King's college London, London, United Kingdom

[2]Medical Center for Molecular Biology, Institute for Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia.

dusko.ilic@kcl.ac.uk

Human pluripotent stem cells (hPSC), both embryonic and induced (hESC/iPSC), provide an unlimited source of cells for in vitro modeling of skin diseases. This ability allows researchers to study rare and complex skin diseases despite the limited availability of sample material. hPSC enable drug discovery and toxicity testing, which can aid in the development of better therapies for skin diseases. They also offer the potential for personalized medicine for patients with rare genetic skin diseases.

Differentiation of hPSC into keratinocytes is a complex process. Several groups worldwide, including ours, have published differentiation protocols. However, the field still struggles to reproduce these protocols and scale them up to industrial level manufacturing.

In this presentation, I will discuss the quality control system we introduced at the different points of differentiation to ensure the reproducibility of the protocol. I will also explain how we used this system in development of a model of a skin disease associated with loss of function of the filaggrin (*FLG*) gene.

Duško Ilic graduated from the Medical School and Molecular Biology at the University of Belgrade, Serbia. He obtained his Ph.D. at the University of Tokyo, Japan. He has worked in both academia and industry in the USA. He was as an Associate Adjunct Professor at the University of California San Francisco, and Research Consultant at the Dermatology Services, Veteran Affairs Medical Center in San Francisco. He served as Director of R&D in StemLifeLine in CA, USA, and was a technical co-founder and advisor to VitroLabs (www.vitrolabsinc.com), in CA, USA. Currently, he is a Professor of Stem Cell Science at the King's College London and the Academic Chair for Student-led Sustainability Innovation at the Circle U (www.circle-u.eu). The focus of his work involves the translational aspects of human reproduction, stem cell research, and the development of sustainable technologies.

Talin2 and KANK2 functionally interact to regulate microtubule dynamics, paclitaxel sensitivity and cell migration

Marija Lončarić[1], Nikolina Stojanović[1], Anja Rac Justament[1], Kaatje Coopmans[1], Dragomira Majhen[1], Jonathan D. Humphries[2], Martin J. Humphries[3], Andreja Ambriović-Ristov[1]

[1]Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia;

[2]Department of Life Science, Manchester Metropolitan University, Manchester, United Kingdom;

[3]Wellcome Centre for Cell-Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom

Andreja.Ambriovic.Ristov@irb.hr

Focal adhesions (FAs) are integrin-containing, multi-protein structures that link intracellular actin to the extracellular matrix and trigger multiple signalling pathways that control cell proliferation,

differentiation, survival and motility. Microtubules (MTs) are stabilised in the vicinity of FAs through interaction with the components of the cortical microtubule stabilising complex (CMSC). KANK (KN motif and ankyrin repeat domains) family proteins within the CMSC, KANK1 or KANK2, bind talin within FAs and thus mediate actin-MT crosstalk. We previously identified in MDA-MB-435S cells, which preferentially use integrin αVβ5 for adhesion, KANK2 as a key molecule enabling the actin-MT crosstalk. KANK2 knockdown also resulted in increased sensitivity to MT poisons, paclitaxel (PTX) and vincristine and reduced migration. Here, we aimed to analyse whether KANK1 has a similar role and to distinguish which talin isoform binds KANK2. We show that KANK1 is not a part of the CMSC associated with integrin αVβ5 FAs and its knockdown did not affect the velocity of MT growth or cell sensitivity to PTX. The talin2 knockdown mimicked KANK2 knockdown i.e. led to the perturbation of actin-MT crosstalk, which is indicated by the increased velocity of MT growth and increased sensitivity to PTX and also reduced migration. We conclude that KANK2 functionally interacts with talin2 and that the mechanism of increased sensitivity to PTX involves changes in microtubule dynamics. These data elucidate a cell-type-specific role of talin2 and KANK2 isoforms and we propose that talin2 and KANK2 are therefore potential therapeutic targets for improved cancer therapy.

Andreja Ambriović-Ristov received her PhD in adenovirus vectored vaccines in 1997. Since 2004, she has expanded her research into the field of integrin-mediated resistance of tumor cells to antitumor drugs. Her current focus of research is the molecular composition of integrin adhesion complexes.

Plenary session 8:

MOLECULAR BIOLOGY IN CLINICAL RESEARCH OF RARE DISEASES

Chair: N. Debeljak, Medical Centre for Molecular Biology

How is the MPN genetics information changing our clinical judgment?

Rajko Kušec

Universtiy hospital Dubrava Zagreb, School of medicine University of Zagreb, Croatia

rkusec@mef.hr

The discovery in 2005 of the JAK2 V617F substitution has considerably changed our approach to the diagnosis of MPN. At the end of 2013, two studies by Klampfl et al. and Nangalia et al. addressed the gap in the molecular characterization of wild-type JAK2/MPL patients with ET and PMF, by describing novel mutations in calreticulin (CALR) in the majority of such patients. These driver gene mutations cover almost 90% of polycythemia vera, essential thrombocythemia and primary myelofibrosis. With the advancement of next generation sequencing additional gene mutations have been described and clinically evaluated with integration into novel molecularly emphasized prognostic scoring systems. However, the latest WHO and ICC classifications point to the necessity to integrate peripheral blood findings and bone marrow morphology with the molecular genetic in refining the clinical MPN subtypes. Additional mutations have established role for risk stratification in MF, but prognostic role and clinical impact in PV, ET and e-PMF remains to be established with the open discussion on the best genetic testing strategy. Therapeutically, we are still lacking the true targeted mutation-specific therapy, but with rapidly emerging drugs directed at aberrant MPN cell signaling pathways or metabolic engines that are being evaluated in clinical trials. However, despite not mutation-specific inhibitor of JAK2, drug ruxolitinib has significantly improved our clinical competence in treating myelofibrosis, a complicated and debilitating disease with clear advantage over previously available therapies in the last 20 years. Overall survival was superior for patients who received initial ruxolitinib therapy, with a median survival of 72 months versus approximately 50 months for the remaining approaches (Masarova L, et al, Cancer, 2023). In summary, genomics is enabling greater precision to establish diagnosis and prognosis in MPN and is becoming valuable monitoring marker which will be particularly important for new targeted therapies.

Rajko Kušec, is specialist in internal medicine, consultant haematologist and professor of medicine at School of Medicine University of Zagreb. Graduated from School of Medicine University of Zagreb with postgraduate study in Clinical haematology at University of Vienna, followed by research in molecular haematology at Nuffield Division of Clinical Laboratory Sciences (NDCLS) at University of Oxford. Professor Kusec is head of Department of haematology and department of molecular diagnostics and genetics at Dubrava university hospital in Zagreb. He is past president of Croatian society of haematology (2016-2022) and member/board member of national and international associations and working groups in clinical and laboratory haematology (e.g. CROHEM, CEMPO, SWG MPN-EHA, MPN-EuroNet, EHOG).

Diagnosis and management of familiar erythrocytosis

Saša Anžej Doma, MD, PhD[1,2], Irena Preložnik Zupan, MD, Prof[1,2]

[1]Hematology department, University Medical Centre Ljubljana, Ljubljana, Slovenia

[2]Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

sasa.anzej.doma@kclj.si

Familiar erythrocytosis is an extremely rare disease which can easily get misdiagnosed among the more frequent and heterogeneous types of erythrocytosis. In particularly, it can be suspected in patients with erythrocytosis since childhood and a positive family history. Specific genetic investigations should be performed after exclusion of other, more common acquired disorders. We usually follow a systematic diagnostic algorithm: after confirming erythrocytosis by at least two blood samples, we continue, according to the clinical picture, either for polycythaemia vera or secondary acquired erythrocytosis investigations. Polycythaemia vera is easily confirmed or excluded by *JAK2* mutation (usually positive) and erythropoietin level testing (subnormal). To characterize erythrocytosis as secondary, we must determine the underlying hypoxic conditions, causative for compensatory erythrocytosis (cardio-pulmonary or kidney diseases, smoking, sleep apnea,…) or conditions where erythrocytosis is a result of ectopic erythropoietin secretion (tumors, renal diseases) or other reasons (androgen use, haemochromatosis). In the third step, we refer patients without a known cause of erythrocytosis (idiopathic erythrocytosis) for genetic testing to identify possible familiar erythrocytosis. Apart from genetic testing we can use haemoglobin electrophoresis to diagnose high-oxygen-affinity Hb variants (limited sensitivity) and blood gas analysis with p50 determination to diagnose high-oxygen-affinity haemoglobinopathies, 2,3-BPG deficiency, methemoglobinemias and *PIEZO1* mutations.

As management of familiar erythrocytosis depends on the underlying causative condition, it is very important to properly classify erythrocytosis. Nevertheless, it is possible that the underlying genetic defect is not identified. All in all; the best treatment approach remains to be established in many patients with familiar erythrocytosis.

Saša Anžej Doma and **Irena Preložnik Zupan** are hematologists covering both malignant and benign hematology. Recently, their focus of research has been on non-clonal erythrocytosis and establishment of a clinical algorithm for erythocytosis characterisation, including recommendations for genetic testing referral.

Ten years of experience using next generation sequencing for diagnosis of rare diseases in Slovenia

Aleš Maver

Clinical Institute of Genomic Medicine,
University Medical Centre Ljubljana, Ljubljana, Slovenia

ales.maver@kclj.si

Rare genetic diseases represent one of the principal public health challenges of modern societies worldwide. They are characterized by a wide heterogeneity of genetic causes and underlying mutational mechanisms, as well as frequently non-specific and overlapping clinical presentations. Next-generation sequencing has transformed the diagnosis of these disorders, particularly with the ability to sequence the complete human exome and genome in a diagnostic setting.

At the Clinical Institute of Medical Genetics, we introduced diagnostic exome sequencing in 2013, making Slovenia one of the pioneering countries in implementing comprehensive sequencing approaches for the routine evaluation of rare disease patients. From the outset, our focus has been on developing internal bioinformatic workflows to facilitate rapid and comprehensive analysis of sequencing data. To maximize the diagnostic yield, we have employed several strategies to detect a broader spectrum of disease-associated variants, including structural, mitochondrial, and non-coding variants, which resulted in a substantial increase in diagnostic rates. Additionally, we have actively pursued the resolution of diseases with unknown genetic causes. Through systematic utilization of international data sharing networks, we have led or participated in the discovery of over 10 novel gene-disease associations. Moreover, as genome-wide sequencing datasets have grown, we have developed local resources encompassing population-specific and disease-associated genetic variations, further enhancing the diagnostic capabilities for genetic disorders in our region.

Our experience with diagnostic next-generation sequencing exemplifies the transformative impact of novel genome sequencing approaches in enabling faster and more efficient diagnosis for Slovenian patients with rare genetic disorders. Furthermore, it has contributed to improving our understanding of the causes and mechanisms underlying human diseases.

Aleš Maver The principal focus of my work is the field of rare and complex human disease genetics. Principally, I have been involved in the application of high-throughput sequencing approaches for clinical diagnostics and research.
Currently, my work is focused on diagnostics based on exome, genome and RNA sequencing. I am particularly enthusiastic about implementing novel approaches to improve genome-level clinical variant interpretation, create resources of national variation and to increase use of data sharing to facilitate the diagnosis of rare genetic disorders.

Analysis and interpretation of next-generation sequencing data in practice

Tadej Pajič, Aleš Maver, Borut Peterlin

Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, 1000 Ljubljana, Slovenia

tadej.pajic@kclj.si

Rare disease diagnostics and disease gene discovery has undergone a remarkable transformation with the advent of next-generation sequencing (NGS). Nonetheless, the task of identifying the precise variant(s) accountable for a specific condition from the immense pool of millions of variants in each individual remains a significant challenge. To address this, a systematic approach involving primary, secondary, and tertiary analysis of NGS data is implemented to enhance sensitivity and minimize the need for labor-intensive expert review.

Primary analysis involves DNA sequence production and initial data quality control. Secondary analysis identifies DNA variants through bioinformatics alignment to a genome reference, variant calling, and additional quality control. Annotation, filtering, and prioritization strategies are then used to establish a minimal set of variants for reviewing using genotype and phenotype-driven analysis. Genotype-driven analysis screens all suspicious genetic variation irrespective of disease association, while phenotype-driven analysis utilizes comprehensive phenotyping of patients, reference genotype–phenotype knowledge, and family clinical data to narrow down the search further. These strategies primarily target single nucleotide variants and small indels in the nuclear genome. By including additional analyses such as copy-number variation detection, nonconsensus

splice defect identification, genomic breakpoint detection, homozygosity mapping, and mitochondrial variant analysis, a more comprehensive understanding of genetic variations and their potential implications in disease can be obtained. Furthermore, the utilization of established local databases that contain comprehensive information regarding both pathological and normal genomic variant variability can significantly enhance the identification of genetic variants associated with diseases distinguishing them from benign variations in the genome. This valuable resource adds another layer of support to the diagnostic process and contributes to more precise and reliable results.

Described clinical NGS workflow results in a high diagnostic outcome (approx. 40% in our lab) and was found to be indispensable in the diagnostic of rare diseases and disease gene discovery. Some of the representative cases will be presented on the symposium.

