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Abstract Book

CoMBoS2

2nd Congress of Molecular Biologist of Serbia

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CoMBoS2

Content

WELCOME SPEECH

Professor Dušanka **Savić-Pavićević**
President of the Serbian Society
for Molecular Biology

Dr. Melita **Vidaković**
President of the Steering Committee
of the Serbian Society for Molecular Biology

Dear colleagues and friends,

On behalf of the Serbian Society for Molecular Biology (MolBioS), we warmly welcome you to Belgrade for the Second Congress of Molecular Biologists of Serbia (CoMBoS2).

The congress is gathering almost 250 participants from 13 countries (Sweden, United Kingdom, Italy, Switzerland, USA, Australia, Hungary, Czech Republic, Romania, Montenegro, Croatia, Bosnia and Herzegovina, and Serbia).

The program covers various fields of Molecular Biology, including Molecular Biomedicine, Molecular Biotechnology and Molecular Cell Biology, and consists of plenary and invited lectures, the MolBioS award winner lecture, poster sessions and the project corner. Special attention is paid to students and young scientists through the MolBioS Student Session, flash presentations and workshops on state-of-the-art molecular biology methods.

We wish you to be inspired by exciting and outstanding lectures given by renowned scientists and experts, exchange ideas, find opportunities for new collaborations, and have good fun.

WELCOME TO

CoMBoS2

CONGRESS ORGANIZERS

Serbian Society for Molecular Biology (MolBioS)

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MolBioS is committed to preserving the memory of the great Serbian scientists who paved the way for fruitful research and education in Molecular Biology in Serbia.

CoMBoS2 is dedicated
to our outstanding teachers
and great scientists

Professor **Ana Savić** (1936-2022)
and Professor **Vladimir Glišin** (1930-2020)

Professor Ana Savić (left photo)
and Professor Vladimir Glišin (right photo)
and the first-generation students
of the study program
Molecular Biology and Physiology
May 1975, Kotor, Yugoslavia

MolBioS

Award Winner

For achievements in the field of Molecular Biology and contribution to its development and promotion in Serbia

Academician **Milena Stevanović** graduated Molecular Biology and Physiology in 1983 at Faculty of the Natural Sciences at the University of Belgrade. She received M.Sc in Molecular Biology and Biochemistry in 1986 and Ph.D. in Biological Sciences in 1990 at the University of Belgrade. She received first post-doctoral training in human molecular genetics at the Imperial Cancer Research Fund (UK) from 1991-1992 and second in Genetics Department at the Cambridge University (UK) from 1992-1994. After returning to Serbia, she become a group leader by founded the Laboratory for Human Molecular Genetics at the Institute for Molecular Genetics and Genetic Engineering, which she has been leading since 1994. Since 1999 she has been participating in the teaching activities and in 2017 she became Full Professor in the field of Biochemistry and Molecular Biology at the Faculty in Biology, University of Belgrade. In 2009 she was elected as corresponding and then in 2015 as a full member of Serbian Academy of Sciences and Arts.

She has performed seminal work on cloning and first characterization of human SOX genes and she has continued research by studying the roles of SOX genes in maintaining pluripotency, cell fate determination, differentiation and oncogenesis. Currently, she is leading the EU funded project STREAMLINE focused on neurodevelopmental disorders (NDDs) in patients with 22q11.2 microdeletion syndrome and development of modern technologies for modelling NDDs using induced pluripotent stem cells and organoids.

She has been principal investigator on 17 projects (10 national and 7 international) and she has published more than 100 papers in peer reviewed journals.

Milena
Stevanović

Adrian J **Harwood**

Adrian J Harwood is Co-Director of Cardiff University's Neuroscience and Mental Health Innovation Institute (NMHII), a multidisciplinary research centre of neuroscientists, psychologists, human geneticists, and clinicians. His research studies patient derived and CRISPR-engineered induced pluripotent stem cells (iPSCs) to study psychiatric disorders, including those associated with Rare Genetic Syndromes. These activities include projects with the pharmaceutical industry and the founding of MeOmics, a university spinout company based on large-scale patient iPSC-based neuronal assays. He chairs MINDDS, a research network to bring together European researcher in the NDD, and currently Chairs MINDDS-connect, which aims to develop a federated data platform for building patient meta-cohorts of CNV carriers with an associated NDD.

Radoje (Rade) **Drmanac**

Radoje (Rade) Drmanac is one of the founders of the field of Genomics and serial inventor including the process of massively parallel sequencing (MPS) using DNA nanoarrays. Currently he is CSO at Complete Genomics, Inc. (CGI) he co-founded in 2005 in Silicon Valley for efficient whole genome sequencing using DNBSEQ, an advanced MPS technology based on PCR-free DNA nanoballs, and LFR/stLFR, a MPS co-barcoding technology for haplotype phasing (Science 2010, Nature 2012, Genome Research 2015, Genome Research 2019). CoolMPS is his latest MPS invention where base-specific antibodies read DNA sequence (BioRxiv 2020).

CGI was acquired by BGI (China) in 2013 and Dr. Drmanac now serves as CSO of MGI, a life-science tool spinout from BGI. MGI/CGI is first to sequence human genome for $100 in 2020. Earlier, he co-founded Callida Genomics in 2001 to advance MPS and Hyseq (1994) to discover novel genes. Prior to that he was a group leader at Argonne National Labs (1991-1994) within HGProject and postdoc (1989-1990) in ICRF (London). He started his career at the Center for Genetic Engineering (IMGGI) in Serbia (1982-1988). He received Ph.D. 1988 in Molecular Biology at Belgrade University for the first MPS technology (Science 1993, Scientia Yugoslavica 1990, Genomics 1989) and BS in Molecular Biology and Biochemistry in 1981.

Ulrich **Keyser**

Ulrich Keyser was appointed as Assistant Professor in 2007 and is now Professor of Applied Physics at the Cavendish Laboratory, University of Cambridge. His research group consists of 15 members working on elucidating the physics of membrane transport, controlling molecules in nanopores, mimicking, and understanding protein channels. His experimental group uses single molecule techniques, nanopore sensing, DNA (origami) self-assembly, optical tweezers and microfluidics. He was awarded an ERC Starting Grant 2010-2015 and ERC Consolidator Grant (2015-2021). Currently, his lab is working on integrating RNA:DNA nanotechnology with solid-state nanopore based single-molecule biosensing for DNA data storage, studies of RNA structure, and disease detection.

Web: www.keyserlab.org

Snežana **Maljević**

Snežana Maljević is a molecular neuroscientist and ion channel physiologist with a strong focus on ion channels and other genes linked to epileptic disorders. She studied Molecular Biology and Physiology at the University of Belgrade before pursuing a PhD at the University of Ulm in Germany. Following her doctoral studies, she embarked on postdoctoral training and later became a Junior Group leader at the Hertie Institute for Clinical Brain Research in Tübingen. In 2015, the Australian Government awarded Snezana an Endeavour Research Fellowship, which allowed her to join The Florey Institute as a visiting researcher. This led to her recruitment to the Florey in 2016 where she currently leads a team of 20 researchers focusing on the development of stem cell disease models and antisense oligonucleotide therapies. Snezana's ultimate goal is to translate her research findings into novel and effective treatments for patients suffering from epilepsy and related conditions.

Biljana **Ristić**

Biljana Ristić is a Research associate at Institute for Medical Research, University of Belgrade. She obtained a PhD degree in Molecular Medicine at Faculty of Medicine, University of Belgrade studying the antioxidant, photodynamic cytotoxic and antibacterial effects of graphene quantum dots nanoparticles (GQD) in human glioma cells and antibiotic-resistant bacteria. Dr Ristić was awarded by the "Stanka Romac Foundation - FOSTAR" prize for the best PhD dissertation in human molecular genetics and biomedicine in 2018. Her research interests are mainly focused on the examination of biological activity of various nanoparticles and their potential applications in nanomedicine. A significant part of her research included examination of autophagy regulation and role in cancer therapy, nutrient deprivation, neurodegenerative and infective diseases. She is a member of the FENS, FEBS, MolBios, ISOS. Dr Ristić is an author/co-author of 22 SCI-indexed articles with 1054 citations and an h-index 12 (Scopus; July 5, 2023).

Stojan **Perić**

Stojan Perić is employed at the Department for Neuromuscular Disorders of the Neurology Clinic, University Clinical Center of Serbia. He is engaged as a teaching assistant at the Faculty of Medicine, University of Belgrade. Dr Perić defended his doctoral thesis on myotonic dystrophy type 1. Main field of his clinical and scientific work are muscular dystrophies and other myopathies, neuromuscular junction diseases, acquired and inherited polyneuropathies, as well as motor neuron diseases. He has published more than 120 scientific papers. His h-index is 21. He is a member of the Editorial Board of the Journal of the Peripheral Nervous System. He is the winner of the Bruce Schoenberg Award of the American Academy of Neurology for 2015. Dr Perić is a member of the European Academy of Neurology and co-chair of the EAN Panel for Neuropathies. He is a co-chair of the global Myotonic Dystrophy Registry.

Gabriele **Stocco**

Gabriele Stocco has been an Associate Professor of Pharmacology at the University of Trieste since 2019 and Clinical Pharmacology manager at the Institute for Maternal and Child Health I.R.C.C.S. Burlo Garofolo, Trieste since 2023. He graduated with honors in Pharmaceutical Chemistry and Technology from the University of Trieste and received his Ph.D. in Pharmacology from the same university. He completed postdoctoral training at the St. Jude Children's Hospital in Memphis, USA, in the laboratory of Prof. Evans from 2006 to 2011. His scientific activity is evidenced by more than 120 publications in international scientific journals and numerous presentations at national and international conferences. Dr. Stocco is a member of the Italian Society of Pharmacology and the American Society for Clinical Pharmacology and Therapeutics. Research activity is focused on translational studies on pharmacogenomics and personalization of therapy with antimetabolites, glucocorticoids and biologics used in chronic pediatric and oncological diseases, in particular chronic inflammatory bowel disease, acute lymphoblastic leukemia and juvenile idiopathic arthritis.

Branka **Zukić**

Branka Zukić is a Full Research Professor, head of the Group for Molecular Biomedicine at the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade. She received her PhD in Molecular Biology from the University of Belgrade in 2010. Branka Zukić has over 20 years' experience in biomedical research of various diseases, with the focus on individualization of the therapy of childhood acute lymphoblastic leukemia. Her expertise includes the identification and validation of pharmacogenomic and pharmacotranscriptomic markers of response and adverse reactions to drugs. She is adept at designing and conducting translational research, using different traditional and state of the art molecular biology techniques and bioinformatics tools. She has published more than 50 research papers cited for more than 1000 times. Branka Zukić is currently a principal investigator of a HORIZON Europe funded project dedicated to pharmacogenomics at the Western Balkan region.

Mariagrazia **Di Luca**

Mariagrazia Di Luca is a Microbiologist. She got her PhD in "Microbiology and Genetics" (University of Pisa) in 2010. Then, she became Specialist in "Microbiology and Virology" in 2015 working on biofilms associated to chronic rhinosinusitis. In 2016 she joined Trampuz´ group at Charité University Medicine Berlin as responsible for the scientific management of the Biofilm Research Lab working on phage therapy for treating prosthetic joint infections. Since June 2018 Dr Di Luca has been appointed as Assistant Professor at Biology Department of University of Pisa establishing the Phage&Biofilm Lab. Her current research interests include medical biofilms, the development of alternative strategies to target sessile bacteria, *in vitro* studies on the antibacterial activity of new drugs, bacteriophage-bacteria interaction and bacteriophage therapy. Furthermore, she is a founder member of the Italian Group of Viruses of Microbes and she is supporting infectious diseases doctors to develop phage therapy in Italy.

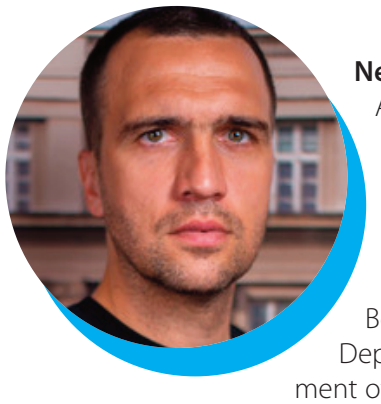
Nataša **Skoko**

Nataša Skoko graduated in Molecular Biology and obtained MSc degree in Molecular Biology and Biochemistry in 2002 at the University of Belgrade. From 2003 to 2006 she was a research fellow in the Molecular Pathology Laboratory at the ICGEB, Trieste working on the molecular mechanisms of pre-mRNA splicing in health and desease and obtained a PhD degree in 2006. From 2006 to 2017 she worked as a research scientist in the Biotechnology Development Unit (BDU) at the ICGEB on the development of biosimilars such as erythropoietin, GCSF, insulin, growth hormone using bacteria, yeast and mammalian cells expression systems. Since 2018 Dr. Skoko is a Head of the BDU and her current interest is focused at development of biosimilars to monoclonal antibodies. Dr. Skoko works in close collaboration with the industrial sector by coordinating the transfer of know-how for the production of biosimilars with aim to increase local pharmaceutical industries capacities in emerging markets. Over the past decade, BDU concluded over 70 collaboration agreements with industrial partners and has trained more than 150 scientists from 22 countries.

Ivica **Dimkić**

Ivica Dimkić is the group leader of the Microbial Biotechnology Group at the Department of Biochemistry and Molecular Biology (FBUB), and his current interest is in developing microbial solutions for sustainable agriculture. His team works with beneficial bacteria and has complementary expertise in biotechnology and collaboration with industrial partners. As a project leader, he has been involved in international, bilateral and national projects, including leading several R&D sector projects for the needs of companies and other institutions, and he is the founder of the start-up company BioCombact. In addition to plenary and guest lectures at scientific congresses, he has lectured at various institutions and has been involved in reviewing numerous scientific papers and projects. He has published 170 bibliographic records, including 70 scientific papers (> 1600 citations, Hirsch index 20, cumulative IF > 200).