Assist. Prof. **Tadej Pajič,** PhD, Clinical Laboratory Geneticist, European Specialist of Laboratory Medicine. My work and research revolve around molecular genetic testing, diagnostics of rare genetic disorders by NGS techniques, prenatal and pre-implantation genetic diagnostics, and clinical biochemistry. I strive to advance our understanding of genetic diseases, and enhance patient care through precise and comprehensive molecular testing approaches. In the field of clinical biochemistry, my interest is in investigating various aspects of biochemical markers and their role in disease diagnosis, prognosis, and treatment monitoring.

Plenary session 9:

MOLECULAR BIOLOGY IN PRECLINICAL RESEARCH OF CANCER

Chair: P. Hudler, Medical Centre for Molecular Biology

APOBEC proteins play an important role in Human papillomavirus infection and oncogenesis

Martina Bergant Marušič[1], Fabio Lapenta[1], Nika Marija Lovšin[2]

[1]University of Nova Gorica, Laboratory for Environmental and Life Sciences, Nova Gorica, Slovenia

[2]University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia

Human papillomaviruses (HPV) cause nearly 5% of all human malignancies, including cervical cancer and head and neck cancer. DNA-editing activity of a group of cytosine deaminases, APOBEC 3 (A3), is part of the innate immune response to viral infections. However, this intrinsic host defence mechanism may also be responsible for long-term host DNA hypermutation and cancer development during persistent infections. Two members of the APOBEC family, A3A and A3B, have been associated with a high mutational burden in HPV-related cancers, whereas the editing-independent activity of the A3 proteins remains largely unknown.

Our research focuses on the editing-independent role of A3 proteins in HPV infection and cell transformation. We analysed the expression profile of head and neck cancer patients from The Cancer Genome Atlas (TCGA) and identified genes that correlated with either A3A or A3B. Genes whose expression correlated with A3A appeared to be generally downregulated in HPV-positive patients. GO Analysis of these genes revealed enrichment of genes associated with epidermal differentiation and innate immunity. Genes correlated with A3B were associated with the cell cycle, chromosome organisation, DNA replication, and DNA repair and were overexpressed in HPV-positive cancer patients. This suggests a distinct role for the two enzymes. A3A is more involved in the antiviral response and therefore mostly present in HPV-negative cancers, whereas

A3B affects the expression of genes involved in cell transformation and is more abundant in HPV cancer patients. HPV host cells HFK (human foreskin keratinocytes) lacking A3A or A3B protein showed altered expression of selected genes correlating with the expression of A3A or A3B proteins and HPV oncoproteins. These cell lines also exhibited altered patterns of cell proliferation, migration, and invasion, confirming the role of editing-independent activity of A3 proteins in cell transformation, particularly in the context of HPV.

Martina Bergant Marušič Her main area of research is human papillomavirus (HPV) biology. She is particularly interested in the molecular mechanisms by which the viral genome is transported from the cell membrane to the nucleus and in host-pathogen interactions in persistent HPV infections.

Tumor antigens at the crossroads of male fertility and cancer therapy resistance – a single cell perspective

Barbara Breznik[1,2,3], Maria Camila Hoyos Sanchez[1,2], Sima Tozandehjani[1,2], Juan Solano[1,2], Klementina Fon Tacer[1,2]

[1]School of Veterinary Medicine, Texas Tech University, Amarillo, Texas, USA

[2]Texas Center for Comparative Cancer Research, Texas Tech University, Amarillo, Texas, USA

[3]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia

fontacer@ttu.edu

Melanoma-associated antigens (MAGE) are the first tumor antigens discovered more than 30 years ago and correlate with the more aggressive disease and resistance to therapy in many solid tumors. Intriguingly, these proteins are normally expressed in male germ cells but get aberrantly activated in cancer. We study the molecular, cellular, and biological functions of MAGE proteins at the crossroads of spermatogenesis and cancer and aim to leverage the insights we learn to advance

cancer treatment. Our preliminary data suggest that MAGEs evolved to protect and finetune the intricate process of male germ cell differentiation, including resistance to nutrient stress during the transition of germ cells through the blood-testis barrier. Furthermore, these protective functions are likely coopted by cancer cells and may trigger therapy resistance. To determine the underlying molecular mechanisms, regulated by MAGE genes in male germ cells and human cancer, we use Mage knock-out mice and 2D/3D cancer cellular models. Single-cell sequencing of testicular cells of animals, exposed to nutrient stress, suggested that several pathways are governed by Mages to protect the integrity of germ cells and male fertility under suboptimal conditions. We also used gain-of-function and loss-of-function models to evaluate the role of MAGEs in cancer growth and therapy response. Our data show that MAGEC2 and MAGEA3/6 promote cancer growth and therapy resistance, respectively, however, the underlying mechanisms are different. This is in line with our preliminary data suggesting that different MAGE genes evolved to protect a distinct subset of the germ cell population against different types of stressors. We are currently investigating the molecular underpinnings of their function which may provide novel therapeutic opportunities, in particular in therapy-resistant patients.

Klementina Fon Tacer is an assistant professor at Texas Tech University School of Veterinary Medicine (TTU SVM) and director of the Texas Center for Comparative Cancer Research (TC3R) in Amarillo, Texas. Dr. Fon Tacer obtained her DVM and PhD degrees at the University of Ljubljana. After postdocs at the UTSW Medical Center in Dallas, Texas, and St. Jude's Children Research Hospital in Memphis, Tennessee, she joined the newly established TTU SVM as a Cancer Prevention and Research Institute of Texas (CPRIT) Scholar to pursue research at the intersection of reproduction and cancer (fontacerlab.org).

Poster

Muscle-specific microRNAs as potential biomarkers in spinal muscular atrophy

Maruša Barbo[1], Gregor Jezernik[2], Damjan Glavač[2, 3], Metka Ravnik-Glavač[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Center for Human Genetics & Pharmacogenomics, Faculty of Medicine, University of Maribor, Maribor, Slovenia

[3]Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

marusa.barbo@mf.uni-lj.si

Spinal muscular atrophy (SMA) is a severe neurodegenerative disease characterized by muscle weakness and paralysis caused by a deficiency of the survival motor neuron (SMN) protein. This deficiency is caused by deletions in the survival of motor neuron 1 (*SMN1*) gene and leads to progressive degeneration of motor neurons. Three drugs (nusinersen, risdiplam, and onasemnogene abeparvovec) have been approved to treat *SMN1* deficiency by increasing the production of the SMN protein. Assessing the efficacy of these treatments remains challenging, however, circulating microRNAs (miRNAs) have emerged as potential biomarkers for measuring disease progression and treatment response in several diseases, including SMA.

We conducted an extensive literature search to identify miRNAs associated with the pathogenesis of SMA and response to treatment with nusinersen. The identified miRNAs were further used to construct miRNA-lncRNA-mRNA axes using the RNA Association Interaction Database (RAID v2). Finally, the molecular interaction networks were visualized using Cytoscape bioinformatics software platform.

Four muscle-specific miRNAs (myomiRs), including hsa-miR-1-3p, hsa-miR-133a-3p, hsa-miR-133b, and hsa-miR-206 have shown promise as potential SMA biomarkers. These myomiRs were found to be frequently elevated in SMA patients compared with healthy controls and their levels decreased after nusinersen treatment. Two axes, hsa-miR-1-3p–GJA1–hsa-miR-206 and hsa-miR-133b–LINCMD1–hsa-miR-133a-3p, were associated with genes involved in neurological inflammatory processes, further highlighting their relevance to SMA.

To validate the use of myomiRs as reliable biomarkers that could significantly improve the clinical management of SMA and support the development of personalized treatment approaches, further studies with larger cohorts of healthy individuals and SMA patients, both before and after treatment, are needed.

Maruša Barbo is a part of the research group of Pharmacogenetics Laboratory (IBKMG, MF, UL, Slovenia) led by prof. Vita Dolžan. Since October 2022, she is a PhD student of the Interdisciplinary doctoral programme, Biomedicine (UL MF), under the supervision of prof. dr. Metka Ravnik Glavač.

Efficacy of selective laser trabeculoplasty may be associated with pharmacogenetic biomarkers of inflammatory and oxidative stress pathways in patients with ocular hypertension or primary open-angle glaucoma

Tanja Blagus[1], Makedonka Atanasovska Velkovska[2], Katja Goričar[1], Barbara Cvenkel[2,3], Vita Dolžan*[1]

[1]University of Ljubljana, Faculty of Medicine, Institute of Biochemistry and Molecular Genetics, Pharmacogenetics Laboratory, Ljubljana, Slovenia

[2]University Medical Centre Ljubljana, Department of Ophthalmology, Ljubljana, Slovenia

[3]University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

tanja.blagus@mf.uni-lj.si

Selective laser trabeculoplasty (SLT) is a well-established method for reducing intraocular pressure (IOP) in individuals with primary open-angle glaucoma (POAG), however, not all patients experience sufficient IOP reduction. We aimed to identify pharmacogenetic biomarkers that can predict the response to SLT treatment in patients with ocular hypertension (OH) and POAG.

This pilot study included 51 treatment naïve patients with either OH or mild to advanced POAG. The response to SLT was evaluated as the reduction of IOP six weeks after the treatment. All participants were genotyped for common genetic variants in inflammatory (*TNF* rs1800629; *IL1B* rs16944, rs1143623; *IL6* rs1800795) and oxidative stress pathway (*GSTM1**0; *GSTT1**0; *GSTP1* rs1695, rs1138272; *SOD2* rs4880; *CAT* rs1001179; *GPX1* rs1050450). Statistical analysis was conducted using logistic regression and ROC curve analysis.

After six weeks of SLT treatment, 39.2% of patients had good treatment outcome (>30% IOP reduction), while 47.1% of had moderate (15-30% reduction) and 13.7% had poor treatment outcome (<15% IOP reduction). Among the clinical parameters, family history of glaucoma was associated with less effective treatment outcomes (P=0.033). Patients carrying at least one polymorphic *IL6* rs1800795 allele were less likely to achieve a good treatment outcome (OR=0.24, 95% CI=0.07-0.81, P=0.021). When clinical and clinical-pharmacogenetic models were developed to predict treatment outcomes, the clinical-pharmacogenetic model (area under the curve (AUC)=0.85, P<0.001) outperformed the clinical model alone (AUC=0.74, P=0.005) in predicting IOP reduction.

Pharmacogenetic biomarkers may predict the response to SLT treatment. The integration of clinical and pharmacogenetic data into predictive models could be used to identify patients with POAG and OH in whom SLT will be the most effective first-line treatment.

Tanja Blagus is a part of the research group of Pharmacogenetics Laboratory (IBKMG, MF, UL, Slovenia) led by prof. Vita Dolžan. Since October 2021, she is a PhD student of the Interdisciplinary doctoral programme, Biomedicine (UL MF).

Whole genome sequencing in patients with hypogonadotropic hypogonadism

Nika Breznik[1], Katarina Trebušak Podkrajšek[2,3], Magdalena Avbelj Stefanija[2]

[1]Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

[2]Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

[3]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

breznik.nika@gmail.com

Hypogonadotropic hypogonadism (HH) is a heterogeneous disorder with variable clinical presentation. Identifying the genetic cause of HH is often challenging, as approximately 50 % of cases remain unexplained.

Whole genome sequencing was performed in 20 subjects from 18 families in whom previous genetic approaches have not been successful. Single nucleotide variants, small deletions, insertions, and copy number variations in selected 366 genes were analyzed with the aim of explaining their clinical picture. Using available genome-wide data from family members, we also examined the intron regions of 34 genes known to be associated with HH. Sanger sequencing was used to confirm the segregation of the identified genetic variants within the families.

Genetic etiology was identified in 30 % of subjects and partially in another 45 % of subjects. We identified novel genetic variants in *ANOS1*, *FGFR1*, *IGSF10*, *NRP2*, and *SPRY4*, all known HH-associated genes. We also identified genetic variants in candidate genes not previously associated with HH. In only two subjects, we were not able to identify genetic variants that could have contributed to the clinical picture of HH. In three subjects from the same family, we detected a deep intronic variant in *FGFR1* that may have contributed to the clinical presentation. However, identifying deep intronic variants is currently a major challenge and to the best of our knowledge, deep intronic variants have not yet been reported in patients with HH.

Nika Breznik is a master's student of Laboratory Biomedicine at Faculty of Pharmacy, and a master's student of Applied Statistics at Faculty of Electrical Engineering. She has gained experience both in clinical diagnostic laboratory, as well as in research establishment through her master's thesis work. Currently, she is engaged in student work in Biologics TRD at Novartis. In October, she will start her studies in the doctoral degree program in Biomedicine, while her research work will take place in the Laboratory for Translational Medical Biochemistry at the Institute of Biochemistry and Molecular Genetics, Faculty of Medicine.

Core-modified estrane derivates as new inhibitors of AKR1C enzymes and their effect on ovarian cancer cell lines

Ajda Godec[1], Maša Sinreih[1], Dániel Aszmann[2], Péter Traj[2], Vivien Resch[3], Gábor Paragi[3,4,5], Erzsébet Mernyák[2], Tea Lanišnik Rižner[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Inorganic, Organic and Analytical Chemistry, University of Szeged, Szeged, Hungary

[3]Department of Medical Chemistry, University of Szeged, Hungary;

[4]Institute of Physics, University of Pécs, Hungary

[5]Department of Theoretical Physics, University of Szeged, Hungary

ag5790@student.uni-lj.si, tea.lanisnik-rizner@mf.uni-lj.si

AKR1C enzymes are involved in chemoresistance, either by metabolising chemotherapeutics or indirectly by eradicating the cellular stress created by chemotherapeutics. These enzymes represent interesting drug targets for chemoresistant cancers. High-grade serous ovarian cancer (HGSOC) is the most common type of ovarian cancer and accounts for approximately 80% of all ovarian cancer deaths. Although HGSOC treatment has advanced significantly over the last decade, the five-year survival rate for OC remains below 30%. Despite high initial response to first line chemotherapy, most patients relapse in 18 months after the treatment due to chemoresistance. Six compounds that can be classified as core-modified estrane derivates (DTP-153, DTP-154, DTP-155, DTP-158, AD-13), the five tetrahydronaphtalen-1-one derivates (AD-4, AD-5, DTP-036, DTP-026, DTP-150, DTP-150-KK) were studied for their effects on AKR1C enzymes and HGSOC cell lines. 13α-Estrone derivatives were more effective inhibitors than tetrahydronaphtalen-1-one derivates. The two most active inhibitors of the AKR1C2 isoenzyme, DTP-036 and AD-4, had an IC_{50} value of 3.3 μM and 14.2 μM, respectively. At 100 μM concentration two of tetrahydronaphtalen-1-one derivates (DTP-154 in DTP-158) also showed more than 50%

inhibition of AKR1C3. The control ovarian cell line (HIO-80) and HGSOC cell lines (COV-362 and OVSAHO) were next exposed to 10 μM and 50 μM inhibitors for 48 hours. Cell viability of the OVSAHO cells was significantly decreased when exposed to 50 μM DTP-153, DTP-155, AD -13, and DTP-150. There were no effects on control cell line HIO-80 and HGSOC cell line COV-362. To conclude, we discovered new selective inhibitors of AKR1C2 isoform and four compounds that decrease viability of the HGSOC cell line OVSAHO.

This study was supported by N1-0234 and J3-2535 grants to T.L.R. from the Slovenian Research Agency and OTKA SNN 139323 to E.M.

Ajda Godec is completing her master's degree in biochemistry at the Faculty of Chemistry and Chemical Technology at the University of Ljubljana. Her master's thesis includes the production of recombinant proteins, the kinetics of enzymes and studies on ovarian cancer cell lines.

Leukocyte telomere length as a biomarker of radiotherapy response in breast cancer

Sara Redenšek Trampuž[1], Ana Trstenjak[2], Vita Dolžan[1], Katarina Trebušak Podkrajšek[3], Tanja Marinko[4,5], Katja Goričar[1]

[1]Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

[3]Laboratory for translational medical biochemistry, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[4]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[5]Institute of Oncology Ljubljana, Ljubljana, Slovenia

katja.goricar@mf.uni-lj.si

Ductal carcinoma in situ (DCIS) is a non-invasive type of breast cancer that is often detected in screening programs. Patients are usually treated with surgery and adjuvant radiotherapy (RT). RT enables good disease control, but also causes adverse events. Several molecular factors could contribute to the inter-individual variability of RT response, including telomere length. Telomeres

are nucleoprotein complexes that protect chromosomes from degradation and studies suggest that their length could influence radiosensitivity of the cells. Our aim was to determine leukocyte telomere length (LTL) dynamics in DCIS patients treated with RT and to evaluate the association of LTL with adverse events of RT.

Our study included 89 DCIS patients treated with adjuvant RT. Skin and cardiac adverse events were evaluated after RT and after 6 months. Genomic DNA was isolated from blood samples before RT, after RT and after 6 months. LTL was determined using monochrome multiplex quantitative PCR and expressed as the ratio between the telomere and housekeeping gene amplicons. Nonparametric tests were used in statistical analysis.

Relative LTL was 0.47 (0.41-0.58) before RT, 0.72 (0.63-0.88) after RT and 0.68 (0.56-0.79) after 6 months. After RT, significant increase in LTL was observed in 91.0% of DCIS patients ($P<0.001$). After 6 months, relative LTL again decreased in 58.4% of DCIS patients ($P=0.043$), but it remained higher than before RT in 77.5% of patients ($P<0.001$). LTL was not associated with radiodermatitis or skin adverse events of RT after 6 months (all $P>0.05$). On the other hand, LTL was associated with cardiac adverse events: patients with longer LTL after RT were more likely to exhibit signs of heart failure assessed using NYHA classification ($P=0.043$).

In conclusion, RT may affect LTL dynamics and could serve as a biomarker of RT response in DCIS patients.

Katja Goričar is an assistant professor at the Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana. Her research work focuses on (pharmaco)genetic, epigenetic and protein biomarkers for personalized medicine, especially in the field of oncology.

An example of the synergy of various diagnostic methods to confirm the clinical diagnosis

Maja Ficko[1], Daša Perko[1], Katarina Trebušak Podkrajšek[1,2], Barbka Repič Lampret[1], Tinka Hovnik[1,2]

[1]Clinical institute for Special Laboratory Diagnostics, University Children's Hospital, UMC, Ljubljana, Slovenia

[2]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

tinka.hovnik@kclj.si

Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD) is the most common congenital disorder of fatty acid metabolism (OMIM # 201450) and is inherited in an autosomal recessive

manner. MCADD is caused by homozygous or compound heterozygous variants in the *ACADM* gene encoding medium chain acyl-CoA dehydrogenase (MCAD) involved in mitochondrial fatty acid β-oxidation. Typical clinical symptoms are hypoketotic hypoglycemia, vomiting, seizures, and coma triggered by fasting, catabolic stress, or common illness.

2-year-old female was admitted to the hospital due to persistent vomiting, dehydration and lethargy caused by a viral infection. Her levels of transaminases and creatinine kinase were markedly elevated. Analysis of organic acids in urine using gas chromatography–mass spectrometry (GC/MS) showed the presence of hexanoylglycine and suberylglycine, typical biomarkers of MCADD. Furthermore, the acylcarnitine profile, determined with tandem mass spectrometry (MS/MS), showed a characteristically elevated octanoylcarnitine (C8), decanoylcarnitine (C10), and decenoylcarnitine (C10:1). However, next-generation sequencing (NGS) identified only one pathologic heterozygous variant in the *ACADM* gene namely c.353G>T, p.Gly118Val, not sufficient to genetically confirm the diagnosis. Nevertheless, the activity of MCAD in the lymphocytes was shown to be below the limit of quantification (<0,02nmol/(min.mg protein). Consequently, we aimed to identify possible pathogenic copy number variant on the second allele of the *ACADM* gene with multiplex ligation-dependent probe amplification (MLPA) analysis, which revealed a heterozygous deletion of exons 1 and 2 of the *ACADM* gene spanning at least 3.9 kb in the chromosomal region 1p31. With this MCADD was confirmed at the molecular level.

Establishing the final diagnosis in neonatal and young children is crucial for further clinical management since early diagnosis reduces deaths and severe adverse events associated with MCADD. The presented case is an illustrative example of combining different diagnostic methods in order to confirm the clinical diagnosis.

Tinka Hovnik is the head of the Cytogenetics Laboratory at the Clinical Institute for Special Laboratory Diagnostics at the University Children's Hospital Ljubljana. She has two specializations: in Medical biochemistry and in Laboratory medical genetics. She is an assistant professor at the Faculty of Medicine, University of Ljubljana. Her professional and scientific interests are cytogenetic and molecular genetic background of rare genetic diseases.

Exploring circadian rhythm disturbances in obstructive sleep apnea: identification of molecular biomarkers from buffy coat and plasma using RNA expression analysis and LC-MS

Maruša Jerše[1], Eva Kočar[1], Cene Skubic[1], Miha Moškon[4], Julija Šmon[1,2], Leja Dolenc Grošelj[2,3], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia

[3]Department of Neurology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[4]Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia

marusa.jerse@gmail.com

Obstructive sleep apnoea (OSA), a common sleep disorder, is associated with increased daytime sleepiness and a higher risk of cardiovascular complications. Current diagnostic tools are polysomnography and polygraphy, but their limited availability leaves a large proportion of patients undiagnosed. Altered circadian genes and hormones in OSA patients suggest the possibility of developing more accessible diagnostic tools for OSA.

Twenty-five adult male and female subjects at high risk for OSA underwent one day hospitalisation, during which peripheral blood samples were collected at 4 time points: 13:00, 19:00, 1:00, and 7:00. Between 22:00 and 6:00, subjects underwent polygraphy for OSA diagnosis, which divided them into patient and control groups based on Respiratory Event Index (REI). mRNA was isolated from the peripheral blood cells (buffy coat), containing mostly while blood cells and platelets. The expression of the core clock circadian genes (*PER1, PER2, PER3, CRY1, CRY2, BMAL, CLOCK*) was measured by quantitative reverse transcriptase-polymerase chain reactions (qPCR). Melatonin and cortisol were extracted from plasma and their levels determined by liquid chromatography-mass spectrometry (LC-MS) using a Shimadzu LCMS-8050.

Our initial results indicate changes of *CRY2* and *BMAL1* gene expression in OSA patients at distinct time points. First results of expression of melatonin and cortisol show decreased melatonin expression in OSA patients at 19.00. Pearson correlation coefficient showed a positive correlation between cortisol levels at 01:00 and REI. Cosinor analysis of melatonin and cortisol confirmed circadian rhythmicity with a 24-hour period.

Our study demonstrates that patients with OSA exhibit altered expression of specific circadian genes in peripheral blood cells from the buffy coat and changed hormone levels in blood plasma, suggesting the potential of using circadian genes and hormones as molecular biomarkers.

Maruša Jerše, a student of general medicine at the University of Ljubljana, is investigating the disruption of circadian rhythm in patients with obstructive sleep apnoea at the Centre for Functional Genomics and Biochips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, in collaboration with the Institute of Neurophysiology, University Medical Center of Ljubljana.

Unravelling transcriptome profiles for addressing non-alcoholic fatty liver disease

Eva Kočar[1], Tadeja Režen[1], Pablo J. Giraudi[2], Silvia Palmisano[2, 3, 4], Claudio Tiribelli[2], Natalia Rosso[2], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Fondazione Italiana Fegato – ONLUS Area Science Park Basovizza, Trieste, Italy

[3]Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy

[4]Surgical Clinic Unit, Cattinara Hospital, ASUGI, Trieste, Italy

eva.kocar@mf.uni-lj.si, tadeja.rezen@mf.uni-lj.si, pablo.giraudi@fegato.it, spalmisano@units.it, ctliver@fegato.it, natalia.rosso@fegato.it, damjana.rozman@mf.uni-lj.si

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in developed countries, associated with risk for other comorbidities. However, the underlying mechanisms determining specific NAFLD stages and predicting disease progression are unclear. We performed transcriptome analysis and statistical modeling to identify novel target genes that can accurately classify stages of NAFLD.

Liver biopsy samples were collected from morbidly obese individuals, who underwent bariatric surgery, with histologically determined fibrosis stages (F0-F4). Gene expression (GE) profiling was conducted using Affymetrix microarrays and statistical analysis (including patients' medical data) was performed with TAC software to identify differentially expressed genes (DEG). Enrichment analysis of KEGG and Reactome pathways was conducted using Enricher and G:profiler tools. DEG along with a subset of genes recently published by others were selected for validation in a new cohort of NAFLD patients. Correlation analysis between GE and fibrosis stage was performed using Orange software.

Several known and novel gene candidates exhibited significant differential expression (FDR ≤ 0.05) across fibrosis stages in the discovery cohort (*e.g. ITGBL1, PTGDS, LUM*). Differential expression of

selected genes was successfully validated using RT-qPCR in both cohorts. Furthermore, BMI, fatty acids, and age had a greater impact on GE than fibrosis stage itself. Correlation analysis between GE and fibrosis stage identified *ITGBL1* as the most promising candidate gene (+0.66). Enrichment analysis of Reactome and KEGG pathways uncovered significant alterations (FDR ≤ 0.05) in metabolic pathways when comparing early to later fibrosis stages in the discovery cohort.

In conclusion, we identified several differentially expressed genes capable of distinguishing between different fibrosis stages, thereby potentially contributing to improved patient stratification and appropriate treatment.

assist. razisk. **Eva Kočar, mag. mol. funkc. biol. is a Ph.D.** student in Biomedicine currently working as a young researcher at the Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, UL MF. Her current research focuses on cholesterol-related factors in metabolic liver disease and SARS-CoV-2 infection.