Nemanja **Mirković**



Nemanja Mirković was born on 12/06/1982 in Belgrade. In 2008, he graduated from the Faculty of Agriculture, University of Belgrade with a degree in Food Technology. In 2016, he defended his dissertation at the Faculty of Agriculture, University of Belgrade, obtaining the title of Doctor of Science - Technological Engineering. From 2011 to 2013, he worked at the Institute of Hygiene and Meat Technology in Belgrade and from 2013 to 2017 at the Faculty of Agriculture, University of Belgrade. From 2017 to 2019, he was employed at the Institute of Molecular Genetics and Genetic Engineering in Belgrade. In 2019, he was hired as a teaching assistant at the Faculty of Agriculture in Belgrade at the Department of Food Microbiology. In 2020, he was elected Assistant Professor at the Department of Food Microbiology, and in 2023 he was appointed Assistant Professor at the Department of Food Microbiology. Dr. Nemanja Mirković was involved in the implementation of 7 projects, including two national, three international and two projects of the Science Fund of the Republic of Serbia. Dr. Nemanja Mirković published and communicated a total of 67 scientific papers, including one PhD thesis. According to the data from SCOPUS, the total number of citations was 206, excluding self-citations, and the h-index was 10.

Jelena **Lozo**

Jelena Lozo is a full professor at the Chair of Biochemistry and Molecular Biology, University of Belgrade - Faculty of Biology. So far she has published 50 scientific papers in international journals, and she is a co-author on one university textbook and one student handbook. Her papers have been cited over 1000 times, and the h-index is 18 (according to the Scopus database). She is engaged in research in the field of interaction between microorganisms and plants from the point of view of biological control in the broadest sense, with special emphasis on the study of bacteria that promote plant growth and help them overcome the effects of drought as abiotic stress. The biochemical and genetic characterization and the determination of the mechanisms of action of antimicrobial compounds with protein character, bacteriocins, is also a scientific field in which she is active. She participates in several international and national scientific projects, is a reviewer for leading scientific journals in various fields of microbiology, is an associate editor of the journal BMC Microbiology and is a member of the Editorial Board of the Archive of Biological Science. She is one of the founders of the Serbian Society for Molecular Biology.

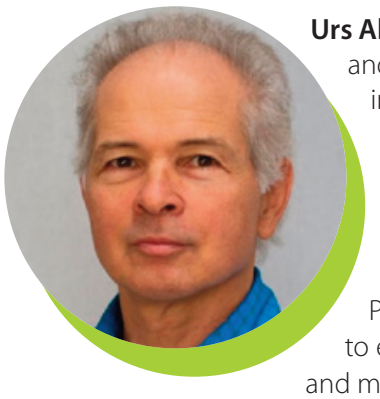
Jasmina **Nikodinović-Runić**

Jasmina Nikodinović-Runić is a full research professor, and an Eco-biotechnology and Drug Development group leader at the Laboratory for Microbial Molecular Genetics and Ecology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade. She conducts research in the field of molecular genetics of bacteria, directs the evolution of enzymes, isolates and characterizes novel biocatalysts, converts petrochemical plastic monomers to biopolymers (PHA), and works on biotechnological process optimizations. Her research interests include microbial biotechnology, biocatalysis, bacterial bioactive secondary metabolites, and novel bio-materials. The group is active in eco-green molecular biotechnologies and in the design and optimization of bioactive molecules. She has co-authored 180 scientific articles, 3 book chapters and holds 5 patents in the field of biotechnology.

Tomasz **Jurkowski**

Tomasz Jurkowski studied Molecular Biology at Warsaw University (PL) (1999-2004). He did his MSc thesis in the laboratory of Dr Janusz Bujnicki (IIMCB, PL) and Dr Monika Radlinska (Warsaw University, PL). Afterwards, he joined the group of Dr Albert Jeltsch at Jacobs University Bremen for his doctoral training, which he completed in 2008. After 4 years of post-doctoral training at Jacobs University in 2012, he joined the Faculty of Chemistry at the University of Stuttgart as a Junior Professor in Biochemistry and Molecular Epigenetics. In 2019 he joined Cardiff University as Senior Lecturer in Mammalian Systems and in 2022 was promoted to Reader. His group employs an interdisciplinary, modular approach to dissect the chromatin networks responsible for establishing and maintaining the epigenetic signals.

Urs **Albrecht**



Urs Albrecht studied Biochemistry at the University of Zürich and subsequently did a PhD in Molecular and Cellular Biology at the University of Bern, working on RNA splicing and studying polyDNA viruses in a parasitic wasp. In 1993 he joined the Department of Biochemistry at Baylor College of Medicine, Houston, Texas, USA, where he performed seminal work on circadian clock genes in humans and mice that earned him a position as Assistant Professor. In 1999 he joined the Max-Planck Institute for experimental Endocrinology in Hannover, Germany, where he established his research group working on circadian clocks. He returned to Switzerland in 2001 as Associate Professor to the Department of Biochemistry at the University of Fribourg where he still leads a research group as full Professor. His research interests are centered around the question how different tissue clocks adjust to environmental cues and how the brain integrated this information to produce coherent systemic and metabolic rhythms.

Matej **Orešič**

Matej Orešič holds a PhD in biophysics from Cornell University (1999; Ithaca, NY, USA). He is professor of medicine, with specialization in systems medicine at Örebro University (Sweden) and a group leader in systems medicine at the University of Turku (Finland). Prof. Orešič's main research areas include exposomics and metabolomics applications in biomedical research and systems medicine. He is particularly interested in the identification of environmental exposures (exposome) and disease processes associated with different metabolic phenotypes and the underlying mechanisms linking these processes with the development of specific disorders or their co-morbidities. Prof. Orešič also initiated the popular MZmine open-source project, which led to the development and release of popular software for metabolomics data processing. As of 2016, he was made a Lifetime Honorary Fellow of the Metabolomics Society. Prof. Orešič currently serves as member of the Board of Directors of the Metabolomics Society and is one of the founders of the Nordic Metabolomics Society, previously serving as its chair of the board. In 2019, he co-chaired the 1st Gordon Research Conference on 'Metabolomics and Human Health' (Ventura, CA, USA). Previously, he also chaired the Keystone Symposium on Systems Biology of Lipid Metabolism (2015; Breckenridge, CO, USA).

Tatjana S. **Kostić**

Tatjana S. Kostić, Ph.D., is a Professor of Animal Physiology at the Faculty of Sciences, University of Novi Sad. She concurrently heads the Department of Animal Physiology and leads the Laboratory for Chronobiology and Aging *(https://www.dbe.uns.ac.rs/nauka/laboratorije/chronage/)*. Tatjana is also a founder and active participant in the Accredited Center of Excellence for Reproductive Endocrinology and Signaling, as well as a member of the Laboratory for Reproductive Endocrinology and Signaling. Tatjana Kostić obtained her Ph.D. degree in Biology at the University of Novi Sad and furthered her expertise as a visiting fellow at the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), USA, from 1999 to 2002. She commenced her career as an Assistant at the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad and now holds the position of Full Professor at the same institution. Tatjana's teaching portfolio spans Animal Physiology, Cellular Signaling, Molecular and Cellular Immunology, Reproductive Physiology/Endocrinology, and Chronobiology, which she imparts at the Bachelor's, Master's, and Ph.D. levels. With a robust focus on Cellular Signaling, Reproductive Endocrinology, Circadian Rhythms, Stress, and Aging, Tatjana's research output includes over 60 peer-reviewed papers. Her contributions have resulted in a h-index of 24 and a citation count of over 1477 in the SCOPUS.

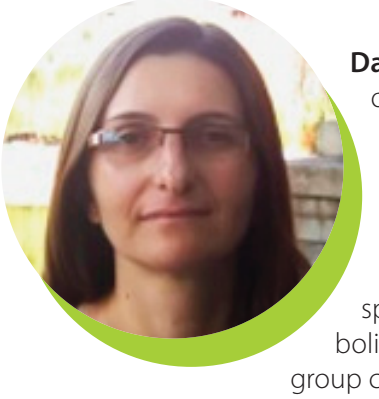
Đorđe **Miljković**

Đorđe Miljković is a research professor (from 2008) at the Institute for Biological Research „Siniša Stanković", University of Belgrade, where he is the head of the Department of Immunology (appointed in 2015) and leader of the Group for Neuroimmunology (from 2010). He obtained his PhD in immunology at the University of Belgrade in 2002. His main research interest is in autoimmunity, multiple sclerosis in particular. His recent and ongoing projects: Cellular and molecular mechanisms of recovery of rats from experimental autoimmune encephalomyelitis, Characterization of cell death mechanisms in the central nervous system of rats suffering from experimental autoimmune encephalomyelitis, Human gut microbiota transfer for novel insights into central nervous system autoimmunity pathogenesis, The role of gut microbiota and gut immune cells in the CNS-directed autoimmunity induced in rats without the use of the complete Freund's adjuvant, Modulation of gut ILC3 by a FFAR2 agonist for the treatment of autoimmune diseases.

Aleksandra **Uskoković**

Aleksandra Uskoković is employed as Principle Research Fellow at the Department of Molecular Biology, Institute for Biological Research, University of Belgrade. She graduated from Department of Biochemistry, Faculty of Chemistry, University of Belgrade, completed her MA thesis at the same faculty and obtained her PhD degree at the Faculty of Biology, University of Belgrade. Aleksandra Uskoković has published over 60 papers in scientific journals with high impact factors. Besides national projects, she participated in the Project of 7th Framework Programme of European Commission (Globaqua) and in several COST actions being a part of management comities. Her scientific interests are focused on the basic mechanisms of DNA demethylation with emphasis on the regulation of TET activity, manipulation of the process of DNA (de)methylation and epigenetic regulation of gene expression in the treatment of diabetes and cancer.

Danijela **Mišić**



Danijela Mišić is the Principal Research Fellow at the Dpt. of Plant Physiology of the Institute for Biological Research "Siniša Stanković"- National Institute of the Republic of Serbia, University of Belgrade. After earning the PhD degree (2009) at the University of Belgrade- Faculty of Biology, she was a postdoctorate fellow at the Wageningen University, The Netherlands (2011-2012). She was a fellow of "Norman Borlaug Fellowship" sponsored by U.S. Department of State (USDA) (2005), and a PIFI fellow of the Chinese Academy of Sciences (CAS) (2019). Danijela is a PI of the multidisciplinary research group working in the area of plant sciences, and covering fields of plant physiology, plant specialized metabolism, plant stress physiology, plant molecular biology, functional genomics, metabolic engineering, biodiversity and conservation, plant-biotic interactions, and plant genetics. The group currently numbers 16 permanent members, and is especially devoted to the investigation of biology, chemistry, ecology and biotechnology of iridoids- and phenolics-rich plant species.

Abstracts

Abstracts

PLENARY LECTURES

THE STORY OF THE SOX GENES: FOR BETTER OR FOR WORSE…

Milena Stevanović[1,2,3]

[1]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Biology, Belgrade, Serbia;
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The *SOX* genes encode a group of transcription factors showing diverse expression patterns during development and acting as key regulators of diverse cellular processes. SOX transcription factors are involved in multiple events from the maintaining of stem cells pluripotency and cell fate decision to driving terminal differentiation of cells into specialized cell types. During adulthood SOX transcription factors control various physiological processes. Mutations in *SOX* genes have been associated with severe clinical disorders, while deregulation of their expressions cause a broad range of pathological condition. Accumulating evidence suggests that SOX proteins act as oncogenes and recent evidence points toward pro-proliferative, pro-survival and/or anti-differentiation roles of the SOX proteins.

The results of long-term research of the structure, regulation of expression and the function of selected *SOX* genes will be presented. It will include data obtained by studying the roles of *SOX* genes in *in vitro* neural differentiation of pluripotent embryonal carcinoma cells, as well as interaction of SOX transcription factors with signalling pathways active during neurogenesis and oncogenesis. Special focus will be made on ongoing research focused on the roles of *SOX* genes in promotion of malignant phenotype of cancer cells and maintaining of cancer stem cells. The interplay of SOX transcription factors and microRNAs in the brain under physiological and pathological conditions, along with crosstalk between *SOX* genes and long non-coding RNAs in glioblastoma will be discussed. The role of SOX transcription factors in ageing and age-related diseases will be outlined.

Key words: *SOX* genes; neural differentiation; signalling pathways; cancer stem cells; ageing

MIRNA REGULATION OF THE NEURODEVELOPMENT OF HUMAN IPSC, AND WHAT THIS MAY MEAN FOR NEUROPSYCHIATRIC DISORDERS

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The risk genetics for neurodevelopmental disorders (NDD), such as intellectual disability (ID), ADHD, ASD and schizophrenia offers a route to identify the biological causes of these psychiatric conditions. Cell biology studies based on patient iPSC and/or CRISPR engineering of human iPSC are a major opportunity to correlate NDD risk genes to neurodevelopment and subsequent human neuronal function, but this is confounded by polygenicity of genetic risk in the general patient population. However, patients carrying chromosomal structural variants (SVs), have a much higher penetrance at a single gene locus and hence a strong correlation between psychiatric risk and the identifiable mutation. These easily translate to human iPSC studies, and present powerful cases in which to study the cell biology of psychiatric disorders.