Characterization and comparative analysis of 3D spheroid models in high-grade serous ovarian cancer: insights into enhanced tumor phenotype and altered proliferation dynamics

Vesna Kokondoska Grgič[1,2], Katja Kološa[3], Tea Lanišnik Rižner[1], Maša Sinreih[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Kemomed, Research & Development

[3]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology

v.kokondoska@kemomed.si, masa.sinreih@mf.uni-lj.si

Traditional studies on high-grade serous ovarian cancer utilize 2D cell lines, which only partially resemble the tumor's spatial characteristics. Spheroids mimic the spatial structure of tumors more closely, allowing for better representation of biochemical changes associated with the loss of cellular polarity and acquisition of migratory and invasive properties known as epithelial-mesenchymal transition (EMT). In our study, we aimed to establish 3D spheroids using well-characterized HGSOC cell lines OVCAR-4, Kuramochi, OVSAHO, and COV362, and compare their characteristics with 2D models. Our 3D spheroid models exhibited consistent morphological

features, with diameters ranging from 400 to 1400 µm, and roundness values exceeding 0.85 for all cell lines. Subsequently, we assessed the expression of 22 genes associated with EMT, extracellular matrix proteases, angiogenesis-related factors, mutation markers, and proliferation markers. As expected, we observed higher expression levels of EMT markers *VIM* and *SLUG* in the spheroids, indicating a more invasive phenotype. Moreover, increased *WNT11B* expression in spheroids suggested reduced attachment to the extracellular matrix. After establishing a protocol to dissociate spheroids into single-cell suspensions for flow cytometry analysis, we examined the expression of the proliferation marker Ki67 after 7 days maintenance of COV362. We observed that in 2D cell cultures, approximately 14.5% of cells exhibited Ki67 expression. In contrast, the proportion of Ki67-positive cells in 3D cultures was 35.5%. These results suggest a higher proliferative activity in the 3D cell culture, indicating the potential impact of the 3D model on cell proliferation dynamics. Our findings underscore the greater suitability of HGSOC spheroids compared to 2D cell lines. We are currently conducting functional studies to explore differences in cell proliferation, migration, invasiveness, and response to chemotherapy agents between 3D and 2D models.

This study is supported by L4-4565 grant to M.S. from the Slovenian Research Agency.

Vesna Kokondoska Grgič is a biotechnology professional and project manager at Kemomed. With a master's degree in Biotechnology and ongoing Ph.D. studies, she specializes in cell and tissue characterization. Vesna is dedicated to promoting science and providing support to researchers in the region.

Enhanced FREM2 protein expression in glioblastoma and astrocyte cells following temozolomide exposure

Gloria Krapež[1], Mojca Katrašnik[1], Neja Šamec[1], Alja Zottel[1], Ivana Jovčevska[1]

[1]CFGBC, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

gloria.krapez@mf.uni-lj.si

Glioblastoma (GBM) is an incurable primary brain tumor, affecting approximately 5 per 100,000 people annually worldwide. Its prognosis is bleak, with a life expectancy of less than 18 months, underscoring the critical need to comprehend GBM pathophysiology. Recent findings at our

laboratory have indicated the overexpression of FRAS1 related extracellular matrix protein 2 (FREM2) on the plasma membrane of GBM cells, correlating with cell motility and migration. Literature also suggests a potential correlation between FREM2 and GBM progression after treatment with the commonly used chemotherapeutic drug, temozolomide (TMZ). To investigate the role of FREM2 in TMZ resistant glioblastoma further, we examined the impact of TMZ treatment on FREM2 gene and protein expression levels in GBM cells and astrocytes. Our primary objectives were to establish TMZ-resistant GBM cell lines and astrocytes, quantify changes in FREM2 gene and protein expression levels between TMZ-resistant and TMZ-sensitive cells, and determine the relationship between FREM2 gene expression and GBM cell resistance to TMZ. To accomplish this, we exposed two stem-like (NCH644, NCH421K) and two differentiated (U87MG, U251MG) GBM cell lines, as well as astrocytes, to increasing concentrations of TMZ (1 to 50 μM) for five weeks. Metabolic and apoptotic assays were conducted weekly to monitor cell viability during the induction of resistance. All cell lines demonstrated survival at the maximal concentration (50 μM) of TMZ for one week, and an overall increase in FREM2 protein levels was observed in TMZ-treated cells compared to non-treated controls. However, mRNA expression did not exhibit a consistent pattern. Only NCH644 cells displayed an upregulation in relative FREM2 mRNA expression levels following TMZ treatment. Additionally, quantitative polymerase chain reaction (qPCR) and western blotting revealed higher expression of FREM2 gene and protein in stem-like GBM cells compared to differentiated GBM cells and astrocytes.

Gloria Krapež is a second-year PhD student at the Center for Functional Genomics and Biochips. As a young researcher she is focused on understanding the pathophysiology of glioblastoma, treatment of glioblastoma with nanobodies and exploring the role of a potential glioblastoma biomarker FREM2.

Study on the influence of estrogens on the responsiveness of an ovarian cancer cell line to carboplatin

Tinkara Kreft[1], Nika Marolt[1], Tea Lanišnik Rižner[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

e-mail: tea.lanisnik-rizner@mf.uni-lj.si, tinkara.kreft@gmail.com

High-grade serous ovarian cancer (HGSOC) is the most lethal form of ovarian cancer, mainly because of late diagnosis and development of resistance to platinum-based chemotherapy. Estrogens can promote cell proliferation via estrogen receptors (ERs) or show genotoxic properties through their metabolites; however, the exact role of estrogens in HGSOC pathogenesis remains unclear.

The aim of this study was to investigate the role of estrogens in the chemoresistance of the HGSOC cell line OVCAR-3. First, we established a carboplatin-resistant cell line OVCAR-3/R by prolonged carboplatin exposure and confirmed its higher resistance compared to the parental cell line by determining IC_{50} values. We then performed targeted transcriptomics analysis on both cell lines by qPCR to compare the expression of genes involved in estrogen biosynthesis, metabolism, and estrogen action. With western blotting (WB), we evaluated the expression of two ERs (ERα and GPER) and steroid sulfatase (STS), the main enzyme of the estrogen biosynthesis pathway. With LC-MS/MS we assessed the capacity of OVCAR-3 to form active estrogens from the precursor estrone sulfate (E1-S). Finally, we investigated the effects of estrogens (estradiol, ethinylestradiol and equilin) on cell proliferation and sensitivity to carboplatin.

We found a significant increase in *ESR1* expression in OVCAR-3/R *vs* OVCAR-3. The mRNA levels of ERs were relatively low, whereas WB showed high protein levels of GPER. STS expression was also confirmed at the protein level. E1-S metabolism study showed that OVCAR-3 cell line does not metabolize E1-S to active estrogens. This might be due the formation of less active STS variants. Natural and synthetic estrogens had no significant effects on OVCAR-3 cell viability or sensitivity to carboplatin. Further studies are ongoing to better understand the role of estrogens in HGSOC chemoresistance, which may contribute to development of effective targeted therapies.

This study was funded by J3-2535 grant to T.L.R. from the Slovenian Research Agency.

Tinkara Kreft Biotechnology student at the Biotechnical Faculty, University of Ljubljana, currently working on her Master thesis under the mentorship of prof. dr. Tea Lanišnik Rižner and work supervision of Nika Marolt.

Variants identified in Slovenian patients with hereditary erythrocytosis

Aleša Kristan[1], Petra Hudler[1], Tadeja Režen[2], Jure Stojan[2], Tanja Kunej[3], Rok Količ[4], Andrej Vuga[4], Saša Anžej Doma[5], Irena Preložnik Zupan[5], Tadej Pajič[6], Aleš Maver[6], Nataša Debeljak[1]

[1]Medical Centre for Molecular Biology, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[3]Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Domžale, Slovenia

[4]Kemomed Research and Development, Kemomed Ltd., Ljubljana, Slovenia

[5]Department of Haematology, University Medical Centre Ljubljana, Ljubljana, Slovenia

[6]Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia

alesa.kristan@mf.uni-lj.si

Hereditary erythrocytosis is a rare genetic haematological disorder presented as increased number of erythrocytes that can lead to severe thromboembolic complications. The cause of erythrocytosis remains unknown in the majority of the cases, suggesting that the aetiology of the erythrocytosis is extremely heterogeneous and difficult to diagnose. However, identifying the specific cause of erythrocytosis is important for proper management of the patient.

The aim of the study was to identify genetic cause of erythrocytosis in Slovenian patients suspected of hereditary erythrocytosis and elucidate the role of novel variants in the development of the disease. Targeted NGS covering 39 erythropoiesis-associated genes was applied for genetic analysis of 31 patients and their healthy relatives. Selected variants were further functionally assessed with 3D protein model, protein expression analysis and luciferase reporter assay.

A known pathogenic *EPAS1* variant c.1609G>A p.(Gly537Arg), responsible for the development of hereditary erythrocytosis type 4 (ECYT4), was identified in one patient. Genetic screening also revealed four variants of unknown significance (VUS) in the genes *EPAS1*, *EGLN1*, *JAK2*, and *SH2B3*. Additionally, a high proportion of patients were carriers for two *HFE* variants, suggesting a possible association between the *HFE* and erythrocytosis. VUS in the *EGLN1* gene, c.1072C>T p.(Pro358Ser), was observed in strong co-segregation with the disease in one family and was further functionally assessed. The selected variant was positioned in an active site of the EGLN1 hydroxylase and expression analysis showed significantly decreased protein level of variant in comparison to the wild-type EGLN1 protein. Both results implicate reduced activity of the protein. However, luciferase reporter assay did not confirm the effect on impaired EGLN1 activity. Therefore, further tests are undergoing to confirm the pathological impact of this variant.

Aleša Kristan received a Master's degree (M.Sc) in Biotechnology in year 2018. Currently she is a PhD student at the Medical Centre for Molecular Biology, Institute of Biochemistry and Molecular Genetics and involved as a researcher in the applicative research project Genomic of erythrocytosis.

Clinical features and genetic background of the rare retinal degenerative disease: Macular Telangiectasia type 2 (MacTel 2)

Ajda Kunčič[1], Mojca Urbančič[2], Darja Dobovšek Divjak[2], Petra Hudler[1], Nataša Debeljak[1]

[1]Medical Centre for Molecular Biology, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Ophthalmology, University Medical Centre Ljubljana, Ljubljana, Slovenia

ajda.kuncic@mf.uni-lj.si

Macular Telangiectasia type 2 (MacTel 2) is a slowly progressive bilateral disease of the macula affecting patients over the age of 40 years. The disease is equally prevalent worldwide with no racial predilection. It usually starts in the temporal parafovea, vision loss occurs due to photoreceptor atrophy. The prevalence has been estimated to be 0.0045 to 0.1% in four population-based studies [1,2]. Typical clinical findings include reduced retinal transparency, crystalline deposits, retinal pigment plaques, foveal atrophy, mildly ectatic capillaries, and blunted venules. Subretinal neovascularization may develop and further reduce vision. The cause of the disease is still unknown. A genetic predisposition has been proposed through the relatives' studies. However, the genetic background is complex and much remains undiscovered. The objective of this study was to retrospectively analyze and characterize the clinical features of Slovenian patients with MacTel 2. Additionally, we investigated published literature and data from databases to define metabolic pathways and genes associated with the disease. A total of 48 patients, with a mean age of 68 years, have been diagnosed at the Department of Ophthalmology, UMC Ljubljana. To date, two genome-wide association studies (GWAS) were conducted on 1067 patients [3]. These studies have identified 22 single nucleotide variants (SNVs) in genes implicated in the retinal vascular diameter, glycine/serine metabolic pathway, and sphingolipid and cholesterol metabolism. Consistent with these findings, patients have significant deficiency of blood serine and glycine, and elevated blood alanine and deoxysphingolipids. Here we will elucidate clinical features, genetic background, and metabolic profile of patients with MacTel 2 to enable future ground for early diagnosis and personalized treatment.

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Ajda Kunčič
PhD student (young researcher) at the University of Ljubljana, Faculty of Medicine, Institute of Biochemistry and Molecular Genetics, Medical Centre for Molecular Biology.

Fungal extracellular particles role in adaptation to environmental stress

Teja Lavrin[1], Samo Hudoklin[2], Peter Veranič[2], Ana. Plemenitaš[1], Metka Lenassi[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

teja.lavrin@mf.uni-lj.si

Fungal extracellular vesicles are important for intercellular communication in pathogenicity, but their role in adaptation to environmental stress is lacking. To address this, we isolated and characterized extracellular particles (EPs) released from *Hortaea werneckii*, a model extremotolerant fungus known to adapt to high osmolarity conditions.

Specifically, *H. werneckii* was cultured in defined media with or w/o melanin biosynthesis inhibitor and 3M NaCl. EPs were isolated from conditioned media by combining ultracentrifugation with separation on density gradient or size exclusion chromatography. EPs morphology was characterized by electron microscopy, concentration and size by nanoparticle tracking analysis and molecular content by immunoblotting and spectrophotometry. The role of EPs was evaluated by *in vitro* functional assay in different conditions.

We successfully optimized isolation of EPs from non-stressed *H. werneckii* cultures. Micrographs showed heterogeneous nature of EPs, which also included vesicles with cupshaped morphology. *H. werneckii* EPs were released to high concentrations in culture media (1.7×10^9 EPs/mL) and had an average mode diameter of 97 nm. They carried typical EVs marker proteins α-tubulin and GAPDH, but additionally packed Hog1, the main kinase in osmotic stress response. Further separation of EPs based on density or size showed heterogeneous population that can be stratified into 3 subpopulations: vesicles, vesicles with bound melanin, and melanin particles. Importantly, low nutrient availability, high osmolarity and absence of melanin affected EP subpopulations. Functional assays showed that the presence of melanin in EPs improved growth of *H. werneckii* culture exposed to high osmolarity.

To conclude, extremotolerant *H. werneckii* releases EPs enriched in melanin and protein Hog1, which contribute to osmotic stress adaptation.

Teja Lavrin is a young researcher in the Laboratory for Extracellular Vesicle Research. Study interests: HIV Nef protein and Nef-carrying extracellular vesicles and their role in HIV-associated neurocognitive disorders; fungal EVs in relation to pathology and ecology.