Most studies have focused of the function of protein coding genes, however genetic defects could also arise due to dysregulation of gene regulation. We have shown that for Kleefstra Syndrome (KS), which is due to the loss of the histone 3 dimethyl transferase ehmt1, in vitro neurodevelopment causes up-regulation of miRNA and suppression of NRSF/REST function, which leads to premature neurodifferentiation. Using KS as a model, we have identified key miRNA drivers of neurodevelopment and shown that targeted intervention of these miRNA can restore the normal developmental programme. I will describe our current work to reveal an extensive miRNA regulatory network, dysfunction of which directly impinges on NDD.

STUDYING MOLECULAR BIOLOGY OF CELLS AND MONITORING CELL HEALTH WITH UNLIMITED DNA SEQUENCING

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Understanding and monitoring molecular biology of our cells is critical for advanced healthcare focused on disease prevention. Large-scale massively parallel sequencing (MPS) is the most efficient way to comprehensively monitor molecular processes in tissues or individual cells.

After 35 years since MPS invention (IMGGE, Belgrade, 1988) the efficiency of this technology is reaching unbelievable levels. MGI/Complete Genomics is now offering sub $100 30x whole genome sequencing (WGS) using an advanced MPS platform based on PCR-free (clonal error free) DNA nanoball (DNB) technology. Genome sequencing is now affordable and highly beneficial test especially for newborns.

Using DNB technology, a $10 genome ($0.1/Gb) is achievable, and it will enable practically unlimited sequencing. With such low-cost sequencing, a broadly accessible complete, accurate and haplotype phased 100x+ personal WGS at under $100 will become a new standard for this ultimate genetic test. Furthermore, billions of affordable and highly informative omics tests as annual checkups of our molecular health will become routine in the future healthcare. Examples of such tests are deep plasma cell-free DNA sequencing, gene expression in 10K single-cell PBMCs, deep gut and mouth microbiome sequencing, and quantifying plasma proteome by sequencing DNA barcodes linked to corresponding antibodies. By accumulating large data sets of such molecular and related health/disease phenotypes we can train artificial intelligence (AI) systems for generating actionable reports. An efficient and accurate AI interpretation of such tests will help with affordability and their broad use forming a basis for extending our healthy and productive lives for all, hopefully over 100 years.

RNA DETECTION USING NANOPORES: FROM ISOFORM ANALYSIS TO DISEASE DIAGNOSTICS

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Rapid identification of RNA molecules is a major challenge in biotechnology. This is driven by the discovery of RNAs that control cellular function ranging in length from a few to 1000s of nucleotides. Here we design three-dimensional nucleic acid constructs that enable the identification of short and long RNA molecules and nanopore readout.

First, we describe the identification of transcript isoforms at the single-molecule level using solid-state nanopore microscopy. We refold target RNA into RNA identifiers with designed sets of complementary DNA strands. Each reshaped molecule carries a unique sequence of structural (pseudo)colours. The sequence of structural colours of RNA identifiers enables simultaneous identification and relative quantification of multiple RNA targets without prior amplification. RNA IDs discriminate circular and linear transcript isoforms in a one-step, enzyme-free reaction in a complex human transcriptome using single-molecule read-out. We will show recent results on analysing transcription termination and introduce a methodology to count CTG repeats in RNA.

In the second part, we use designed DNA identifier that allows the multiplexed identification of short RNA molecules. We demonstrate the power of the approach by identifying common viruses and their variants with a nanopores microscope. Finally we show bacterial disease identification with single-base pair resolution with advanced RNA:DNA nanotechnology and solid-state nanopore sensing.

Abstracts

Session MOLECULAR BIOMEDICINE

ANTISENSE THERAPIES FOR NEUROGENETIC DISORDERS

Snežana Maljević[1]

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Antisense oligonucleotides (ASOs) are synthetic, short strands of nucleic acids that bind to a specific RNA target through Watson-Crick base pairing to modulate the expression of a targeted gene on the RNA level. By binding to messenger RNA, ASOs can prevent the production of harmful proteins, correct aberrant splicing, or increase the production of beneficial proteins. Following decades of efforts, several ASO therapies have been approved or have entered clinical trials for rare monogenic CNS diseases including spinal muscular atrophy, amyotrophic lateral sclerosis, Huntingtin's disease, and Multiple Sclerosis.

Over the past two decades, the progress in sequencing technology has led to a significant rise in the identification of genetic alterations associated with various brain disorders. Among these, > 800 genes have been linked to a heterogeneous group of conditions known as developmental and epileptic encephalopathies (DEE) and characterised by persistent seizures, developmental and cognitive delay, autism spectrum disorder and behavioural and movement problems. Our team has been focusing on the preclinical development of ASO therapies for DEE disorders with poor prognoses and unmet clinical needs. In this presentation, I will explore the implications of genetic variations linked to different forms of DEE and provide examples of how genetic changes can disrupt brain function. Various approaches employed to investigate the effects of DEE variants on multiple levels, including protein analysis, cell-autonomous studies, and neuronal network analysis will be discussed. Finally, I will address the development of ASO treatments that specifically target genes or disease mechanisms in several forms of DEE.

GRAPHENE QUANTUM DOTS PROTECT SH-SY5Y NEURONAL CELLS FROM SNP-INDUCED APOPTOTIC DEATH

Biljana Ristić,[1,2] Matija Krunić,[2] Verica Paunović,[2] Mihajlo Bošnjak,[2] Gordana Tovilović-Kovačević,[3] Nevena Zogović,[3] Aleksandar Mirčić,[2] Irena Vuković,[2] Ljubica Harhaji-Trajković,[3] Vladimir Trajković[2]

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Introduction: We examined the molecular mechanisms of graphene quantum dot (GQD)- mediated protection of SH-SY5Y human neuroblastoma cells from oxidative/nitrosative stress induced by iron-nitrosyl complex sodium nitroprusside (SNP).

Methods: GQD was produced by electrochemical oxidation of graphite and characterized by AFM, UV-VIS and FTIR spectroscopy. The antioxidant activity of GQD in cell-free conditions was assessed by DPPH, NBT and EPR analysis. The neuroprotective potential of GQD was determined by cell viability assays MTT, CV. Flow cytometry was used to assess markers of apoptosis and GQD scavenging of intracellular ROS/RNS as well. Cellular internalization of GQD was determined using TEM.

Results: GQD prevented SNP-induced apoptosis, caspase activation and mitochondrial depolarization in neuroblastoma cells. Although GQD diminished the NO levels in SNP-treated cells, NO scavengers displayed only a slight protection. GQD significantly protected SH-SY5Y cells from neurotoxicity of light-exhausted SNP, incapable of producing NO, implying that protective mechanism is independent of NO-scavenging. GQD reduced SNP-triggered increase in intracellular levels of ROS, particularly •OH, $O2^{\bullet-}$ in cells and cell-free condition. Nonselective antioxidants, •OH scavengers and iron chelators, mimicked GQD cytoprotection, indicating that GQD protect cells by neutralizing •OH generated in the Fenton reaction. Cellular GQD internalization was required for optimal protection since the removal of extracellular GQD by extensive washing partly diminished their protective effect, suggesting that GQD exerted neuroprotective effect intra- and extracellularly.

Conclusion: By demonstrating that GQD protect neuroblastoma cells from SNP-induced apoptosis by •OH/NO scavenging, our results suggest that GQD could be valuable candidates for treatment of neurodegenerative diseases associated with oxidative/nitrosative stress.

Key words: graphene quantum dots, sodium nitroprusside, neuroprotection, antioxidants, neurons

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GENETIC SPECIFICITIES OF MUSCULAR DYSTROPHIES IN SERBIA

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Muscular dystrophies are group of rare genetically heterogeneous disorders. However, overall prevalence of these disorders is up to 25 per 100 000 inhabitants and they have significant impact on patients, their families and society. As in other countries, in Serbia the most common type of muscular dystrophy in adults is myotonic dystrophy type 1 (DM1) caused by CTG repeat expansion in noncoding sequences of the *DMPK* gene. In the last years myotonic dystrophy type 2 caused by CCTG repeat expansion in the *CNBP* gene becomes better recognized and almost as common as DM1 in Serbia and other Central and Eastern European countries. The second most common muscular dystrophy in adults is facioscapulohumeral muscular dystrophy with complex genetic basis. The most common type of limb-girdle muscular dystrophy in Serbia is calpainopathy inherited in autosomal recessive manner. All our patients carry founder c.550delA mutation in the *CAPN3* gene in a heterozygous state. The most common distal myopathy in Serbia is an autosomal recessive titinopathy. All Serbian titinopathy patients carry stop-gain c.107635C>T variant in the *TTN* gene in a homozygous or a heterozygous state. Dystrophinopathies are the most common muscular dystrophies in children. Methods of the next generation sequencing are very helpful to make distinction between clinically similar myopathies. Making proper genetic diagnosis of muscular dystrophy is of crucial importance for disease prognosis, genetic advice and causal therapy. Number of novel therapies have been registered for hereditary muscle diseases, or they are investigated in clinical trials in humans.

Key words: muscular dystrophy; myotonic dystrophy; limb-girdle muscular dystrophy; distal myopathy; dystrophinopathy

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PHARMACOGENOMIC AND PHARMACOKINETIC APPROACHES FOR PRECISION THERAPY IN PEDIATRIC PATIENTS

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Precision therapy uses individual phenotypes and genotypes obtained through molecular profiling to tailor the right therapeutic strategy for a patient and ensure optimal therapeutic response. This approach can be particularly useful in pediatric patients due to the unique drug disposition associated with patient growth and development. Therapy with immunosuppressive and anti-leukemic agents in children shows significant interindividual differences in efficacy and safety associated with pharmacogenomic and pharmacokinetic profiles that could be exploited for precision therapy. Our team has identified and validated several innovative pharmacogenetic and pharmacokinetic assays for precision therapy in children requiring thiopurine antimetabolites and anti-TNF biologics. For thiopurines, we have identified PACSIN2 variants associated with severe mercaptopurine gastrointestinal toxicity in children with acute lymphoblastic leukemia and azathioprine efficacy in children with inflammatory bowel disease; the molecular mechanism of this association is still unclear and may involve modulation of autophagy or extracellular vesicle release. For anti-TNF, we validated infliximab and adalimumab serum concentrations during induction therapy as predictors of sustained efficacy in children with inflammatory bowel disease and identified FCGR3A variants as associated with infliximab concentrations, anti-infliximab antibody production and efficacy. Clinical translation of this promising set of pharmacogenomic and pharmacokinetic assays is currently being tested to improve the efficacy and safety of antimetabolites and anti-TNF therapy in children with inflammatory bowel disease. Innovative approaches to precision medicine can be achieved by extending pharmacogenomic markers to epigenetics, such as DNA methylation or non-coding RNA profiles, and by developing patient-specific models based on induced pluripotent stem cells or organoids.

PRECISION MEDICINE AND COVID-19: IMPORTANCE OF HOST GENOME PROFILING

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Introduction: The clinical picture and the course of the disease in COVID-19 patients, caused by coronavirus SARS-CoV-2, vary from asymptomatic to fatal outcome. As the same agent cause the disease, the individual genomic profile of the patient could contribute to better understanding of this phenomenon. The current knowledge about genetic markers responsible for a wide range of clinical pictures, as well as possible application of individualized treatment, will be presented.

Methods: Variants in genes responsible for predisposition and response to SARS-CoV-2 infection, pharmacogenetic variants related to drugs used in the treatment of COVID-19, nutrigenetic markers in genes relevant for the metabolism of the micronutrients (vitamin D, selenium and zinc) were investigated using GWAS, PCR and sequencing. Genotype data were extracted from database previously obtained using TruSight One Gene Panel (Illumina).

Results: Eleven pharmacogenomics markers in 7 pharmacogenes relevant for COVID-19 treatment and 10 variants affecting the structure and/or function of proteins important for susceptibility and resistance to SARS-CoV-2 infection were identified. Several variants in genes related to micronutrients were associated with severe COVID-19. Moreover, GWAS detected a significant genetic signal associated with COVID-19 related pneumonia.

Conclusion: Multidisciplinary approach, modern sequencing technologies, comprehensive studies with well-characterized patients' groups, as well as the design of robust bioinformatics tools, enable identification of novel human genetic markers associated with COVID-19. Newly gained knowledge will empower the development of the targeted therapy, as well as the implementation of nutrigenomics/pharmacogenomics, leading to the application of precision medicine in the treatment of COVID-19 patients.

Key words: COVID-19; precision medicine; pharmacogenomics; nutrigenomics; GWAS

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22Q11.2 DELETION SYNDROME AS A TOOL FOR MODELLING AND RESEARCH OF NEURODEVELOPMENTAL DISORDERS

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Introduction: Neurodevelopmental disorders (NDDs) are a group of complex and heterogeneous disorders that include autism spectrum disorders, intellectual disability, schizophrenia and bipolar disorder. However, underlying pathophysiological mechanisms are mostly unknown. In order to get better understanding of the underlying mechanisms and to discover potential therapeutics we have focused our research on 22q11.2 Deletion Syndrome (22q11.2DS), caused by microdeletion of the region q11.2 of chromosome 22 and associated with a high risk for NDDs.

Methods: To study molecular mechanisms underlying intrafamilial phenotypic variability, we have identified families with the inherited form of 22q11.2DS with the aim of conducting the following analyses: whole genome sequencing in order to detect additional genetic variation(s) present in the affected child; generation of induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells; analysis of the effects of 22q11.2 microdeletion on neural differentiation including organoids as 3D model system; transcriptome analysis of iPSC-derived neurons and astrocytes to determine differentially expressed gene sets and dysregulated pathways; and testing the metabolic changes and drug responsiveness of neurons and astrocytes by high-throughput cell-based assays.

Results: Peripheral blood mononuclear cells of the families with inherited form of 22q11.2DS were reprogrammed and established iPSCs were characterized. Generated iPSCs will be subjected to the further analyses.

Conclusion: Currently, most of the treatments of NDDs are symptom-based due to limited understanding of underlying pathophysiological mechanisms. It is expected that patient-derived iPSCs will enable a deeper understanding of unique disease mechanisms and may also provide a significant contribution in preclinical drug development.

Key words: iPSCs; transcriptome analysis; neural differentiation; organoids; drug responsiveness

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LONG-TERM THREE-DIMENSIONAL GLIOBLASTOMA CELL CULTURE MODEL FOR DRUG RESPONSE STUDIES

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Introduction: Glioblastoma, although not the most common, is one of the deadliest human cancers. Despite the implementation of the Stupp protocol in clinical practice, which prolongs patients' survival for only 2.5 months, there have been no significant advances in glioblastoma treatment. Therefore, it is imperative to better understand the mechanisms behind glioblastoma behavior for more efficient treatment in the future. Conventional two-dimensional cell cultures provide an affordable and easy-to-perform *in vitro* system but fail to recapitulate glioblastoma's complex tumor structures and microenvironmental conditions, which often results in a lack of translation to clinical settings. In contrast, three-dimensional (3D) cancer models can advance the understanding of cancer biology and have the potential to revolutionize the development of new drugs and predict their clinical efficacy.

Methods: We have developed a novel long-term 3D glioblastoma model with potential applications in preclinical studies. Using alginate fibers, this model allows the cultivation of U87 glioblastoma cells for extended periods, lasting up to 28 days, which corresponds to a clinically relevant treatment cycle.

Results: This model was used to validate hypothesized optimal temozolomide scheduling for glioblastomas generated via mathematical modeling. The results indicated that increasing the time spacing between doses of TMZ may reduce toxicity, delay the development of drug resistance, and potentially improve survival outcomes.

Conclusion: In conclusion, the establishment and utilization of advanced 3D glioblastoma models offer significant opportunities to advance our understanding of glioblastoma biology and improve treatment outcomes.

Key words: Glioblastoma; Temozolomide; 3D cancer model; drug resistance

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NEUROFILAMENT AS A BIOMARKER OF RESPONSE TO GENETICALLY DESIGNED THERAPIES FOR SPINAL MUSCULAR ATROPHY

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Considering the substantial impact of genetic therapies for spinal muscular atrophy (SMA), longitudinal follow-up of patients undergoing treatment is crucial to effectively monitor treatment response. While functional rating scales are commonly used as primary outcome measures, they may not fully capture all the therapeutic benefits. To address this limitation, the phosphorylated neurofilament heavy chain (pNF-H) protein has emerged as a promising biomarker for evaluating treatment response. pNF-H is a neuron-specific filament that exhibits increased levels in the cerebrospinal fluid (CSF) and plasma in the presence of neuronal degeneration. Our study includes individuals treated with Nusinersen (CSF and plasma samples) and Risdiplam (plasma), as well as age- and sex-matched control subjects (CSF and plasma). By examining the dynamics of pNF-H levels in these groups, we sought to identify significant differences indicative of treatment response. Before treatment, SMA individuals typically exhibit higher levels of pNF-H compared to non-SMA individuals. Elevated levels of pNF-H are associated with more severe clinical manifestations of the disease. During Nusinersen treatment, a notable decline in pNF-H levels during the first 2 months can be observed. Current findings suggest that genetic therapies have a notable impact on reducing pNF-H levels over time. By examining the changes in pNF-H levels, our study offers valuable insights into the underlying biochemical alterations associated with these therapies. Furthermore, it supports the use of pNF-H as a complementary measure to functional rating scales and as a potential biomarker for evaluating treatment effectiveness and monitoring disease progression in SMA.

Key words: Spinal muscular atrophy; Neurofilament; Nusinersen; Risdiplam

Acknowledgements: This study was financially supported by the Association SMA Serbia.

NON-CODING RNAS AS POTENTIAL BIOMARKERS FOR GESTATIONAL DIABETES

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Due to their regulatory role in modulating key signaling pathways involved in glucose homeostasis, insulin sensitivity, oxidative stress response and inflammation, non-coding RNAs (lncRNAs) were recognized as plausible candidates for novel biomarkers of gestational diabetes mellitus (GDM). In our approach aimed at analyzing the GDM-associated changes in the expression of microRNAs and long non-coding RNAs in peripheral blood mononuclear cells (PBMCs), we selected mir-27a, miR-340, miR-222, as well as *H19, MEG3* and *MALAT1*, as target RNA molecules. Expression levels were tested for correlations with clinical parameters of glucose and lipid status, as well as with indicators of oxidative stress and inflammation. In our study group, miR-27a and *MALAT1* were downregulated, while *H19* was significantly upregulated in GDM. The expression level of miR-27a-3p exhibited correlation with a number of blood parameters relevant for the oxygen transport, glycaemic and lipid status, while miR-222-3p was indicative of lipid status and iron status in healthy pregnant women. The expression of miR-340-5p correlated with lipid parameters, while MALAT1 positively correlated with NRF2 expression in both GDM patients and controls. The results obtained illustrate the potential of PBMC-derived microRNA miR-27a-3p and lncRNAs H19 and MALAT1 to serve as diagnostic biomarkers of GDM. All three selected microRNAs, on the other hand, may help in assessing the metabolic status relevant for the pregnancy, while *MALAT1* may serve as an indicator of the increased oxidative stress.

Key words: lncRNA; microRNA; gestational diabetes; lipid status; oxidative stress

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INTEGRATIVE AND FUNCTIONAL MULTI-OMICS APPROACH FOR UNTANGLING THE GENETIC BACKGROUND OF CONGENITAL ANOMALIES OF THE KIDNEY AND URINARY TRACT

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Introduction: Congenital anomalies of the kidney and urinary tracts (CAKUT) are a diverse spectrum of malformations with complex etiology tailored by the interplay between genetic, epigenetic and environmental factors. Thus, to untangle the molecular mechanisms of CAKUT, integrative approach of different –omics data has to be employed. In the present topic the journey of CAKUT investigation by our group and the impact on understanding the molecular mechanisms of CAKUT will be discussed.

Methods: Transcriptome data was obtained from ureter samples of CAKUT patients and controls by Illumina iScan microarray. Integrated bioinformatic analysis has been employed for identification and characterization of differentially expressed genes (DEGs) and putative regulatory miRNAs. Subsequently, a pipeline for comprehensive identification and functional characterisation of miRNA genes most frequenly affected by known, rare CNVs associated with CAKUT was developed. Ultimately, we have designed cell lines which depict the genomic alteration in CAKUT patients, covering the commonly affected miRNAs.

Results: Differentially expressed genes have been associated with major networks relevant to CAKUT while hsa-miR-144, located in CNV-rich region, was identified as potentially key regulator of DEGs. Network analysis of the miRNAs most frequently affected by rare CNVs has revealed the dominant regulation of hsa-miR-484 and hsa-miR-185-5p. The *in vitro* model which depicts the heterozygous deletion of the *MIR484* impies that rare CNVs affect the corresponding miRNA expression and subsequently dysregulates miRNA target genes.

Conclusion: The integration of DEG-miRNA axis further expanded on CNV alterations of miRNA genes is proven to be a comprehensive tool for investigation of complex CAKUT etiology.

Key words: CAKUT; CNV; microRNA; Microarray; Bioinformatics analysis

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RARE METABOLIC DISEASES IN THE GENOMICS ERA

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Introduction: All inborn metabolic diseases are rare, having a prevalence less than 1:2000. Vast majority of them are monogenic and finding pathogenic genetic variants is needed to set the correct diagnosis, enable adequate treatment and provide genetic counseling to members of affected family. This study is an overview of genomic studies of rare metabolic diseases in Serbia.

Methods: Since 2005, more than 300 patients suspected to have a rare metabolic or neurometabolic disease have been analyzed using sanger sequencing, clinical-exome sequencing, whole-exome sequencing or whole-genome sequencing in order to find disease-causing or disease-modifying variants. Novel variants were characterized using *in silico* modelling or in *in vitro* eukaryotic assays (standard or CRISPR/Cas9 developed).

Results: Disease-causing variants were found in more than 60 different genes associated with a metabolic or neurometabolic disease. The most frequent disease was phenylketonuria (109 patients), followed by glycogen storage disease Ib (30 patients), while majority of diseases is seen only in a single patient. More than 40 new genetic variants were comprehensively characterized *in silico* or *in vitro*. For the first time, candidate modifiers (*SHANK* gene family) were identified in a group of phenylketonuria patients with an unusual phenotype.

Conclusion: In the genomics era, next-generation sequencing significantly shortens time to diagnosis and allows studying genetic modifiers of monogenic diseases and genotype-phenotype correlation. Furthermore, characterization of novel genetic targets boosts development of precision medicine.

Key words: rare diseases; next-generation sequencing; genomics; precision medicine

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MOLECULAR DIAGNOSIS AND TREATMENT OF DIFFUSE GLIOMAS IN SERBIA

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Introduction: Serbia is identified as the fifth leading country by age-standardized mortality rate (ASMR) (6.85 per 100, 000 population) of brain and central nervous system (CNS) cancers. Highly infiltrative, intracranial neoplasms, adult-type diffuse gliomas are frequently recurrent, and mostly incurable. This study is an attempt to overview and improve diffuse glioma diagnosis and treatment in Serbia.

Methods: Our single-institution study was performed on 66 diffuse glioma patients from the Neurosurgery Clinic in Niš. The impact of clinicopathological (age, the extension of resection, type of chemotherapy), and molecular characteristics (isocitrate dehydrogenase gene 1 and 2 (*IDH1/2*) mutation status and O6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation status) on overall survival (OS) was statistically evaluated. *IDH1/2* mutations were revealed by Sanger sequencing, *MGMT* promoter methylation was estimated by methylation-specific polymerase chain reaction (MSP) and quantitative MSP (qMSP).

Results: Univariate linear regression analysis of clinicopathological parameters showed strong statistical significance for OS. Therapy with Temozolomide (TMZ) was correlated with the longest OS (14.64 months; $p < 0.001$). Methylation of *MGMT* promoter was determined by MSP in 36 *IDH1 – wild type* glioblastoma (19 (52.8%) positively methylated and 17 (47.2%) unmethylated). Further reduction and homogenization of the cohort to 17 samples (> 50 years, maximal resection, *IDH1 – wild type*) showed significant correlation with OS ($p \sim 0.05$). However, qMSP analysis revealed 23 tumor samples (54%) that were positively methylated without association with OS ($p = 0.15$).

Conclusion: MGMT prognostic value should be further evaluated on larger cohort and assessed more precisely before final decision about translation into clinical practice.

Key words: diffuse glioma; *IDH1*; *MGMT*; MSP; qMSP

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NOVEL ARYL HYDROCARBON RECEPTOR MODULATOR PROMOTES IMMUNOSUPRESSIVE IMMUNE RESPONSE BY STIMULATING T REGULATORY CELLS IN THE GUT

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Introduction: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor which is highly expressed in mucosal tissues - by epithelial cells and immune cells such as Th17 $CD4^+$ and T regulatory cells (Treg). Besides its function of clearing environmental pollutants from the body, it was also revealed that AhR has immunoregulatory effects, thus becoming a potential therapeutic target for modulating the immune response. For that purpose we tested a novel synthetic AhR modulator under the code name C43.
Methods: CYP1A1 (downstream effector of AhR) activation was tested by the EROD assay. Sort-purified $CD4^+$ cells from mesenteric lymph nodes (MLN) were treated with C43 for 24 h. Zebrafish embryos were used to test the toxicity of C43. Male C57BL/6 mice orally received C43 (10 mg/kg) for 5 consecutive days, after which MLN were harvested. Phenotype and function of the cells were analyzed by flow cytometry.
Results: C43 showed mild AhR agonistic activity. After treating the sort-purified $CD4^+$ cells with C43, there was a shift in the Th17/Treg ratio in favour of the latter. C43 showed no signs of toxicity when tested on zebrafish embryos. MLN cells from mice that received C43 revealed a shift in the Th1/Treg ratio in favour of Tregs, with a documented rise of the portion of Tregs that expressed CYP1A1 in comparison with the control group of mice.
Conclusion: C43 can modulate the immune response through the intestine by promoting the immunosuppressive Treg population.

Key words: AhR; immunomodulation; gut immunity; Treg; CYP1A1

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IDENTIFICATION OF POTENTALLY CAUSAL VARIANTS FOR MYASTHENIA GRAVIS: A BIOINFORMATICS-DRIVEN FINE-MAPPING APPROACH COMBINED WITH GENETIC ASSOCIATION STUDY

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Introduction: Genome-wide association studies (GWAS) identify genomic loci that contain genetic determinants of complex diseases. Subsequent functional genomic approaches, such as bioinformatic fine-mapping and transcriptome-wide association studies (TWAS), can reveal potentially causal single nucleotide variants (SNVs) that can be tested on patient samples. We applied this approach to study causal SNVs for acetylcholine receptor (AChR) seropositive myasthenia gravis (MG). We focused on *CHRNA1* and *CHRNB1* loci, coding AChR subunits, and *CTLA-4* locus, coding protein transmitting an inhibitory signal to T cells.

Methods: *CHRNA1* was fine-mapped by PAINTOR using data from GWAS summary statistics, 1000 genome and RegulomeDB. Alongside, rs4151121 identified by TWAS in *CHRNB1*, and *rs231735 and rs231770* identified by fine-mapping in *CTLA-4* were studied. SNVs were genotyped using allele discrimination assays in 447 Serbian AChR-MG patients (183 early-onset and 264 late-onset) and 447 sex- and age-matched controls.