Treatment with PCSK9 inhibitors affects expression of microRNAs in patients with very high lipoprotein(a) levels

Tina Levstek[1,2], Tina Karun[1], Nina Jadrič[1], Nik Podkrajšek[1], Andreja Rehberger Likozar[3], Miran Šebeštjen[3,4,5], Katarina Trebušak Podkrajšek[1,2]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

[3]Department of Vascular Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

[4]Department of Internal Medicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[5]Department of Cardiology, University Medical Centre Ljubljana, Ljubljana, Slovenia

tina.levstek@mf.uni-lj.si

There is growing evidence that dysregulation of microRNAs (miRNAs) contributes to the development and progression of atherosclerosis. Additionally, miRNAs are involved in the regulation of proprotein convertase subtilisin-kexin type 9 (PCSK9), one of the regulators of low-density lipoprotein cholesterol. We aimed to identify the expression of miRNAs previously reported to be involved in PCSK9 regulation and to evaluate the changes in inflammatory parameters after treatment with PCSK9 inhibitors (PCSK9i).

A total of 69 patients with stable coronary artery disease (CAD) after premature myocardial infarction were included in the study. All patients had extremely elevated lipoprotein(a) levels and received a PCSK9i. Clinical and laboratory parameters were measured before and after six months of placebo and after six months of treatment period. In addition, 16 age- and sex-matched control subjects were included. RNA was isolated from plasma samples. Expression of selected miRNAs was determined by qPCR.

PCSK9 levels were significantly higher in control subjects compared with patients. After 6 months of treatment, total serum PCSK9 levels increased significantly. The expression of miR-191-5p was significantly lower, and the expression of miR-224-5p and miR-483-5p was significantly higher in patients compared with control subjects. Using linear regression, the expression of miR-483-5p significantly predicted the serum PCSK9 level at baseline. After the 6-month treatment period, the expression of miR-191-5p and miR-483-5p significantly increased. Consistent with previous studies, no significant change in the levels of hs-CRP, TNF-α, and IL6 was observed after 6 months of treatment.

Our findings provide evidence for the involvement of miR-483-5p in the regulation of circulating PCSK9 *in vivo*. Moreover, the observed differences in the expression of miR-191-5p, miR-224-5p,

and miR-337-3p between patients and control subjects suggest their possible contribution to the development of CAD.

Tina Levstek is a PhD student at the Faculty of Medicine. She obtained her master's degree in laboratory biomedicine at the Faculty of Pharmacy, University of Ljubljana. She is currently employed as a young researcher at the Institute of Biochemistry and Molecular Genetics at the Faculty of Medicine.

Estrogen metabolism and aldo-keto reductase activity interplay in chemoresistance of ovarian cancer

Nika Marolt[1], Renata Pavlič[1], Marija Gjogorska[1], Alja Zottler[1], Tea Lanišnik Rižner[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

nika.marolt@mf.uni-lj.si; tea.lanisnik-rizner@mf.uni-lj.si

High-grade serous ovarian carcinoma (HGSOC) is the most common ovarian cancer (OC) that usually develops resistance to chemotherapeutics. Estrogens are linked to increased metastatic potential and proliferation of cancer cells. AKR1C enzymes are associated with resistance to chemotherapeutics and may contribute to the growth of hormone-dependent tumors. To date, the role of estrogens and AKR1C in chemoresistance of HGSOC remains unclear. We performed targeted transcriptomics including genes involved in estrogen metabolism, steroid precursor transport, and oxidative metabolism in HGSOC cells (OVSAHO, OVCAR-3, Kuramochi, OVCAR-4, Caov-3 and COV362) with different sensitivity to carboplatin. Results indicate the potential of *ABCG2, ESR2, STS*, *HSD17B14*, *NOQ1*, *GSTP1*, and *AKR1C1* as predictive markers for chemoresistance. LC-MS/MS analysis showed formation of active estrogens only in cells sensitive to carboplatin, except OVCAR-3, possibly due to a short STS isoform, but not in the most resistant cells COV362. To examine the effects of carboplatin resistance on *HSD17B14* and *CYP1A2* expression, associated with prognosis in HGSOC tissues, we established HGSOC cells with higher carboplatin resistance and used publicly available data (PAD). We found the potential of *HSD17B14* as a predictive tissue marker for chemoresistance of HGSOC tumors and as a prognostic biomarker candidate. PAD study revealed moderate to strong positive correlations between gene pairs such as *AKR1C1 – AKR1C3, AKR1C1 – NFE2L2, AKR1C1 – SULT1E1, NOQ1 – HSD17B14, COMT – SULT1A1, ABCG2 – SLC515* in chemoresistant patients and a strong positive correlation between the *CYP1B1 – SULT1E* gene

pair in chemosensitive patients. By using Kaplan-Meier plotter we found that *AKR1C1*, *AKR1C2*, *NFE2L2*, *GPER* and *ABCC4* genes affect survival of serous OC patients. Further studies are ongoing to elucidate the mechanism of the interplay between estrogen metabolism and AKR1C activity in chemoresistance of HGSOC.

Nika Marolt obtained a Master's degree in Biotechnology from the Biotechnical Faculty of the University of Ljubljana. Presently, she is pursuing a PhD in Biochemistry and Molecular Biology, with emphasis in cancer research and chemoresistance at the Institute of Biochemistry and Molecular Genetics.

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Steps towards the establishment of a new human stromal cell line for peritoneal endometriosis

Maja Pušić Novak[1], Helena Ban Frangež[2], Tea Lanišnik Rižner[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Obstetrics and Gynecology, University Medical Centre, Ljubljana, Slovenia

maja.pusic@mf.uni-lj.si

Endometriosis is a chronic gynecological disorder characterized by the presence of endometrial-like tissue outside the uterus cavity. This complex, inflammatory disease affects 10% of women in their reproductive age, or approximately 190 million women worldwide. New experimental models of endometriosis are needed to better understand the pathophysiology of endometriosis and to discover new diagnostic and treatment options for this debilitating disease. The aim of this study was to establish a new in vitro model cell line that can be used to study peritoneal endometriosis at the cellular level. To this end, the protocol for isolation and cell cultivation of endometriotic primary cells from peritoneal endometriosis was optimized. The three steps of isolation and cultivation of primary cells were optimized: 1) content of digestion solution, 2) separation of cells, and 3) batch of fetal bovine serum (FBS) added to the culture medium. Subsequently, the cell morphology and viability of obtained cells were determined. The established protocol for isolation of endometriotic primary cells includes the following steps: mincing the endometriotic

tissue with a scalpel, digestion of tissue samples in a digestion solution containing collagenase type I and DNase I, separation of epithelial and stromal cells with a 70 µm and 40 µm cell strainer and cultivation of cells in DMEM/F12 medium containing 10% FBS. All primary cells obtained were Mycoplasma negative, reached confluence within 2 weeks with high viability and appropriate cell morphology. Future experiments will include complete characterization of the cells by determining their migratory ability, lack of cell senescence, and expression of morphological markers and estrogen-associated genes. After establishing their purity, they will be used for immortalization using the human telomerase reverse transcriptase plasmid (hTERT).

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Maja Pušić Novak is a postdoctoral researcher at the IBKMG, Faculty of Medicine, University of Ljubljana. She received her PhD in the field of biomedicine at University of Zagreb. Currently, her research focuses on discovery of novel biomarkers for non-invasive diagnosis of endometriosis and establishing *in vitro* cell models for endometriosis research.

Gastric cancer associated PLK2 haplotype affects miR-23b-5p binding

Pia Pužar Dominkuš[1], Aner Mešić[2], Petra Hudler[3]

[1]Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3]Medical Centre for Molecular Biology, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

pia.puzar-dominkus@mf.uni-lj.si

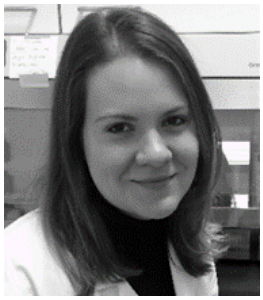
Stomach cancer is globally ranked as the fifth deadliest cancer and represents a high health burden. Various molecular and genetic factors contribute to the development and progression of the disease. The latter include single nucleotide polymorphisms (SNPs) in cell cycle genes that contribute to chromosomal instability. The aim of our study was to analyse the associations between single nucleotide polymorphisms in cell cycle genes with gastric cancer risk and clinicopathological features and to evaluate their functional consequences.

We enrolled 221 patients and 321 controls in the genotyping study. The associations between

SNPs in candidate genes and the risk and clinicopathological features of gastric cancer were analysed with the GLM. We confirmed the binding of candidate miRNA to the polymorphic allele *in vitro* with the luciferase reporter assay. We analysed the differential expression of the candidate miRNA using the TCGA and GEO datasets.

The *PLK2* $C_{rs15009}$-$C_{rs963615}$ haplotype was less frequent in the gastric cancer group compared with the control group (0.337% vs. 0.402%; P_{corr}=0.050). The *PLK2*-rs15009 polymorphic allele (C/G) differentially bound miR-23a-5p and miR-23b-5p. Relative luciferase activity in cells expressing the *PLK2* 3′ UTR with the G variant was decreased by 41% upon co-transfection with miR-23b-5p mimic (P=0.0097). Low miR-23b-5p expression was associated with longer 10-year survival in gastric cancer patients (HR=1.52; *P*=0.007).

PLK2 haplotype $C_{rs15009}$-$C_{rs963615}$ could serve as a risk biomarker, whereas miR-23b-5p expression level could be used as prognostic survival biomarker in gastric cancer patients.



Pia Pužar Dominkuš is a research assistant at the Pharmacogenetics laboratory and habilitated teaching assistant at the Institute of Biochemistry and Molecular Genetics. Her research interests are pharmacogenomics, molecular genetic background of cancer and the role of extracellular vesicles in human disease.

The frequency of connexin 26 (GJB2) genetic variant c.-23+1G>A in Slovenian patients with hearing loss

Emma Ravnihar[1], Inge Sotlar[2], Tina Levstek[2,3], Saba Battelino[4,5], Katarina Trebušak Podkrajšek[2,3]

[1]Bežigrad Grammar School, Ljubljana, Slovenia

[2]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[3]Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

[4]Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia

[5]Department of Otorhinolaryngology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

emma@ravnihar.si

Pathogenic variants of the *GJB2* gene are the most prevalent cause of nonsyndromic hearing loss and are usually associated with childhood-onset hearing loss inherited in an autosomal recessive manner. Substitution c.-23+1G>A (rs80338940) is a common non-coding pathogenic *GJB2* variant in the Caucasian population with hearing loss, with an estimated frequency of 2.3% in Europe. We aimed to investigate the frequency of this variant by TaqMan genotyping in Slovenian patients with hearing loss (n = 408) and control participants with normal hearing (n = 369).

The results showed that only five (0.64%) subjects were heterozygous for variant c.-23+1G>A, whereas 772 (99.36%) subjects had a normal genotype and no homozygous genotypes were detected. Four heterozygous subjects were patients with hearing loss (0.98%) and one was a control participant (0.27%). The frequency of the variant was 0.49% in patients with hearing loss, and 0.13% in control participants. In our study, the difference in the frequency of *GJB2* c.-23+1G>A variant in patients with hearing loss compared to healthy control participants in the Slovenian population was not statistically significant ($p = 0.377$). However, the frequency of the c.23+1G>A variant was considerably higher in Slovenian patients with hearing loss (0.49 %) compared to the general European (non-Finnish) population (0.03%).

The role of the c.23+1G>A *GJB2* variant in the development of hearing loss in the Slovenian population was not as significant as might have been expected from the literature. However, further studies in a larger cohort would be needed to adequately assess the frequency of this variant.

Emma Ravnihar is a graduate of the IB Diploma Programme in Bežigrad Grammar School, where her research work was awarded on National Young Researchers Competition in 2022. Later she participated in research activities at the Institute of Biochemistry and Molecular Genetics of the Faculty of Medicine.

Influence of sterol intermediates: insights from the targeted knockout of CYP51A1 on LEF1-mediated transcriptional activation and cellular functions

Cene Skubic[1], Hana Trček[1], Petra Nassib[1], Andrew Walakira[1], Tadeja Režen[1], Damjana Rozman[1]*

[1]Center for Functional Genomics and Bio-Chips, Institute of Biochemistry and molecular genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

cene.skubic@mf.uni-lj.si

We explored the significance of various sterols which are essential components of cell membranes, and their potential impact on a wide range of cellular processes. The biosynthesis of cholesterol

entails a series of enzymatic reactions that give rise to multiple sterol intermediates. These intermediates, in turn, exert influence over the activation of distinct signaling pathways.

To gain insights into the specific roles of individual sterols accumulated in our cell models, we employed targeted knockouts of consecutive genes encoding enzymes involved in cholesterol synthesis, *CYP51A1*, *DHCR24*, *SC5D*, and *HSD17B7*. Additionally, we conducted targeted metabolomics using LC-MS and performed the transcriptomic analysis. These methods enabled us to determine the distinct effects of specific sterols.

Our findings unveiled that particular sterols exhibit diverse influences on gene regulatory pathways. For instance, early sterols such as 24,25-dihydrolanosterol, were found to promote cell proliferation and cell cycle progression. Conversely, the accumulation of other sterols resulted in reduced proliferation and the promotion of apoptosis and tumor suppressor pathways. Intriguingly, only 24,25-dihydrolanosterol, and no other sterols, activates LEF1 signaling through the NFKB/WNT pathway, thereby affecting downstream signaling events.

By advancing our understanding of the interplay between sterol intermediates and gene regulation, our study highlights the critical role of sterols in governing gene expression and their potential importance in cellular processes such as LEF1 activation and cell cycle control. Moreover, our results strongly suggest that maintaining a balanced ratio of different sterols is crucial for sustaining normal cellular functions.

Assist. Cene Skubic finished masters studies of molecular and functional biology at Biotechnical Faculty, University of Ljubljana and is currently in the final stages of PhD studies at Faculty of Medicine UL under the mentorship of prof. Damjana Rozman. He works within the CFGBC research group, focusing on the novel roles of sterols in gene regulation and cellular processes. He is involved also in the ESFRI – ELIXIR infrastructure, involved in developing the long read sequencing pipelines and LC MS/MS protocols.

Characterizing the effects of lanthanide ions and aromatic substrate/inhibitor on Paraoxonase 1 functionality

Janez Smerkolj[1], Jure Stojan[1], Aljoša Bavec[1], Marko Goličnik[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

janez.smerkolj@mf.uni-lj.si

Paraoxonase 1 (PON1) is a Ca^{2+}-dependent serum enzyme of mammals that belongs to the paraoxonase enzyme family along with paraoxonase 2 (PON2) and paraoxonase 3 (PON3). It is a

43 kDa glycoprotein that hydrolyzes a wide range of compounds, including (thio)esters, lactones, oxidized lipids, estrogen esters, homocysteine thiolactone, phosphorous esters (such as paraoxon, organophosphate pesticides, and nerve gases) and participates in drug metabolism.

Accurate determination of hPON1 concentration in biological samples is methodologically difficult, relatively expensive, and currently unreliable. As a result, in clinical studies, most researchers rely on evaluating the enzyme's specific activity normalized to various body fluid contents. The commonly used substrates in such studies include paraoxon (a toxic organophosphate), phenyl acetate, 4-nitrophenyl acetate, dihydrocoumarin (DHC), and 5-thioalkyl butyrolactone. However, the different substrate-specific kinetic parameters of the enzyme make comparing results from such studies difficult. Furthermore, buffer selection, temperature, ionic strength, and sample collection all have an impact on the kinetic results.

Due to these methodological difficulties, the determination of hPON1 content and activity, despite its potential diagnostic role in many diseases, is currently limited to research laboratories and has not been implemented in routine clinical laboratories. Routine clinical determination of analytes relies on fast, simple, and cost-effective methods. Therefore, we aim to study the effects of replacing Ca^{2+} ions with Ln^{3+} ions on the structure and properties of rePON1. Additionally, the right conditions and interactions of rePON1, Ln^{3+} and aromatic inhibitor 2-hydroxyquinoline (2HQ) might exhibit time-resolved fluorescence spectroscopic properties, which could be further utilized for determining hPON1 concentration in biological samples. Therefore, we are interested to characterize the effects of Ln^{3+} ions and aromatic substrate/inhibitors on PON1 functionality.

Our results of measuring rePON1 activity with time-courses of product formation showed that rePON1 was significantly inactivated during the catalytic DHC turnover. The activity of rePON1 was not lost due to product inhibition or spontaneous inactivation in the sample buffers. The progress curves of DHC hydrolysis by rePON1 indicate self-inactivation during the catalytic turnover of DHC. Additionally, human serum albumin or surfactants protected rePON1 from inactivation during this process. We carried out also a detailed progress curve analysis in the absence and presence of Eu^{3+} and Tb^{3+} ions with ionic radius similar to Ca^{2+} ions. The analysis shows inhibitory effect of Ln^{3+} ions due to possible replacement of catalytic Ca^{2+} ion by lanthanide ions. Together with known inhibitory effect of 2HQ, we are eager to prepare and characterize Ln^{3+}/2HQ/PON1 complexes in further studies.

Epigenetics of the Slovene male suicides

Iris Šalamon Arčan[1], Katarina Kouter[1,2], Mojca Katrašnik[1], Urban Alič[1], Tomaž Zupanc[3], Alja Videtič Paska[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia

[2]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia

[3]Institute of Forensic Medicine, Faculty of Medicine, University of Ljubljana, Slovenia

iris.salamon@mf.uni-lj.si

Suicide is an important public health issue, with more than 700,000 deaths every year. Epigenetic studies offer a promising avenue to explore the links between environmental and biological factors contributing to it.

We investigated three different aspects of the epigenetics (DNA methylation, miRNAs, and histone modification) on different tissue of Slovene male suicides. Firstly, genome-wide differential DNA methylation was determined by reduced representation bisulfite sequencing in hippocampus and Brodmann area 9, followed by targeted sequencing of 8 neuropsychiatric candidate genes in brain regions (hippocampus, insula, amygdala, Brodmann area 46) and blood. Secondly, we investigated miRNA expression in the Brodmann area 10. Based on a prediction algorithm, we have chosen miRNAs that are targeting regulation of the genes *SLC6A4*, *HTR1A*, *BDNF*, *NR3C1*, *ZNF714*, and *NRIP3*. In addition, we investigated several suicide-associated miRNAs on extracellular vesicles (EVs) of the cerebrospinal fluid. Thirdly, the acetylation of lysine 14 on histone 3 (H3K14ac) was determined with chromatin immunoprecipitation and next-generation sequencing.

By genome-wide approach, several differences in methylation level between suicides and controls in both brain regions (> 25% different methylation, q-value < 0.01) were found. Two genes, *ZNF714* and *NRIP3*, were determined also as differentially expressed. By targeted amplicon analysis changes were observed in *NR3C1* (insula), *MAOA* (blood), *SKA2*, *HTR1A*, and *GABRA1* (insula, blood), and *NRIP3* (hippocampus, amygdala). Two of miRNAs tested on Brodmann area 10, miR-4516 and miR-381-3p, showed a trend for statistical significance, and 2 out of 9 in EVs present miRNAs, miR-19a-3p and miR-4516, reached statistical significance. The study of H3K14ac showed an overall decrease in H3K14ac in the hippocampus of suicides. From 293046 peaks, 1682 peaks reached statistical significance (q-value ≤ 10^{-5}).

Epigenetics is rather novel filed in research of suicide and psychiatry in general, but represents an important insight in molecular mechanism that lie behind these complex disorders.

Iris Šalamon Arčan, young researcher and a PhD student at the Center for Functional Genomics and Bio-chips. I am investigating the contribution to the suicidal phenotype of miRNAs from the cerebrospinal fluid and histone tail modifications from hippocampus in a group of assoc. prof. dr. Alja Videtič Paska.

Identification and characterization of novel glioblastoma biomarkers for non-invasive liquid biopsy

Neja Šamec[1], Ivana Jovchevska[1], Alja Zottel[1], Metka Novak[2], Barbara Breznik[2], Polonca Mali[3], Klara Železnik[3], Andrej Porčnik[4], Marija Vidmar Skoblar[5], Jernej Kovač[6], Blaž Vrhovšek[6], Tine Tesovnik[6]

[1]Institute of Biochemistry and Molecular genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]National Institute of Biology, Ljubljana, Slovenia

[3]Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

[4]Department of Neurosurgery, University Medical Centre Ljubljana, Ljubljana, Slovenia

[5]Institute of Oncology Ljubljana, Ljubljana, Slovenia

[6]Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

neja.samec@mf.uni-lj.si

Glioblastoma, the most common and lethal form of brain tumor, poses challenges in early diagnosis due to non-specific symptoms, its aggressive nature and lack of biomarkers. To overcome this, identification of non-invasive liquid biopsy biomarkers is essential for diagnostic and therapeutic purposes. A cDNA library was obtained from RNA, isolated from 13 post-mortem normal brain tissues and 10 glioblastoma tissues using the NEBNext rRNA depletion kit. Library quality was assessed using a Bioanalyzer, and sequencing was performed on the NovaSeq 6000 system (Illumina). Bioinformatics analysis of next-generation sequencing mRNA data was conducted using the SnakePipes tool, incorporating Cutadapt for sequence trimming. The Salmon Pseudo-alignment algorithm was employed for quantification and counting of mRNA sequences, and the R DEseq library was used to calculate basic differential expression metrics. Genes with log2FC > 1 and log2FC < 1, along with an adjusted p-value < 0.05, were considered significantly differentially expressed. Through this analysis, we identified 5132 differentially expressed genes in glioblastoma samples. Notably, EMILIN, TUBB6, PLA2G2A, FMOD, and CHRDL2 emerged as the most promising overexpressed genes for detection in liquid biopsies. By combining the results of earlier studies from Jovcevska *et al* (1, 2), Zottel *et al* (3) and Vidak *et al* (4), we propose a comprehensive approach for the diagnosis and monitoring of glioblastoma. Combining biomarkers from the glioblastoma tissue using a reverse proteomic approach with camelid nanobodies, (ALYREF, DPYSL2, FREM2, TUFM, TRIM28, VIM, CRMP1, SPRY1, NAP1L1), and the identified overexpressed genes, EMILIN, TUBB6, PLA2G2A, FMOD, and CHRDL2, obtained from RNAseq, holds great promise in enabling early detection and non-invasive monitoring of glioblastoma. This research enhances clinical outcomes by offering accessible biomarkers for timely interventions and personalized treatment strategies in glioblastoma patients.

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Assist. dr. Neja Šamec current position is Postdoctoral Researcher at the CFGBC. Her research interests are proteomics and transcriptomics of glioblastoma, production and characteristics of nanobodies as a tool to obtain new biomarkers and liquid biopsy of glioblastoma.

Genetic variability in glucocorticoid pathway and disease severity in COVID-19 patients

Patricija Štampar[1], Tanja Blagus[1], Petra Bogovič[2], Gabriele Turel[2], Franc Strle[2], Vita Dolžan[1]

[1]Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

patricija.stampar@mf.uni-lj.si

Introduction: COVID-19 disease may manifest in various symptom severity, ranging from mild to severe or critical. When the body responds to the viral infection, some patients produce an excessive amount of pro-inflammatory cytokines, resulting in a phenomenon known as a cytokine storm. Glucocorticoids, possessing anti-inflammatory and immune-suppressive properties, are the primary treatment option for managing severe cases of COVID-19. This study investigated associations of glucocorticoid pathway polymorphisms with disease severity and need for intensive care unit (ICU) treatment.

Methods: In total 107 COVID-19 patients treated with dexamethasone were genotyped for *NR3C1* (rs6198, rs33388, rs33389), *CYP3A4* (rs35599367, rs2740574), *CYP3A5* (rs776746), GSTP1 (rs1695,

rs1138272), *GSTM1/GSTT1* deletions, and *ABCB1* (1045642, rs1128503, rs2032582). Statistical analysis employed logistic regression, Mann-Whitney, and Fisher's tests.

Results: We recruited 69.2% male patients and 30.8% females, with median age 62 years (26–85). Only 1.9% of the patients exhibited moderate disease, while the majority (83.0%) experienced severe symptoms, and 15.1% faced critical conditions. *CYP3A4* rs35599367 carriers had higher odds of critical disease (OR = 6.69, 95% CI = 1.22–36.75, p = 0.029) and ICU treatment (OR = 10.22, 95% CI = 1.79–58.27, p = 0.009). *GSTP1* rs1138272 carriers had a higher odds of ICU treatment (OR = 4.88, 95% CI = 1.33–17.87, p = 0.017), while *NR3C1* rs33388 carriers had lower odds (OR = 0.15, 95% CI = 0.03–0.79, p = 0.025).

Conclusions: Glucocorticoid pathway polymorphisms relate to disease severity and treatment response in COVID-19. The associations found between *CYP3A4* rs35599367, *NR3C1* rs33388, *GSTP1* rs1138272 and studied outcomes may suggest the potential influence of these polymorphisms on the severity and course of the COVID-19 disease.

Patricija Štampar has M.S. degree in Molecular and Functional Biology. She started her PhD in 2021 at the Faculty of Medicine, UL, the interdisciplinary program of Biomedicine. During her PhD, she is focusing on exploring biomarkers that could predict the outcome of COVID-19, under the supervision of prof. Vita Dolžan.

A study of androgen synthesis in endometrial cancer model cell

Lea Šturm[1], Marija Gjorgoska[1], Tea Lanišnik Rižner[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

lea.sturm@mf.uni-lj.si, tea.lanisnik-rizner@mf.uni-lj.si

Endometrial carcinoma (EC) is one of the most common gynecological malignancy, which mostly occurs after menopause. Depending on the histological characteristics, EC has been divided into type 1 and type 2. Type 1 is usually low grade, hormone-receptor positive and associated with obesity and metabolic syndrome. Type 2 is associated with non-endometrioid histology, worse prognosis and it's usually hormone-receptor negative and more likely to metastasize.

It is well known that estrogens play pivotal role in the development and progression of EC. Estrogens promote cell proliferation, which leads to a greater chance of DNA replication errors. The impact of androgens on the formation and progression of EC is not yet fully understood. Epidemiological evidences suggests that there is increased risk of EC associated with elevated blood concentrations of androgens. Other studies have shown that AR-dependent signaling inhibits cell proliferation and that androgens could play a protective role in the development and progression of EC.