Results: *CHRNA1* rs35274388 was fine-mapped as a potentially causal variant (PIP2=92%) exhibiting transcription factor binding and chromatin accessibility peaks. *CHRNA1* rs35274388 minor allele A and *CHRNAB1* rs4151121 minor allele G increased the risk for late-onset MG (OR=1.669, 95% CI=1.05-2.638, p=0.027, p10e^6 permutation=0.031 and OR=1.322, 95% CI=1.063-1.644, p10e^6 permutation=0.014, respectively). On the other hand, *CTLA-4* rs231735 recessive genotype TT decreased, while rs231735-rs231770 haplotype GC increased the susceptibility to early-onset MG (OR=0.548, 95% CI=0.339-0.888, p=0.014, p10e^6 permutation=0.014 and OR=1.360, p=0.027, p10e^6 permutation=0.027, respectively).

Conclusion: *CHRNA1* rs35274388 and *CHRNAB1* rs4151121 loci could be causal genetic factors for late-onset MG while *CTLA-4* rs231735 and rs231770 could be causal genetic factors for early-onset MG in Serbian population.

Key words: myasthenia gravis; GWAS; fine-mapping; *CTLA-4*; *CHRNA1/B1*

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EXPRESSION OF CATION-HANDLING PROTEINS IN THE HEART OF METABOLICALLY COMPROMISED RATS SUBJECTED TO WALNUT-ENRICHED DIET

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Introduction: Intracellular transport of cations in the cardiomyocytes is essential for the functioning of the heart. Due to the high content of polyunsaturated fatty acids, we hypothesize that walnuts have the capacity to affect cation transport in the heart.

Methods: Healthy and metabolically compromised rats drinking 10% fructose solution, were subjected to a diet supplemented with 2.4 g of walnuts for six weeks. The expression of cation-handling proteins in the heart was determined by the Western blot method.

Results: Fructose-rich diet increased the level of the a1 subunit of Na^+/K^+-ATPase and the phosphorylation of ERK1/2 kinase in the heart of control and walnut-eating rats, while elevated levels of LTCCa, NCX1, and Maxi Ka were observed only in rats whose diet did not contain walnuts. On the other hand, walnuts significantly increased the content of LTCC, NCX1, Maxi Ka, Kir6.1 and SUR2B, but only in the heart of fructose-naive rats. The walnut diet-induced increase in LTCC and NCX1 expression in healthy rats may indicate intense cardiac calcium turnover, while the effect on Kir6.1 and SUR2B subunits suggests stimulation of K_{ATP} channel transport in the cardiac vasculature. In animals that drank fructose, walnuts increased only Akt kinase phosphorylation, which may be a part of the antiarrhythmic mechanism of decreasing cation currents in cardiomyocytes.

Conclusion: The effects of walnuts on the cation-handling proteins in the heart, mostly limited to metabolically intact animals, suggest the possible use of a walnut-supplemented diet in the prevention rather than the treatment of cardiological channelopathies.

Keywords: walnut; fructose; heart; cation transport; protein kinases

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IMMUNOHISTOCHEMICAL EVALUATION OF FIBROSARCOMA METABOLISM, PROLIFERATION, ANGIOGENESIS AND APOPTOSIS AFTER TREATMENT WITH METFORMIN COMBINATIONS IN HAMSTERS

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Introduction: The NF-kB transcription factor regulates cell proliferation, development, apoptosis and response to oxidative stress. Metformin may have an important antitumor role by down regulating the activity of NF-kB. The aim: Detecting an anticancer effect of metformin in combinations with other repurposed drugs, already registered for other indications, which may be immediately applied and clinically investigated in oncology, reducing the time and cost of research for new cancer treatments.

Methods: Immunohistochemistry was performed for tumors treated by dual drug combinations containing metformin with 2-Deoxy-D-glucose, deoxycholic acid, caffeine, itraconazole, nitroglycerin or disulfiram. The drugs were applied in Syrian golden hamsters (6 animals per group) with inoculated BHK21/C13 fibrosarcoma in doses equivalent to usual human doses, <50 % LD_{50}. The anticancer effects were assessed by immunohistochemical markers of glucose metabolism (GLUT1), NO metabolism (iNOS), tumor proliferation (Ki-67, PCNA), neoangiogenesis (CD34, CD31) and apoptosis (COX4, Cytochrome C). Also, biophysical characteristics of fibrosarcoma, animal blood samples and the toxicity on main organs were analyzed.

Results: Treatments significantly ($P<0.05$) reduced tumor glucose metabolism, NO metabolism, proliferation, neoangiogenesis and enhanced apoptosis, without toxicity and influence on biochemical blood and hematological tests.

Conclusion: Administration of metformin in two-drug combination with 2-Deoxy-D-glucose, deoxycholic acid, caffeine, itraconazole, nitroglycerin or disulfiram may be recommended for further clinical investigations in oncology.

Key words: fibrosarcoma; metformin; repurposed drugs; hamsters

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RESCUING HAMSTER FIBROSARCOMA GROWTH BY STIMULATION OF DIFFERENT PROONCOGENIC SIGNALING PATHWAYS RELATIVE TO REPURPOSED ANTICANCER DRUG MECHANISMS

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Introduction: Many drugs registered for various non-oncological indications influence tumor metabolic processes, signaling pathways, enzymes, proteins, tumor receptors and genes that regulate proliferation, neoangiogenesis, apoptosis and necroptosis, without affecting these activities in healthy cells. The aim: Detecting underlying anticancer mechanism of metformin in two-drug combinations with other repurposed drugs (2-Deoxy-D-glucose, deoxycholic acid, caffeine, itraconazole or disulfiram) by rescuing BHK-21/C13 hamster fibrosarcoma growth with glucose, vitamin C, nitroglycerin or mebendazole.

Methods: The anticancer mechanisms of examined drug combinations, <50% LD_{50} (equivalent to usual human dose), were determined by rescuing fibrosarcoma growth with addition of aforementioned agents in treatment. Immunohistochemical markers (Ki-67, PCNA, CD34, CD31, GLUT1, iNOS, COX4, Cytochrome C) in control and experimental groups were assessed 19 days after BHK-21/C13 tumor inoculation. Tumors were excised, measured and blood collected. Biophysical, pathohistological, toxicological, hematological, biochemical and statistical analyses were performed.

Results: Only addition of NF-kB stimulator mebendazole to effective two-drug combinations containing metformin rescued cancer growth, indicating that this pathway may be responsible for antitumor action.

Conclusion: NF-kB signaling pathway downregulation plays an essential role among anticancer mechanisms of investigated metformin combinations in hamster fibrosarcoma treatment.

Key words: fibrosarcoma; hamster; repurposed drugs; anticancer mechanism

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CHALLENGES ASSOCIATED WITH MOLECULAR CONFIRMATION OF ALVEOLAR ECHINOCOCCOSIS FROM FORMALIN-FIXED PARAFFIN EMBEDDED (FFPE) TISSUE SAMPLES

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Introduction: Diagnostic of alveolar echinococcosis (AE), parasite-borne zoonosis, is based on pathohistological and molecular evidence. Etiological agent *Echinococcus multilocularis* (EM), cestode, persists mainly in red foxes as final and wild rodents as intermediate hosts. Humans are infected by the accidental ingestion of embrionated eggs, resulting in cyst formations, without protoscolices, localized primarily in the liver. The infection has a long course and mimics liver cancer, with metastatic spreading, diffuse infiltration, and fibrosis. Since 2015, AE is present in foxes and jackals in endemic region of Srem, where the first human case was recently registered.

Methods: Pathological examination was performed on liver tissue, while ELISA and Western blot were accomplished on the blood sample. For molecular analysis by conventional PCR, total DNA was extracted from FFPE tissue samples using two different commercial kits with particular modifications. PCR product of 200 bp was sequenced.

Results: Pathohistological analysis determined AE, also proved by Elisa and Western blot. Molecular identification was achieved only for 2 out of 45 FFPE samples, when PCR amplification of parasitic DNA was observed utilizing EM-H17/EM-H15 primers. Employing BLASTn alignment, it was revealed that obtained nucleotide sequence may represent a novel 12S rRNA gene variance.

Conclusion: The key step in EM molecular diagnostic was parasitic DNA extraction, regarding the DNA quality and quantity. Manufacturer's procedure was modified: 1.5-2 times increased amount of tissue subjected to DNA extraction, plus doubled amount of proteinase K. Besides, it's crucial that FFPE tissue sample contains nuclei observed by pathologist, not only dead hyaline membranes.

Key words: Alveolar echinococcosis; pathohistological analysis; DNA extraction; PCR

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TELOMERE LENGTH IN INTESTINAL BOWEL DISEASE AND COLORECTAL CANCER

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Introduction: Intestinal bowel disease (IBD) is a term used to describe two principal conditions: Crohn's disease (CD) and ulcerative colitis (UC). It is well known that chronic inflammation of the gastrointestinal tract (GIT) increases the risk for colorectal cancer (CRC) development. Our aim was to estimate the telomere length in all three conditions and evaluate its predictive value.

Method: Blood and GIT tissue samples were collected from young and elderly patients with chronic abdominal pain and swelling symptoms in University Children's Hospital "Tiršova" and University Medical Center "Zvezdara", respectively. GIT tissue was submitted to pathohistological analysis to set the diagnosis (CD, UC, CRC). Peripheral blood leukocytes were used as a source of DNA for telomere length estimation. Relative telomere to single copy gene (T/S) ratio was determined by qPCR.

Results: Our study showed that young patients suffering from CD (8.43; 0.51-23.88), as well as elderly participants with CRC (1.74; 0.01-5.21) had significantly shorter telomeres, compared to controls (11.75; 6.11-29.88). No statistical significance was observed in the UC group (9.41; 5.55-20.28). It seems that mechanisms triggering CD and CRC have a more profound influence on telomere length versus UC driven effects. Results obtained from the CRC group can partially be explained by higher age, although the influence of various genotoxic agents cannot be excluded.

Conclusion: This study suggests that a telomere shortening can be observed in IBD, with more obvious effects in CD patients. Also, it can be concluded that leukocyte telomere length might be a good predictive parameter for CRC development.

Key words: Crohn's disease; ulcerative colitis; colorectal cancer; telomere length

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EXPRESSION ANALYSIS OF LONG NONCODING RNAS *H19* AND *MEG3* IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF WOMEN WITH GESTATIONAL DIABETES

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Introduction: Dysregulation of mechanisms related to activities of long non-coding RNAs (lncRNAs) is associated with various human pathologies, including gestational diabetes mellitus (GDM). Since lncRNAs expressed from imprinted genomic regions are involved in the metabolism and cell growth, *H19* and *MEG3* were selected for the evaluation of potential biomarker properties in GDM.

Methods: The expression levels of *H19* and *MEG3* in peripheral blood mononuclear cells (PBMCs) obtained from patients with GDM (n = 40) and normoglycaemic controls (n = 40) were assessed by quantitative real-time polymerase chain reaction. Furthermore, the expression levels were tested for correlations with certain clinical parameters.

Results: The expression of *H19* was significantly lower in patients with GDM, compared to controls (p = 0.015), with an average fold change of 1.7. However, no correlations with parameters associated with glucose or lipid status were found for *H19* expression. The expression of *MEG3*, on the other hand, was not associated with GDM diagnosis, but it was inversely correlated with the level of cholesterol and LDL in GDM patients.

Conclusion: The results obtained support the potential diagnostic biomarker property of PBMC-derived lncRNA *H19* in GDM, while *MEG3* may assist in evaluating the patient's lipid status in pregnancies complicated with GDM.

Key words: lncRNA; *H19*; *MEG3*; gestational diabetes; lipid status

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NOVEL MULTIPLEX SNAPSHOT REACTION DESIGNED FOR DETECTION OF EIGHT FOUNDER MUTATION ASSOCIATED WITH SINGLE-GENE DISORDERS IN SERBIAN ROMA POPULATION

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Introduction: The Roma represent the most widespread ethnic minority in Europe with unique genetic structure shaped by the string of population bottlenecks and founder effects during their migrations from Indian subcontinent towards Europe. Several founder disease-associated mutations have been identified until now, however they have not been fully characterized in Roma population in Serbia.

Methods: We designed a novel SNaPshot multiplex reaction which targets the most common founder mutations in Roma population associated with eight monogenic disorders: hereditary motor and sensory neuropathy (HMSN) type Lom and Russe, autosomal-recessive cerebellar ataxia 3 (ARCA3), congenital myasthenic syndrome (CMS), nonsyndromic hearing loss (NSHL), limb girdle muscle dystrophy type 2C (LGMD2C), congenital cataracts, facial dysmorphism and neuropathy (CCFDN) and galactokinase deficiency (GALK). Genomic DNA from 228 individuals and 5 undiagnosed patients was isolated from buccal swabs. Affected regions of genes associated with the diseases (*NDRG1*, *HK1*, *CTDP1*, *SGCG*, *GALK1*, *CHRNE*, *GJB2* and *ANO10*) were analyzed using SNaPshot method, while Sanger sequencing method was used for confirmation of detected mutations.

Results: We found 59 mutated alleles among investigated samples, out of which the most frequent was the one in *GJB2* gene. All 5 patients were successfully diagnosed, three with HMSN-Lom, one with CMS and one with HMSN-Russe. The latter was the first case of this disease reported in our country.

Conclusion: This multiplex represents great method for carrier-testing programs, which in addition to community-based education could improve poor health status of the socially marginalized Roma population and reduce the occurrence of the analyzed monogenic disorders.

Key words: founder mutations; Roma population

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COMPARISON OF TWO METHODS FOR DNA EXTRACTION FROM FORMALIN FIXED AND PARAFFIN-EMBEDDED TISSUES

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Introduction: DNA extraction from formalin fixed and paraffin-embedded tissue (FFPE) is a critical step for many molecular techniques. Unbuffered, fixative solution (pH<1) with high concentration of formaldehyde reduces both the amplifiable quantity and length of extracted DNA.