The aim of this study was to evaluate the metabolism of androgens in EC model cell lines and a control cell line. Cell lines were treated with different steroid precursors in the synthesis of active classical androgens and 11-oxyandrogens. Metabolites were extracted by solid-liquid or liquid-liquid extraction and then detected by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Based on the results, we found differences in metabolism between model cell lines. The highest levels of active classical and 11-oxy androgens were observed in well-differentiated cell lines RL95-2 and Ishikawa, while the lowest levels were found in KLE, which represents poorly differentiated EC. From the obtained results, we conclude that androgens may have an inhibitory effect on the development of EC but further research is needed to provide additional evidence.

This study was supported by J3-2535 grant to T.L.R. from the Slovenian Research Agency.

Lea Šturm: has a master's degree in pharmacy and she wrote her master's thesis at the Institute of Biochemistry and Molecular Genetics. Now she works as a researcher in the Laboratory for molecular basis of hormone-dependent diseases and biomarkers.

Exploring the circRNA transcriptome in hepatocellular carcinoma through long-read nanopore sequencing

Hana Trček[1], Rok Razpotnik[1], Blaž Trotovšek[2], Mihajlo Đokić[2], Miha Petrič[2], Boštjan Plešnik[2], Arpad Ivanecz[3], Damjana Rozman[1], Tadeja Režen[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Division of Hepatobiliary Surgery and Liver Transplantation, Department of Abdominal Surgery, University Medical Center Ljubljana, Ljubljana, Slovenia

[3]Department of Abdominal and General Surgery, University Medical Center Maribor, Maribor, Slovenia

hana.trcek@mf.uni-lj.si

Due to a notable surge in the incidence and mortality rate of hepatocellular carcinoma (HCC), which accounts for more than 90% of primary liver cancers, HCC is becoming a major global health problem. Current diagnostics are very limited, which often results in detection at an advanced stage. Despite the great interest in circular RNAs (circRNAs) in cancer research, including HCC pathology in the past decade, transcriptomic data is still very limited. These studies were performed exclusively on samples from Chinese and Singaporean patients, where viral infections are still the main risk factors for HCC. Furthermore, only Illumina and microarray technologies were used, which present limitations in the precise length and sequence determination of circRNAs.

Considering the above-mentioned limitations, we aim to obtain transcriptomic data on a European population of HCC patients, using long-read Nanopore sequencing. To do that, we will sequence enriched circRNAs from tumor and adjacent non-tumor liver tissue from 10 HCC patients (20 samples in total). Sequencing of circRNAs presents challenges, such as a small proportion of circRNAs, requiring their enrichment prior to sequencing, and lack of poly(A) tail, required for library preparation. To overcome this, we will follow a protocol, where first circRNAs will be enriched, then linearized and finally polyadenylated. This will allow us to prepare cDNA libraries and sequence samples using the Nanopore cDNA-PCR Sequencing Kit on GridION.

We believe that by analyzing circRNA transcriptome data from the European population of HCC patients (without HBV or HCV etiology) our study will significantly contribute to the understanding of the association between etiology, molecular subtypes, and circRNA expression in HCC.

assist. razisk. **Hana Trček,** mag. mol. funkc. biol. PhD student of Biomedicine, currently working at the Centre for Functional Genomics and Bio-Chips at the Institute for Biochemistry and Molecular Genetics, Faculty of Medicine, UL. Her current research work focuses on the oncogenic potential of circular RNAs in hepatocellular carcinoma.

Circulating extracellular vesicles as predictors of antidepressant response

Alja Videtič Paska[1], Matea Nikolac Perkovic[2], Gordana Nedic Erjavec[2], Tina Milos[2], Lucija Tudor[2], Suzana Uzun[3,4], Ninoslav Mimica[3,4], Katarina Kouter[5], Iris Šalamon Arčan[1], Mojca Katrašnik[1], Nela Pivac[2], Aleš Oblak[6], Jurij Bon[6,7]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Laboratory for Molecular Neuropsychiatry, Division of Molecular Medicine, Ruder Boskovic Institute, Zagreb, Croatia

[3]Department for Biological Psychiatry and Psychogeriatrics, University Psychiatric Hospital Vrapce, Zagreb, Croatia

[4]School of Medicine, University of Zagreb, Zagreb, Croatia

[5]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[6]University Psychiatric Clinic Ljubljana; Ljubljana, Slovenia

[7]Department of Psychiatry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

alja.videtic@mf.uni-lj.si

Slovenian-Croatian multidisciplinary research project combines basic, preclinical and clinical research, including partners from the academic community (Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia, and University Foundation San Pablo CEU, Spain), research institute (Ruđer Bošković Institute, Croatia), and public health facilities (University Psychiatric Clinic Ljubljana, Slovenia, and University Hospital Centre Zagreb, Croatia). Project incorporates cutting-edge metabolomics approach and miRNA profiling in order to identify novel biomarkers that could help clinicians to tailor treatment strategies in depression for individual patients. Currently available therapy for depression involves pharmacotherapy combined with psychotherapy, while dealing with poor response/nonresponse and a frequent discontinuation of treatment. The objective of the study is to give better insights into the efficacy and molecular mechanisms behind the effects of a widely used antidepressant (duloxetine), and compare this to the mechanism behind the effects of alternative methods of treatment in patients with treatment-resistant depression (Transcranial Magnetic Stimulation (TMS), Bright light Therapy (BTL), esketamine treatment). The study will focus on circulating extracellular vesicles (EVs) as easily obtainable and non-invasive biomarkers. We aim to measure the dysregulation of epigenetic markers - the EV miRNA expression, and to determine metabolic alterations in four groups of patients (duloxetine vs. BLT vs. TMS vs. esketamine). Another goal is to identify specific metabolic and miRNA signatures of depression by comparing groups of patients diagnosed with

depression (non-treatment resistant and treatment resistant depression) with an appropriate healthy control group. We expect that new biomarkers identified in this project will help determine treatment efficiency in depression and predict good or poor response to treatment, as a key step towards the inevitable personalized and effective medicine approach.

Assoc. prof. dr. Alja Videtič Paska is a researcher who has been investigating the molecular basis of psychiatric disorders and suicidal behavior for more than a decade. With her findings, she contributes significantly to the creation of a mosaic of knowledge, especially about genetics and epigenetics. She has already published more than thirty articles, and in addition, she also passes on her knowledge to younger generations through her pedagogical work.

Identification of candidate miRNAs and their expression in blood plasma and CSF samples of Alzheimer's disease patients

David Vogrinc[1], Milica Gregorič Kramberger[2,3,4], Andreja Emeršič[2], Saša Čučnik[2,5,6], Katja Goričar[1], Vita Dolžan[1]

[1]Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Neurology, University Medical Centre Ljubljana, Ljubljana, Slovenia,

[3]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[4]Karolinska Institutet, Department of Neurobiology, Care Sciences and Society, Division of Clinical Geriatrics, Huddinge, Sweden

[5]Department of Rheumatology, University Medical Centre Ljubljana, Ljubljana, Slovenia

[6]Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

david.vogrinc@mf.uni-lj.si

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases. It often begins with mild cognitive impairment (MCI) and some molecular processes precede the onset of clinical symptoms. Cerebrospinal fluid (CSF) biomarker analysis is important in establishing clinical diagnosis, but it requires an invasive procedure to obtain the fluid. There is thus an increasing

need to identify non-invasive biomarkers to support early clinical diagnosis of AD. As miRNAs are important regulators of gene expression and highly stable in body fluids, they bear a great potential as potential novel circulating biomarkers in AD.

With a combined approach of literature screening and database mining, we aimed to identify target miRNAs that have previously been found in body fluids of AD patients. Additionally, we performed enrichment for miRNA-target gene interactions to associate list of identified AD risk genes with target miRNAs. Subsequently, we evaluated the expression of candidate miRNAs (miR-146a-5p, miR-451a, miR-30c-5p, miR-375-3p, miR-107, miR-193-3p and miR-29c-3p) in CSF and blood plasma of AD and MCI patients. We also assessed the associations of investigated miRNAs with CSF biomarker levels.

No significant differences in expression levels between MCI and AD patients were observed for selected miRNAs in either plasma or CSF (all $p>0.05$). In the entire cohort, higher expression of plasma miR-30c-5p was associated with higher CSF total tau ($p=0.035$). In AD patients, higher expression of plasma miR-146a-5p was associated with lower CSF $A\beta_{42/40}$ ratio ($p=0.027$). Additionally, higher expression of CSF miR-146a-5p was associated with higher CSF p-tau181 ($p=0.042$), while higher expression of CSF miR-107 was associated with higher CSF total tau ($p=0.025$). On the other hand, higher CSF miR-29c-3p expression was associated with lower $A\beta_{42/40}$ ratio ($p=0.023$).

In conclusion, we showed the potential of investigated miRNAs as non-invasive biomarkers of cognitive decline.

David Vogrinc is a PhD student at the Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana. He is involved in the research of biomarkers in neurodegenerative diseases. His main focus is on genetic variability as well as small non-coding RNAs and their role in Alzheimer's disease and mild cognitive impairment.

Extracellular vesicle-bound miRNAs as potential biomarkers for noninvasive detection of kidney transplant rejection

Lan Vukolić[1*], Tjaš Žvar[1*], Marija Holcar[1], Katja Goričar[1], Miha Arnol[2,3], Metka Lenassi[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Nephrology, Division of Internal Medicine, University Medical Center Ljubljana, Ljubljana, Slovenia

[3]Department of Internal Medicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

metka.lenassi@mf.uni-lj.si

Kidney transplantation is the most effective therapy for end-stage kidney disease, but unrecognized and untreated rejection injury can contribute to decreased allograft function and survival. Traditional biomarkers for monitoring kidney allograft lack sensitivity and specificity, while histopathologic characterization of kidney biopsies is an invasive and costly procedure with risk of complications. Therefore, novel non-invasive biomarkers are needed to allow frequent monitoring and earlier detection of kidney allograft injury. The aim of this study was to evaluate micro RNAs (miRNAs) associated with extracellular vesicles (EVs) as biomarkers for non-invasive detection of kidney transplant rejection.

We enriched EVs from urine of well-characterized kidney transplant recipients undergoing allograft biopsy, characterized their miRNA cargo using qPCR and evaluated its association to allograft injury. According to histological assessment of the allograft, patients were stratified into normal histology (N = 16), non-rejection injury (N = 13) and rejection injury (N = 19) groups. Patient groups differed significantly in biopsy type (surveillance vs. for-cause), time from transplantation to biopsy, donor specific antibodies, serum creatinine and estimated glomerular filtration rate (all $P < 0.021$). Patients with non-rejection or rejection injury had higher normalized urinary EV concentrations ($P = 0.019$) compared to those with normal histology. Higher normalized miR-155 ($P = 0.003$) and miR-223-3p ($P = 0.051$) expression tended to be associated with lower concentrations of urinary EVs, with miR-223-3p detected at higher levels in patients with rejection injury ($P = 0.035$). Normalized miR-155 ($P = 0.012$) and miR-181a ($P = 0.004$) were associated with the type of biopsy, while miR-223-3p was associated with presence of donor specific antibodies ($P = 0.024$).

Urinary EV-associated miR-223-3p is a potential noninvasive biomarker of kidney transplant rejection, but further studies are needed.

Lan Vukolić and **Tjaš Žvar** are forth year medical student at UL Faculty of Medicine. In the last year, they have worked on the student research project focused on extracellular vesicles as potential biomarkers of kidney transplant rejection, under the supervision of Prof. Miha Arnol, MD, PhD, and Assoc. Prof. Metka Lenassi, PhD.

Exosomes as anti-vimentin nanobody delivery system for glioblastoma targeting

Sara Colja[1], Ivana Jovčevska[1], Neja Šamec[1], Rok Romih[2], Alja Zottel[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[2]Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

alja.zottel@mf.uni-lj.si

Glioblastoma, a highly lethal cancer, necessitates the development of innovative and effective therapeutic approaches. One promising approach is nanobodies, nanosized biologics with beneficial properties. Nanobodies are capable of targeting intracellular proteins, but to increase their efficacy, a delivery system is required. In our study, we investigated the potential of small extracellular vesicles as a delivery system for the anti-vimentin nanobody Nb79. We delivered Nb79 into small extracellular vesicles by three methods: Incubation with glioblastoma cells, passive delivery into isolated small extracellular vesicles, or sonication of isolated small extracellular vesicles. Small extracellular vesicles were isolated from cell medium by ultracentrifugation on a sucrose cushion. The size distribution and average size of sonicated and non-sonicated small extracellular vesicles were analyzed by nanoparticle tracking analysis. Successful incorporation of Nb79 into small extracellular vesicles was confirmed by Western blot and electron microscopy. The effect of small extracellular vesicles on cell survival was investigated using WST-1 reagent. Sonication proved to be a successful method for obtaining Nb79-loaded small extracellular vesicles, as confirmed by Western blot and electron microscopy. Moreover, the small extracellular vesicles showed an effect on cell viability. Small extracellular vesicles lacking Nb79 increased the survival of U251 and NCH644 cells by 20-25%, whereas Nb79-loaded small extracellular vesicles decreased the survival of NCH421k cells by 11%. Our results indicate that sonication is a suitable method to introduce nanobodies into exosomes, and that these small extracellular vesicles can in turn decrease cell survival. This method holds potential for several applications, including targeted delivery systems for other protein-based drugs.