Methods: Four FFPE tissues, belonging to the same person, were first rinsed with xylene and ethanol solution. DNA was extracted from FFPE tissues using two extraction methods - *QIAquick PCR Purification Kit* and *QIAamp DNA FFPE Tissue Kit*. Extracted DNA was quantified using *Investigator QuantiplexPro Kit*. Analysis was performed using *Investigator 24plex QS, GlobalFiler and NGM detect* Kits, and ABI 3500 Genetic Analyzer.

Results: DNA extracts from both isolation protocols were degraded. Higher DNA quantity was detected in samples isolated by *QIAamp DNA FFPE Tissue Kit*. Using *Investigator 24plex QS Kit,* amplification of 200-300 bp fragments was detected after both isolation methods, but higher RFU values of amplicons were detected after *QIAamp DNA FFPE Tissue Kit* extraction. Furthermore, samples isolated with this kit were selected for PCR amplification with *GlobalFiler and NGM detect*. By combining results of above mentioned PCR kits, STR profile with 20 loci was obtained.

Conclusion: The results revealed that DNA extraction using *QIAamp DNA FFPE Tissue Kit* resulted in significantly improved quantity of amplifiable DNA, also in a shorter period of time knowing that the protocol for isolation of DNA by *QIAquick PCR Purification Kit* lasts more then 48, and by *QIAamp DNA FFPE Tissue Kit* only few hours.

Key words: FFPE tissue; DNA extraction; STR loci

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SUGARS AND THEIR SUBSTITUTES INCREASE PATHOGENICITY OF PSEUDOMONAS AERUGINOSA

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Introduction: Different sugars are commonly used in the diet, but little is understood about the various effects of human health that they can affect. Hence, the impact of sugars and their substitutes used in diet on the development of virulence in *Pseudomonas aeruginosa* PAO1 was investigated. Sugars (fructose, demerara, coconut sugar, and cane sugar) and sugar substitutes (erythritol and stevia) were selected. The genes from three *P. aeruginosa* QS networks (las - lasI, lasR; rhl - rhlI, rhlR; PQS - pqsA, mvfR) were used for RT-qPCR analysis in order to investigate whether the expression of these genes changes. In this work, the focus is on the expression of genes involved in QS and the ability to form biofilms (a type of structured community of microorganisms that is attached to the surface and connected by an exopolysaccharide matrix), as well as determining minimal inhibitory concentration of antibiotics in presence of tested compounds.

Methods: Microdiltuion assay, Antibiofilm assay, RT- qPCR

Results: In the presence of tested sugars and their substitutes, the minimum inhibitory concentration of commercial antibiotics increased, as well as the percentages of biofilm formation (for instance, the percentage of biofilm formation is 171% in the presence of coconut sugar). Furthermore, exposure of *P. aeruginosa* to tested compounds caused the greatest increase in expression of virulence associated with the *lasI* and *pvdF* genes.

Conclusion: More awareness and research is needed to highlight the effects sugars can have on *P. aeruginosa* and to propose new strategies to reduce this negative aspect.

Key words: sugars; sugar substitutes; virulence factors; *Pseudomonas aeruginosa*, gene expression

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UNVEILING THE MOLECULAR TANGO: SPECIFIC PAIRS OF PROTEIN CO-AGGREGATE IN THE BRAINS OF INDIVIDUALS WITH MENTAL ILLNESS

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Introduction: Disrupted proteostasis is an emerging area of research in major depressive disorder (MDD), with several proteins, including CRMP1, DISC1, NPAS3, and TRIOBP-1, having been identified as forming aggregates in the brains of psychiatric patients. Our aim is to investigate the extent to which these proteins "co-aggregate" together within the same individuals.

Methods: Insoluble protein fractions were isolated from post-mortem insular cortex samples obtained from MDD patients, Alzheimer's disease patients, suicide victims, and control individuals. Western blotting was performed to identify insoluble proteins indicative of aggregation. Co-aggregation was explored by systematically testing pairwise expression of these proteins in SH-SY5Y neuroblastoma cells, followed by immunofluorescent microscopy examination.

Results: Multiple insoluble proteins were observed in the brains of many individuals, suggesting aggregation of multiple proteins. However, cell culture analysis demonstrated consistent co-aggregation only between DISC1 and CRMP1, as well as DISC1 with full-length TRIOBP-1, and not other pairs of proteins tested.

Conclusion: While multiple proteins often aggregate in the same individuals, this seems to be mainly due to a combination of shared underlying factors leading to parallel aggregation, with only specific pairs of proteins interacting to form co-aggreges. Further studies, including replication and investigation in additional brain regions, are necessary to elucidate the mechanisms underlying this phenomenon.

Key words: protein aggregation; suicide; major depressive disorder; DISC1; CRMP1

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GENETIC PREDISPOSITION OF SUICIDAL BEHAVIOR: VARIANTS IN *GRIN2B*, *GABRG2*, AND *ODC1* GENES IN SUICIDE ATTEMPT AND COMPLETED SUICIDE IN TWO BALKAN POPULATIONS

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Introduction: Suicidal behavior ranges between suicidal ideation and completed suicide. Completed suicide accounts for over 700,000 deaths worldwide, while attempted suicide is 20 times more frequent. Genetic background is an important factor contributing to suicidal behavior, and candidate genes linked to several neurotransmitter systems have been investigated. Alternations in glutamate, γ-aminobutyric acid (GABA) and polyamine systems have been detected in suicidal behavior. Our aim was to differentiate genetic predispositions underlying two different types of suicidal behavior, attempted and completed suicide, in two Balkan populations.

Methods: The study sample included 173 suicide attempters with comorbid psychiatric disorders (major depressive disorder, bipolar affective disorder, or schizophrenia), 216 non-suicidal psychiatric patients and 172 healthy controls from Serbia, and 333 suicide completers and 356 non-suicidal autopsy controls from Slovenia. Variants in the genes *GRIN2B* (rs2268115 and rs220557), *GABRG2* (rs424740), and *ODC1* (rs1049500 and rs2302614) were genotyped by TaqMan assays and analyzed using PLINK.

Results: The CA genotype of rs220557 in the *GRIN2B* gene increases the risk for completed suicide (OR=1.51, p=0.021), and particularly violent suicide (OR=1.49, p=0.037), compared to controls. In the *ODC1* gene, the CA genotype of rs2302614 decreases the risk for completed suicide compared to suicide attempt (OR=0.32, p=0.012). Marginally, the AC haplotype for variants rs1049500-rs2302614 in the *ODC1* gene decreases the risk for completed suicide compared to suicide attempt (OR=0.50, p=0.052).

Conclusion: Specific genetic variants of the glutamate and the polyamine systems are differently distributed among diverse suicidal phenotypes, thus providing further information on the implication of these systems in suicidality.

Key words: suicidal behavior; genetic variant; *GRIN2B*; *GABRG2*; *ODC1*

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CAFFEIC ACID IMPAIRS ENDOVASCULAR DIFFERENTIATION OF TROPHOBLAST CELLS

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Introduction: Caffeic acid (CA) is a polyphenolic compound found in fruits, vegetables and beverages used in everyday diet. Although CA exhibits various physiological effects including antioxidant and cytoprotective activities there is limited information on its effects in pregnancy. In this study we investigated CA influence on processes important for early pregnancy such as trophoblast endovascular differentiation, endothelium replacement and invasion as well as its effects on the expression of molecular mediators involved in these processes.

Methods: Human extravillous trophoblast HTR-8/SVneo cells treated with 10 μM or 100 μM CA were used in the experiments. Tube formation assay, co-culture with the human umbilical vein endothelial cells (HUVECs) and assessing *CDH2* mRNA levels in treated HTR-8/SVneo cells by qPCR were used to evaluate CA effects on trophoblast endovascular differentiation. Furthermore, we investigated trophoblast invasive properties by HTR-8/SVneo spheroid invasion assay in Matrigel and integrin α1 and β1 protein expression by CELISA after 24h CA treatment.

Results: CA treatment significantly decreased tube length and number of branching points after 5h. Following 24h incubation with CA, capacity of HTR-8/SVneo cells to integrate into HUVECs' monolayer was significantly decreased as well as expression of *CDH2* mRNA in HTR-8/SVneo treated cells, cadherin important for trophoblast-endothelium interaction. On the other hand, CA stimulated trophoblast invasion without affecting protein levels of integrin α1 and β1 subunits, mediators of this process.

Conclusion: Health-promoting potential of CA has been shown in different (patho)physiological processes. Nevertheless, there could be a risk of CA consumption in early pregnancy as our results indicated.

Key words: caffeic acid; endovascular trophoblast; trophoblast-endothelium interaction; *CDH2*; trophoblast invasion

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EXPLORING THE IMPORTANCE OF MGMT PROMOTER METHYLATION STATUS IN ELDERLY COHORT OF DIFFUSE GLIOMA PATIENTS

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Introduction: This study focused on a homogeneous group of 24 newly diagnosed diffuse glioma patients who were over 50 years old and underwent surgical resection. The objective was to determine the mutation status of the isocitrate dehydrogenase 1 (*IDH1*) gene and evaluate the prognostic significance of the methylation status of the epigenetic marker O^6-methylguanine-DNA methyltransferase (*MGMT*).

Methods: Exon 4 from the *IDH1* was amplified by PCR and sequenced by the Sanger method. The *MGMT* methylation status was determined by combined methylation-specific PCR (MSP) and Real-time methylation-specific PCR (qMSP).

Results: The combined MSP analysis identified 10 (41.6%) positively methylated samples and 14 (58.4%) unmethylated samples, showing a significant correlation between overall survival (OS) and *MGMT* methylation status ($p \approx 0.05$). The group with hypermethylated *MGMT* had an OS of 9.6 ± 1.77 months, while the unmethylated group had an OS of 5.43 ± 1.04 months.

Conclusion: Based on these findings, the study identified the *MGMT* promoter methylation status as a positive prognostic factor within this specific patient group. However, further validation with a larger population of diffuse glioma patients is necessary to confirm these results.

Key words: glioma; *MGMT*; MSP; prognosis; *IDH1*

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CORRELATIONS OF *CDKN1A* AND *ADAM17* EXPRESSION WITH A CHANGE OF LEFT VENTRICULAR REMODELING ECHOCARDIOGRAPHIC PARAMETERS IN PBMC OF PATIENTS SIX MONTHS AFTER THE FIRST MYOCARDIAL INFARCTION

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Introduction: Myocardial infarction (MI) and consequential ischemia with cardiomyocyte loss are followed by left ventricular (LV) remodeling. LV remodeling is crucial process for cardiac function preservation, although when prolonged it can become maladaptive and lead to impaired systolic function and further cardiovascular complications. Echocardiographic parameters are used as a measure of LV structure and function. *ADAM17* (a disintegrin and metalloprotease domain) and *CDKN1A* (cyclin-dependent kinase inhibitor 1A) have shown regulating role in DNA repair, inflammation, remodeling and fibrosis. The aim of this preliminary study was to investigate the potential effect of *CDKN1* and *ADAM17* mRNA in post MI heart remodeling.

Methods: Sixty four patients with the first MI were prospectively followed-up 6 months after MI. Change (Δ) of echocardiographic parameters within 6 months was calculated as a difference between the value at 6-month follow-up and value at admission. Relative gene expression was detected using the TaqMan® technology. Statistical analyses were done by Statistica 8 software.

Results: We have observed correlation between *CDKN1A mRNA* expression and change of LV end-diastolic diameter (ΔLVEDD, R=0.3, p=0.01) and LV end-systolic diameter (ΔLVESD, R=0.3, p=0.02), but not with LV ejection fraction and stroke volume. *ADAM17* expression was not in correlation with analyzed parameters of LV remodeling. However, *CDKN1A* and *ADAM17* mRNA expression in PBMC six months after MI were positively correlated (R=0.6, p<0.001).

Conclusion: Preliminary results suggest that *CDKN1* has a role in post MI LV remodeling, correlating with changes in echocardiographic parameters of LV structure. The validation on a larger sample size is required.

Key words: myocardial infarction; LV remodeling; *CDKN1A*; *ADAM17*; gene expression

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MIR200A AND MIR210 AS POTENTIAL MARKERS IN DETECTION OF ENDOMETRIAL ADENOCARCINOMAS

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Introduction: Endometrial cancer (EC) is the most common type of gynecological cancer with estimated crude incidence rates of 33.9 in Serbia during 2020. MicroRNA molecules have emerged as biomarkers with strong diagnostic potential and an essential role in the regulation of tumor suppressors and oncogenes in cancers. Since about 80% of EC are adenocarcinomas (EAC), our work was focused on examining miR-200a and miR-210 which could detect this type of cancer in early stages.

Methods: Total RNA was isolated with RNA Extracol from 34 tissues of EAC in IA, IB and II stage and 16 non-pathological endometrial tissues for the control group. Reverse transcription was performed using NG dART RT kit and stem-loop primers for miRNAs and U6-snRNA endogenous control. SG/ROX onTaq qPCR Master Mix was used in real-time qPCR to quantify its expression in each sample.

Results: Our results showed significantly higher expression of miR-200A and miR-210 with fold change of 26.73±12.07 ($p<0.001$) and 19.18±8.03 ($p=0.006$) in EAC. A significant difference in microRNA expression was not obtained by comparing cancer histology, stage, and grade. ROC curve analysis showed that miR-200 has significant potential in discriminating EAC from control samples with AUC of 0.836, while miR-210 showed a slightly lower value of 0.741. The combination of these two microRNAs showed even stronger discriminating power compared to individual miRNAs with AUC score of 0.855, sensitivity of 82.35% and specificity of 81.25%.

Conclusion: Aberrant expression of miR-200A and miR-210 has good diagnostic potential in detecting endometrial adenocarcinoma at early-stage.