Alja Zottel received PhD degree in Biochemistry and Molecular Biology from the Faculty of Medicine, UL, Slovenia in 2021. She is currently a postdoc researcher at the Center for Functional Genomics and Biochips at the Faculty of Medicine and holds a habilitation as teaching assistant.

Integrative computational modeling to identify genes relevant in hepatocellular carcinoma

Andrew Walakira[1], Cene Skubic[1], Nejc Nadižar[1], Damjana Rozman[1], Tadeja Režen[1], Miha Mraz[2], Miha Moškon[2]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana,

[2]Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia,

andrew.walakira@mf.uni-lj.si

Hepatocellular carcinoma (HCC) contributes about 90% of liver cancer cases. Metabolic associated fatty liver disease is now a major risk factor. Hence, there is an urgent need for non-invasive

diagnostics and effective treatments. We combined integrative modeling and survival analysis to identify novel genes relevant in HCC.

We did a meta analysis of gene expression between tumor and nontumor tissue in six publicly available data sets namely; GSE19665, GSE39791, GSE41804, GSE57957, GSE64041, GSE84402, GSE84598, HCCDB15, using random effects modeling in R software. Furthermore, we integrated GSE39791 dataset into the Human-GEM metabolic model using the protocol described in Walakira et al., 2021. Finally, we assessed the prognostic potential of selected genes using the Kaplan–Meier plotter (KM plotter) (Lánczky & Győrffy, 2021), at default settings. Furthermore, we assessed the potential of the selected genes to classify between tumor and nontumor tissue samples from an independent RNA-Seq dataset, the HCCDB18 dataset, which was not previously used in this analysis.

This study used 8 datasets for the meta-analysis, 286 patients in total, 79% males and 21% females. 690 genes were significantly differentially expressed ((|log2FC|) >= 1 and adjusted BH p-value < 0.05). 30 KEGG pathways were found to be enriched, mainly involved in metabolism, signaling, synthesis of biomolecules, mineral absorption and immunity. Genes namely; *ACSL1, ACSM3, C7, C8 A, CYP3A43, DAK (TKFC), GABRP, HAO1, HBG1, IYD, OXT, P2RY13, PIPOX, PROZ, RDH5, ACSL4,* and *COX7B2* were identified as relevant in HCC showed prognostic potential (except *COX7B2* and *ACSL4)*, log rank p-value < 0.05. Using the Random Forest algorithm, transcriptomics data for the 17 genes effectively classified tumor and nontumor samples in HCC, median MCE: 3.4%, median AUC: 98%.

We identified genes that are relevant in HCC and could be used as a prognostic signature, and hence will be validated further in a human cohort.

Lánczky, A., & Győrffy, B. (2021). Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. *J Med Internet Res 2021;23(7):E27633 Https://Www.Jmir.Org/2021/7/E27633*, *23*(7), e27633. https://doi.org/10.2196/27633

Walakira, A., Rozman, D., Režen, T., Mraz, M., & Moškon, M. (2021). Guided extraction of genome-scale metabolic models for the integration and analysis of omics data. *Computational and Structural Biotechnology Journal*, *19*, 3521–3530. https://doi.org/10.1016/J.CSBJ.2021.06.009

Andrew Walakira is an early stage researcher (ESR) on the TRANSYS EU Horizon project. My mentors are Prof. dr. Damjana Rozman and Assoc. prof. Miha Moškon. My research is aimed at identifying personalized molecular signatures for modulating metabolic associated Fatty Liver Disease and Hepatocellular Carcinoma

06.07.2023 DAY 1

8.00 – 8.30 **Registration**

8.30 – 9.00 **Opening of the symposia and welcome address**

9.00 – 10.30 **Plenary session 1:**
ENZYME RESEARCH FROM BENCH TO BEDSIDE

Chair: A. Bavec, Laboratory of enzyme research

9.00 – 9.10 **Aljoša Bavec** (IBKMG, Faculty of Medicine, University of Ljubljana):
Laboratory of enzyme research: current projects and future perspectives

9.10 – 9.40 **Zrinka Kovarik** (Institute for Medical Research and Occupational Health, Zagreb, Croatia):
The toxicity of organophosphorus compounds can be reduced by cholinesterase activity

9.40 – 10.10 **Alessandro Pesaresi** (Istituto di Cristallografia - C.N.R, Trieste, Italy):
Structural and mechanistic enzymology of acetylcholinesterase at the crossroads between Ljubljana and Trieste

10.10 – 10.30 **Boštjan Petrič** (IBKMG, Faculty of Medicine, University of Ljubljana):
How does paraoxonase 1 function in cerebrospinal fluid? Some findings from a study of Alzheimer's dementia patients

10.30 – 11.00 **Coffee break**

11.00 – 12.30 **Plenary session 2:**
PHARMACOGENETICS AND PERSONALIZED MEDICINE

Chair: V. Dolžan, Pharmacogenetics laboratory

11.00 – 11.10 **Vita Dolžan** (IBKMG, Faculty of Medicine, University of Ljubljana):
Pharmacogenetics Laboratory: current projects and future perspectives

11.10 – 11.40 **Magnus Ingelman Sundberg** (Karolinska institutet, Stockholm, Sweden):
The missing heritability in pharmacogenomics

11.40 – 12.10 **Julia C. Stingl** (University Clinic Aachen, Germany):
Safety in polymedication and pharmacogenetics based precision dosing: the clinical perspective of drug-drug-gene interactions

12.10 – 12.30 **Sara Redenšek Trampuž** (IBKMG, Faculty of Medicine, University of Ljubljana):
Biomarkers of neurodegeneration: towards personalized management of Alzheimer's and Parkinson's disease

12.30 – 14.00 **Lunch break**

14.00 – 15.30 **Plenary session 3:**
EXTRACELLULAR VESICLES - NEW SOURCE OF BIOMARKERS OF DISEASE

Chair: M. Lenassi, Laboratory for extracellular vesicle research

14.00 – 14.10 **Metka Lenassi** (IBKMG, Faculty of Medicine, University of Ljubljana):
Laboratory for extracellular vesicles: driving fundamental and biomarkers discoveries of EVs

14.10 – 14.40 **Benedetta Bussolati** (University of Torino, Italy):
Stem cell-derived extracellular vesicles as therapeutic and diagnostic tools in kidney diseases

14.40 – 15.00 **Miha Arnol** (University Medical Centre Ljubljana):
Molecular biomarkers in kidney transplantation

15.00 – 15.15 **Marija Holcar** (IBKMG, Faculty of Medicine, University of Ljubljana):
Variability of blood-derived extracellular vesicles in healthy humans

15.15 – 15.30 **David Badovinac** (University Medical Centre Ljubljana): Predicting surgical resectability of pancreatic cancer based on plasma extracellular vesicle characteristics

15.30 – 16.30 **Poster session with coffee**

16.30 – 18.00 **Plenary session 4:**
TRANSLATIONAL MEDICAL BIOCHEMISTRY

Chair: K. Trebušak Podkrajšek, Laboratory for translational medical biochemistry

16.30 – 16.40 **Katarina Trebušak Podkrajšek** (IBKMG, Faculty of Medicine, University of Ljubljana):
Laboratory for translational medical biochemistry: current projects and future perspectives

16.40 – 17.10 **Bojan Vujkovac** (General Hospital Slovenj Gradec):
Fabry nephropathy in focus: diagnostics and management

17.10 – 17.40 **Albina Nowak** (University Hospital Zürich, Switzerland):
Novel biomarkers of Fabry nephropathy

17.40 – 18.00 **Tina Levstek** (IBKMG, Faculty of Medicine, University of Ljubljana):
Genetic and epigenetic biomarkers for the development and progression of Fabry nephropathy

07.07.2023 DAY 2

8.30 – 10.00 **Plenary session 5:**
FUNCTIONAL GENOMICS AND SYSTEMS MEDICINE

Chair: D. Rozman, Centre for functional genomics and Biochips

8.30 – 8.45 **Damjana Rozman, Tadeja Režen** (CFGBC, IBKMG, Faculty of Medicine, University of Ljubljana):
Centre for functional genomics and bio-chips – from omics to systems medicine and translational research

8.45 – 9.10 **Ruchi Bansal** (University of Twente, The Netherlands):
(Nano)Technologies empowering understanding and treatment of liver diseases

9.10 – 9.30 **Dubravka Švob Štrac** (Inštitut Ruđer Bošković, Zagreb, Croatia):
In search for biomarkers of neuropsychiatric disorders: new perspectives on early diagnosis and personalized therapy

9.30 – 9.45 **Tadej Battelino, Jernej Kovač** (University Medical Centre and Faculty of Medicine, University of Ljubljana):
Slovenian Reference Genome Project

9.45 – 10.00 **Vladka Čurin Šerbec** (Blood Transfusion Centre, Ljubljana):
Biobanking in Slovenia

10.00 – 10.30 **Coffee break**

10.30 – 12.00 **Plenary session 6:**
MOLECULAR MECHANISMS AND BIOMARKERS IN HORMONE-DEPENDENT DISEASES

Chair: T. Lanišnik Rižner, Laboratory for molecular basis of hormone-dependent diseases and biomarkers

10.30 – 10.40 **Tea Lanišnik Rižner** (IBKMG, Faculty of Medicine, University of Ljubljana):
Integrative approach: from steroid hormones to multiomics biomarker discovery

10.40 – 11.05 **Jerzy Adamski** (Helmholtz Zentrum München, Germany; Yong Loo Lin School of Medicine, National University of Singapore, Singapore; IBKMG, Faculty of Medicine, University of Ljubljana):
Metabolomics studies of mechanisms and biomarkers of complex diseases

11.05 – 11:30 **Karl Storbeck** (Stellenbosch University, SAR):
Biosynthesis, metabolism and bioactivity of 11-oxygenated androgens

11.30 – 11.45 **Marija Gjorgoska** (IBKMG, Faculty of Medicine, University of Ljubljana):
11-oxyandrogens in endometrial and ovarian cancers

11.45 – 12.00 **Luka Roškar** (General Hospital Murska Sobota; Faculty of Medicine, University of Ljubljana):
Models including angiogenic factors as candidate biomarkers of endometrial cancer

12.00 – 13.00 **Lunch break**

13.00 – 14.30 **Plenary session 7:**
MOLECULAR BIOLOGY AND CLINICAL RESEARCH ON RARE DISEASES OF THE SKIN

Chair: M. Liović, Medical Centre for Molecular Biology

13.00 – 13.10 **Mirjana Liović** (MCMB, IBKMG, Faculty of Medicine, University of Ljubljana):
Medical Centre for Molecular Biology: current projects and future perspectives

(MCMB): current projects and future perspectives

13.10 – 13.25 **Mirjana Liović** (MCMB, IBKMG, Faculty of Medicine, University of Ljubljana):
Skin fragility in epidermolysis bullosa and novel therapy approaches development

13.25 – 13.40 **Polona Zakošek** (Debra Slovenia):
Patient association DEBRA Slovenia

13.40 – 14.05 **Duško Ilić** (Kings College London, UK):
Stem cells in treatment of rare diseases of skin, from disease modelling to therapy

14.05 – 14.30 **Andreja Ambriović Ristov** (Rudjer Bošković Institute, Zagreb, Croatia):
Talin2 and KANK2 functionally interact to regulate microtubule dynamics, paclitaxel sensitivity and cell migration

14.30 – 15.30 **Poster session with coffee**

15.30 – 16.30 **Plenary session 8:**
MOLECULAR BIOLOGY IN CLINICAL RESEARCH OF RARE DISEASES

Chair: N. Debeljak, Medical Centre for Molecular Biology

15.30 – 15.50 **Rajko Kušec** (School of Medicine, University of Zagreb, Croatia):
How is the MPN genetics information changing our clinical judgment?

15.50 – 16.10 **Irena Preložnik Zupan / Saša Anžej Doma** (University Medical Centre Ljubljana):
Diagnosis and management of familial erythrocytosis

16.10 – 16.20 **Aleš Maver** (University Medical Centre Ljubljana):
Next generation sequencing for diagnosis of rare diseases in Slovenia

16.20 – 16.30 **Tadej Pajič** (University Medical Centre Ljubljana):
Analysis and interpretation of next-generation sequencing data in practice

16.30 – 17.30 **Plenary session 9:**
MOLECULAR BIOLOGY IN PRECLINICAL RESEARCH OF CANCER

Chair: P. Hudler, Medical Centre for Molecular Biology

16.30 – 16.50 **Martina Bergant** (Laboratory for Environmental and Life Sciences, University Nova Gorica):
APOBEC proteins play an important role in Human papillomavirus infection and oncogenesis

16.50 – 17.10 **Ario de Marco** (Laboratory for Environmental and Life Sciences, University Nova Gorica):
Combination of pre-immune libraries and tailored panning design allows for the selection of ligands with pre-definite binding characteristics

17.10 – 17.30 **Klementina Fon Tacer** (Texas Center for Comparative Cancer Research, USA):
Tumor antigens at the crossroads of male fertility and cancer therapy resistance – a single-cell perspective

17.30 **Closing of the symposium**

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