Key words: Biomarkers; endometrial cancer; expression; miR200A; miR210

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TOWARDS THE XENO-FREE AND CHEMICALLY DEFINED CONDITIONS FOR 3D VASCULAR NETWORK FORMATION IN VITRO

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Introduction: One of the main challenges for tissue engineering is generation of vascularized tissues. Complex systems of endothelial cells (ECs) and stromal cells (SCs) embedded in 3D hydrogels are used for vascularization *in vitro*. Different biomolecules, such as growth factors (GFs), added to the cell culture medium might further promote the vascular network formation. Yet, precisely defined and controllable conditions should be established for potential generation of vascularized organoids or clinically relevant tissue constructs.

Methods: EC spheroids were co-cultured with different SCs in 3D Matrigel or xeno-free hydrogels. Effects of GFs (IGF, FGF2, EGF, VEGF) and their combinations were tested. Vascular network and SC distribution were evaluated using confocal microscopy. Area covered with hydrogel and network length were analyzed.

Results: We observed a significant difference in hydrogel remodeling, SC distribution and network formation when the 3D constructs were cultured in the medium supplemented with particular GFs compared to the medium with high serum concentration. Despite the broad use of VEGF in vascularization field, VEGF alone did not lead to any significant changes in analyzed parameters. It was the synergistic effect of GFs that had positive effect on network formation in our system. This effect was observed regardless of the tested SC type. Based on these findings, we designed the chemically defined medium that supported the EC network formation in xeno-free hydrogel.

Conclusion: We gained information about the potency of certain GFs to support vascular network formation. Employment of chemically defined and xeno-free conditions led to successful network formation.

Key words: vascularization; co-culture; growth factors; hydrogels; xeno-free

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MAP KINASES ACTIVATE TFEB/FOXO-DEPENDENT AUTOPHAGY INVOLVED IN PHORBOL MYRISTATE ACETATE-INDUCED MACROPHAGE DIFFERENTIATION OF HL-60 LEUKEMIA CELLS

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Introduction: Autophagy has been shown to participate in the differentiation of hematopoietic and leukemic cells. We investigated the mechanisms of autophagy action in the differentiation induced by PKC activator phorbol myristate acetate (PMA) in HL-60 acute myeloid leukemia cells.

Methods: The macrophage markers CD11b, CD13, CD14, CD45, EGR1, CSF1R, and IL-8 were assessed by flow cytometry and RT-qPCR. Autophagy was monitored by RT-qPCR analysis of autophagy-related (*ATG*) gene expression, LC3-II/p62 immunoblotting, beclin-1/Bcl-2 interaction, nuclear translocation of TFEB and FOXO1/3. The activation of MAP kinases, ERK and JNK was assessed by immunoblotting. Pharmacological inhibition and RNA interference were used to determine the role of MAP kinases and autophagy in HL60 cell differentiation.

Results: PMA-triggered differentiation of HL-60 cells into macrophage-like cells was confirmed by elevated expression of macrophage markers CD11b, CD13, CD14, CD45, EGR1, CSF1R, and IL-8. The induction of autophagy was demonstrated by accumulation/punctuation of LC3-II, and the increase in autophagic flux. PMA also increased nuclear translocation of TFEB, FOXO1/3, as well as the expression of several *ATG* genes in HL-60 cells. PMA stimulated the phosphorylation of ERK and JNK via PKC-dependent mechanism. Pharmacological or genetic inhibition of ERK or JNK suppressed PMA-triggered nuclear translocation of TFEB and FOXO1/3, *ATG* expression, dissociation of beclin-1 from Bcl-2, autophagy induction, and differentiation of HL-60 cells into macrophage-like cells.

Conclusion: Our study revealed the involvement of ERK and JNK in TFEB/FOXO-dependent autophagy and differentiation of HL60 cells, indicating MAP kinase-mediated autophagy as a possible target in differentiation therapy of AML.

Key words: leukemia; autophagy; differentiation; ERK; JNK

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SPECTROPHOTOMETRIC DETERMINATION OF THE INFLUENCE OF SIOFOR ON AMYLASE ACTIVITY

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Introduction: Siofor is used to lower the blood glucose in those with type 2 diabetes. It is also used as a second-line agent for infertility in those with polycystic ovary syndrome. The use of metformin reduces body weight in people with type 2 diabetes in contrast to sulfonylureas, which are associated with weight gain. Some evidence shows that metformin is associated with weight loss in obesity in the absence of diabetes.

Methods: The spectrophotometric method was used in this study. Absorbance was recorded at a wavelength of 546 nm. The influence of siofor on amylase enzyme activity in phosphate buffer was monitored.

Results: The obtained results show that the reaction follows the Michaelis-Menten model. Siofor inhibited amylase and bound to the active site of the enzyme. Using the Lineweaver-Burk equation, the values of kinetic parameters of maximum velocity and Michaelis-Menten constant were calculated.

Conclusion: By binding sioforan to the active site on the enzyme, metabolic processes in the body are inhibited, and therefore the development of the disease.

Key words: metformin; siofor; amylase; inhibition

PROGNOSTIC SIGNIFICANCE OF CEBPA MUTATIONS IN PATIENTS WITH NORMAL-KARYOTYPE - ACUTE MYELOID LEUKEMIA

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Introduction: *CEBPA* gene encodes a transciptional factor with an essential role in granulocyte differentiation process. In acute myeloid leukemia (AML), mutations in *CEBPA* gene have been associated with favorable outcome and up to now only the presence of double mutated *CEBPA* gene ($CEBPA^{dm}$) was included in WHO classification. Prognostic influence of *CEBPA* mutations in C-terminal (bZIP) region recently have been proposed as a marker for better overal survival (OS), higher probability of achieving complete remission (CR) and a lower risk of relapse. Since AML with normal karyotype (AML-NK) is a group with intermediate prognosis with the need for new prognostic markers, we analyzed the influence of bZIP *CEBPA* mutations as an additional molecular marker in Serbian AML-NK patients.

Methods: *CEBPA* mutational screening was performed using a multiplex polymerase chain reaction–based fragment length analysis. A total of 61 bone marrow samples were collected from *de novo* AML-NK patients.

Results: In our analysis, frequency of *CEBPA* mutations in Serbian AML-NK patients was 15% (12/61 patients). Six out of 12 patients had mutation in bZIP region ($CEBPA^{bZIP+}$). All six $CEBPA^{bZIP+}$ patients (100%) achieved CR after induction chemotherapy versus 62% of $CEBPA^{bZIP-}$ patients. $CEBPA^{bZIP+}$ patients showed a significantly longer OS ($CEBPA^{bZIP+}$ 31.5 months vs $CEBPA^{bZIP-}$ 10 months) and disease free survival (DFS) ($CEBPA^{bZIP+}$ 30 months vs $CEBPA^{bZIP-}$ 10.5 months).

Conclusion: Our analysis of Serbian AML-NK patients showed $CEBPA^{dm}$ was not associated with better prognosis but our results indicate that $CEBPA^{bZIP+}$ status is a good candidate for a prognostic molecular marker.

Key words: acute myeloid leukemia with normal karyotype; *CEBPA*; molecular marker

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DETECTION OF PRELEUKEMIC CLONES IN NEONATAL BLOOD SPOTS OF CHILDREN WITH B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Childhood B-cell precursor acute lymphoblastic leukemic (BCP-ALL) can be traced back to birth using leukemic clone-specific immunoglobulin heavy chain (*IGH*) rearrangements, implying prenatal origin of this disease. The aim of this study was to analyze neonatal blood spots (Guthrie cards) of childhood BCP-ALL patients for the presence of clonotypic *IGH* rearrangements.

Methods: The study enrolled 24 patients aged 1 to 9.6 years. Based on the sequences of *IGH* rearrangements identified in diagnostic lymphoblasts, 2 patient-specific primers were designed for each patient and used in semi-nested PCR for the detection of preleukemic clones at birth.

Results: Clonotypic *IGH* rearrangements were detected in neonatal blood spots of 54.2% of patients. In two cases that had double *IGH* rearrangements at diagnosis, only one rearrangement was present at birth, while in the third case both leukemic rearrangements were detected in neonatal blood. Guthrie card-positive findings were significantly more frequent in children ≤5 years of age than in older children (p=0.011). Regarding patients' characteristics at birth and at diagnosis, Guthrie card-positivity was not associated with sex, birth weight and mother's age, as well as with white blood cell count, percentage of bone marrow blasts, immunophenotype and the presence of *ETV6/RUNX1* and *TCF3/PBX1* fusion genes at diagnosis.

Conclusion: Our study confirms that a large proportion of childhood BCP-ALL originates *in utero*, regardless of the molecular subtype defined by chromosomal aberrations. The latency period to the overt leukemia depends on the presence of preleukemic clone at birth, as well as on the postnatal transforming genetic events.

Key words: childhood acute lymphoblastic leukemia; prenatal origin; Guthrie cards; immunoglobulin heavy chain rearrangements

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PROGNOSTIC SIGNIFICANCE OF THE LONG NON-CODING RNA MALAT1 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: The long non-coding RNA (lncRNA) MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) dysregulated expression has been reported in a variety of cancers, but has been poorly investigated in chronic lymphocytic leukemia (CLL). The aim of this study was to investigate the expression pattern of lncRNA MALAT1 in CLL, and evaluate its prognostic significance.

Methods: MALAT1 expression was analyzed in peripheral blood mononuclear cells of 114 newly-diagnosed CLL patients and 20 healthy controls by qRT-PCR, and association with clinical and biological features at diagnosis was assessed.

Results: MALAT1 was overexpressed in CLL compared to control samples ($p<0.001$). MALAT1 expression was higher in male patients ($p=0.003$). It showed no correlation with age, leukocyte, lymphocyte and platelet count, and serum β2-microglobulin, but exerted a positive correlation with hemoglobin level ($r=0.315$, $p=0.003$) and a negative correlation with lactate dehydrogenase level ($r=-0.303$, $p=0.004$). MALAT1 expression was higher in Binet A and B patients vs. Binet C patients ($p=0.037$). There was also a trend toward higher MALAT1 expression in patients with favorable (del13q) and intermediate (normal karyotype, trisomy12) cytogenetics in comparison to patients with unfavorable (del11q and del17p) cytogenetics ($p=0.059$). In addition, high MALAT1 levels were associated with CD38-negative status ($p=0.017$), but not with *IGHV* mutational status. While there was no association with the time to first treatment, longer median overall survival in MALAT1 high- vs. MALAT1 low-expressing cases was observed (142 vs. 82 months, log rank $p=0.032$).

Conclusion: LncRNA MALAT1 is up-regulated in CLL. High MALAT1 expression at diagnosis may be associated with better prognosis.

Key words: MALAT1; long non-coding RNA; expression; chronic lymphocytic leukemia; prognosis

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EXOSOMAL PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA) AS PROMISING BIOMARKER OF PROSTATE CANCER – EVIDENCE IN SERBIAN POPULATION

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Introduction: Prostate specific membrane antigen (PSMA) is an integral membrane glycoprotein recognized as an important tumor biomarker for prostate cancer (PCa). PSMA is known to be enriched in exosomes secreted by PSMA-positive PCa cells. Exosomes are small extracellular vesicles (30-100 nm) secreted by all cells including cancer that can transport proteins, nucleic acids and lipids from original cells. Herein, we tested the significance of exosomal PSMA as diagnostic and prognostic PCa biomarker.
Methods: Using commercial kit, the exosomes were collected from plasma samples of patients with PCa, as well as patients with benign prostatic hyperplasia (BPH) as a control group. The shape and size of the exosomes were confirmed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analysis. Immunogold analysis demonstrated that PSMA is localized to a membrane of exosomes isolated from plasma of both group of patients. The expression level of PSMA was detected in sample of plasma exosomes by western blot analysis.

Results: Western blot quantification results suggested a trend of statistical significance (*p=0.086*) in exosomal PSMA expression between PCa and BPH patients. Also, we did not find an association with the value of standard prognostic parameters of PCa, nor with risk for PCa progression.

Conclusion: We strongly believe that the main limitation of the study is a relatively small number of patients, as well as that the findings need to be evaluated in a larger group of participants.

Key words: prostate cancer; prostate specific membrane antigen; exosomes

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IDENTIFICATION OF MICRO RNA FROM COMMON COPY NUMBER VARIANTS AS RISK FACTORS FOR CAKUT

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Introduction: Congenital anomalies of the kidney and urinary tracts (CAKUT) are a diverse spectrum of defects with complex etiology and not fully explained genetic background. miRNA-containing copy number variants (CNVs) are described as genetic risk factor for the disease development. We aimed to identify miRNAs with the maximum regulatory coverage of previously reported differentially expressed genes in CAKUT tissue compared to controls and bioinformatically characterize a set of these miRNAs which are located in common CNVs.

Methods: Differentially expressed genes were identified from ureter tissue transcriptome open data GSE83946 from 15 CAKUT patients and 7 healthy controls, generated in house previously. miRPathDB v2.0 was used for identification of miRNAs with maximum coverage of DEGs (miRNAs which complimentarily regulate all DEGs). Mapping of maximum coverage miRNAs onto common CNVs (frequency >0.2) was performed using UCSC genome browser and gnomAD database. miRNA mapping common CNVs were further bioinformatically analyzed using miRPathDB v2.0.

Results: In a maximum coverage set of 50 miRNAs interacting with DEGs in CAKUT, we have identified 3 miRNA genes located in the common CNVs (hsa-miR-663b, hsa-miR-3180-3p and hsa-miR-1302). Using Reactome database we identified all three miRNAs to be significantly enriched in the pathway Neuronal System: -log(p-value)>2.326 for hsa-miR-1302; -log(p-value)>1.556 for hsa-miR-3180-3p; and -log(p-value)>1.703 for hsa-miR-663b.

Conclusion: CAKUT is characterized with variable penetrability and expressivity and often followed with other comorbidities such as neurodevelopmental disorders. miRNAs involved in DEG networks and prone to CNV effects could present modulating factors of the disease phenotype. Further studies should provide additional evidence about hsa-miR-1302, hsa-miR-3180-3p and hsa-miR-663b involvements in CAKUT etiology.

Key words: CAKUT; CNV; microRNA; Microarray; Bioinformatic analysis

Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia, PROMIS, #6066923, miFaDriCa, and Serbian Ministry of Education, Science and Technological development.

SELECTION OF MARKERS FOR AGE-RELATED METHYLATION CHANGE IN DNA FROM HUMAN BLOOD SAMPLES

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Introduction: Epigenetics plays an important role in aging, and methylated DNA (mDNA) has been recognized as the most valuable age-predictive marker in the field of forensic DNA phenotyping. Numerous predictive models based on the theory of „epigenetic clock" have already been established. Using samples from Serbian population, in this study we aim to define mathematical model for chronological age estimation, which would be routinely used in forensic investigations.

Methods: DNA extracted from the blood was quantified and subjected to bisulfite conversion, then amplified by PCR. PCR fragments were, then, analyzed by SNaPshot.

Results: The 48 blood samples from both male and female volunteers aged from 22 to 70 years were analyzed. Using 8 newly designed SNaPshot multiplex primers sets, and one multiplex primer set from previously published data, mDNA level of 40 CpG sites located in promotor regions of 7 genes (*ELOVL2*, *FHL2*, *TRIM59*, *KLF14*, *C1orf132*, *PDE4* and *EDDARAD*) has been analyzed in duplicate. Shapiro-Wilik test showed that distribution of mDNA level in some CpG cites was normal and in others, the distribution was not normal. Paired-t test and Wilcoxon Signed Rank test showed that there was not statistically significant difference between two SNaPshot mDNA measurements ($p>0,05$). Using Pearson and Spearman correlation coefficient we calculated the correlation between chronological age and change of mDNA level in each CpG cite.

Conclusion: 10 CpG cites that showed strong correlation (rho > 0,85) were considered as the most informative and selected for further analyses.

Key words: DNA methylation; Age estimation; SNaPshot; DNA Phenotyping; Forensic genetics

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CAN PHARMACOGENETIC VARIANTS IN *TPMT*, *MTHFR* AND *SLCO1B1* GENES BE USED AS POTENTIAL MARKERS OF OUTCOME PREDICTION IN SYSTEMIC SCLEROSIS PATIENTS?

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Introduction: Systemic sclerosis (SSc) is a rare autoimmune disorder that affects connective tissues and has the highest morbidity and mortality among rheumatologic diseases. Clinical presentations as well as disease progression are highly heterogeneous between patients, implying a strong need for individualization of therapy.

Methods: Four pharmacogenetic variants, namely *TPMT* rs1800460, *TPMT* rs1142345, *MTHFR* rs1801133 and *SLCO1B1* rs4149056 were tested for association with severe disease outcomes in 102 patients with SSc from Serbia treated either with immunosuppressants azathioprine (AZA) and methotrexate (MTX) or with other types of medications. Genotyping was performed using PCR-RFLP and direct Sanger sequencing. R software was used for statistical analysis and development of polygenic risk score (PRS) model.

Results: Association was found between *MTHFR* rs1801133 and higher risk for elevated systolic pressure in all patients except those prescribed with MTX, and higher risk for kidney insufficiency in patients prescribed with other types of drugs. In patients treated with MTX, variant *SLCO1B1* rs4149056 was protective against kidney insufficiency. For patients receiving MTX a trend was shown for having a higher PRS rank and elevated systolic pressure.

Conclusion: Our results open a door wide for more extensive research on pharmacogenomics markers in patients with SSc. Altogether, pharmacogenomics markers could predict the outcome of patients with SSc and help in prevention of adverse drug reactions.

Key words: Systemic Sclerosis; Pharmacogenetics markers; Methotrexate; Azathioprine; Rheumatologic Dis-eases; Personalized therapy

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APPLICATION OF CRISPR/CAS9 TECHNOLOGY FOR *IN VITRO* DISEASE MODELLING IN GLYCOGEN STORAGE DISEASE TYPE IB

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Introduction: Glycogen storage disease type Ib (GSD-Ib) is an autosomal recessive disorder characterized by fasting hypoglycemia and the accumulation of glycogen in the liver, kidneys and intestinal mucosa. Recent studies revealed that chronic endoplasmic reticulum (ER) stress and increased apoptosis play a role in the progression of disease manifestations. Although dietary control is commonly utilized to manage hypoglycemia, there is still a lack of effective pharmacological therapy. Therefore, the establishment of proper model system is essential for testing novel treatment approaches.

Methods: To create GSD-Ib *in vitro* model system, CRISPR/Cas9-knockout (KO) method was used to introduce a deletion in *SLC37A4* gene in the FlpInHEK293 cells. Characterization of CRISPR/Cas9-KO model system was performed using Sanger sequencing, RT-qPCR and Western Blot. Additionally, the expression analysis of ER stress and apoptotic markers was performed.

Results: Sanger sequencing confirmed the presence of c.14_100del in *SLC37A4* gene. The expression level of *SLC37A4* was decreased to 26.8% in the *SLC37A4*$^{-/-}$ cell line compared to the *SLC37A4* wild-type along with Western blot analysis, which confirmed reduced target protein level in *SLC37A4*$^{-/-}$ clones. Furthermore, ER stress (*ATF4*, *DDIT3*, *HSPA5*, *XBP1s*) and apoptotic (*BCL2*, *BAX*, *CASP3*, *CASP7*) markers expression levels were significantly altered in *SLC37A4*$^{-/-}$ clones compared to wild-type, which proved that we created a suitable GSD-Ib *in vitro* model system.

Conclusion: Utilizing CRISPR/Cas9 technology, we established cellular GSD-Ib model system that mirrors increased ER stress and apoptosis. This model system could be used to facilitate a comprehensive understanding of disease mechanisms and enable testing of potential treatment effectiveness.

Key words: CRISPR/Cas9 knockout; Glycogen storage disease type Ib; *in vitro* disease modelling; endoplasmic reticulum stress; apoptosis

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ESTABLISHMENT OF INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS WITH 22Q11.2 DUPLICATION SYNDROME AS A MODEL SYSTEM FOR STUDYING NEURODEVELOPMENTAL DISORDERS

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Introduction: Neurodevelopmental disorders (NDDs), such as autism spectrum disorders (ASD), schizophrenia, and intellectual disability, represent important public health challenge in modern societies with a prevalence of about 10 to 15% of all births and the tendency of increasing worldwide. They are caused by disruption of early brain development. Treatments of NDDs are focused on symptoms due to a limited understanding of underlying pathophysiological mechanisms. Individuals with the 22q11.2 Duplication Syndrome (22q11.2dup), caused by heterozygous 22q11.2 microduplication, have an elevated risk of developing NDDs. Literature data revealed that ASD is detected in 14-25% of patients with 22q11.2dup while schizophrenia is less common in these patients than in the general population, suggesting that 22q11.2 duplication might be protective against schizophrenia.

Methods: Genomic and clinical findings in patients with 22q11.2dup were analyzed and peripheral blood mononuclear cells of patients with 22q11.2dup were reprogrammed.

Results: We formed a cohort of 8 patients with 22q11.2dup. The majority of patients in our cohort have microduplication of approximately 3Mb (80%). Also, the majority of them are familial cases and in 67% of cases, the 22q11.2 microduplication is inherited from the mother. Congenital heart defects were detected in 25% of our patients, while all tested patients have facial dysmorphism. iPSCs were generated from three patients with a familial form of 22q11.2dup and their mothers.

Conclusion: A cohort of patients with 22q11.2dup is formed and iPSCs were generated which can be used as a model system for studying NDDs.

Key words: 22q11.2 Duplication Syndrome; neurodevelopmental disorders; iPSCs; familial cases

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TWO MAIN SKELETAL MUSCLE MOLECULAR PHENOTYPES OF MOUSE DM1 MODELS: A COMPARATIVE TRANSCRIPTOMIC ANALYSIS

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Introduction: Myotonic dystrophy type 1 (DM1) is a rare, incurable multisystemic disease, with the main symptoms being skeletal muscle weakness, atrophy, and myotonia. It is caused by CTG expansion in the 3' UTR of the *DMPK* gene whose RNA acquires toxic functions and sequesters MBNL proteins, resulting in globally altered RNA metabolism. To better understand the DM1 transcriptome, we systematically analyzed gene expression in the skeletal muscles of various mouse DM1 models.

Methods: We retrieved 13 publicly available RNA-seq datasets from mouse models expressing expanded CTG repeats (HSALR, CTG480KI, TREDT960I) and *Mbnl* knockout models (SKO, DKO, TKO). Our bioinformatic pipeline with unified parameters consisted of preprocessing, differential expression (DESeq2), gene network analysis (WGCNA), comparison of gene network interactions with the STRING database, and network node enrichment analysis (Cytoscape).

Results: In models expressing CTG repeats, the average number of up-regulated genes was 787, compared to 676 in Mbnl knockout models, while there was 642 and 380 down-regulated genes, respectively (log2FC>1, padj>0.05). Both model groups had network modules whose nodes were enriched for muscle and secretory functions (FDR<0.05). There were modules related to immune response, lipid transfer, and insulin in models expressing repeats and modules related to immunoglobulins and extracellular matrix in knockout models.

Conclusion: Gene expression patterns separated *Mbnl* knockouts from models expressing CTG repeats that had a greater number of smaller functionally distinct network modules. Our results revealed novel pathway changes in DM1 skeletal muscles, among which immunological and secretory are particularly interesting as molecular targets for further investigation.

Key words: Myotonic dystrophy type 1; comparative transcriptomics; DM1 mouse models; gene co-expression networks

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METHYLATION PROFILE ANALYSIS OF DNA-HALO STRUCTURE BY SYNCHROTRON-BASED FTIR SPECTROSCOPY

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Introduction: DNA methylation is a major regulator of transcriptional activity and alongside other epigenetic modifications it introduces specific level of chromatin complexity. Fourier transform infrared (FTIR) spectroscopy is a rapid, non-destructive, and label-free technique for identifying subtle changes in all bio-macromolecules, and it has been used as a method of choice for studying DNA conformation. The present study was designed to explore the use of synchrotron-based FTIR spectroscopy to monitor the subtle changes on molecular level regarding the DNA methylation status of cytosine in the whole genome.

Methods: For FTIR-based DNA methylation analysis *in situ*, DNA-HALO samples were prepared using slightly modified methodology for nuclear HALO preparations where DNA-HALOs are liberated of any protein residues but preserve higher order chromatin structure.

Results: Using FTIR spectroscopy we analysed and compared methylation profiles of isolated genomic DNA and DNA-HALO samples. DNA-HALO structure shows more distinct peaks in fingerprint region of spectra. DNA-HALO structure is more accurate for detecting bonds in unmemthylated cytosine as specific infrared peaks are defined as vibrations of bonds in unmethylated cytosine at 1151 cm^{-1} and 1357 cm^{-1}. The ratio of integrated area under the peak 1151 cm^{-1} over integrated area under the peak that represents PO_2^- backbone vibrations can be used to assess level of unmethylated cytosine and thus methylation rate in the DNA-HALO samples.

Conclusion: This study demonstrates potential of FTIR spectroscopy to detect DNA methylation in DNA-HALO samples more precisely compared to classical DNA extraction procedure that yield unstructured whole genomic DNA.

Key words: HALO structure; FTIR spectroscopy; DNA methylation; Epigenetic marks; Chromatin

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ASSOCIATION OF *IL2RA* RS2104286 VARIANT WITH ADAPTIVE IMMUNITY GENE EXPRESSIONS LOCATED IN 17q12-21 CHROMOSOMAL REGION IN MULTIPLE SCLEROSIS PATIENTS

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Introduction: The *IL2RA* rs2104286 (A>G) variant is a risk factor for multiple sclerosis (MS), located in an STAT5 super-enchancer region. The G allele shows a protective effect toward MS susceptibility by lowering *IL2RA* expression in naive CD4+ T cells and likelihood of lymphocyte activation through ligation of IL2. Adaptive immunity relevant genes, including *ORMDL3*, *GSDMB* and *IKZF3* are located in 17q12-21 chromosomal region that is associated with risk for asthma and MS. IL2 treatment of mouse CD4+ T lymphocytes causes lower *Ormdl3* and *Ikzf3* expression, while *ORMDL3* downregulation causes higher IL2 expression in Jurkat T cells. Lower expressions of *ORMDL3* and *GSDMB* in PBMCs were associated with MS. We analyzed the association of *IL2RA* rs2104286 variant with *ORMDL3, GSDMB* and *IKZF3* mRNA expression levels in PBMCs of relapsing-remitting (RR) MS patients.

Methods: Association of *IL2RA* rs2104286 with gene expression was investigated according to the dominant genetic model (AA *vs*. AG + GG) in 66 RR MS patients. Genotyping and gene expression analysis were done by TaqMan® methodology.

Results: The protective G allele was associated with higher levels of *ORMDL3* ($p = 0.014$, fold change = 1.33, Student's T test) and *IKZF3* ($p = 0.023$, fold change = 1.38, Student's T test) mRNA expression, while there was no significant association with *GSDMB* mRNA expression ($p = 0.5$, Mann-Whitney U test).

Conclusion: Our results suggest that *IL2RA* rs2104286 G allele affects *ORMDL3* and *IKZF3* mRNA expression, which could influence the dynamics of T cell activation toward lower activation. Further research is required.

Key words: *IL2RA*; *ORMDL3*; *GSDMB*; *IKZF3*; MS

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EFFECTS OF MELDONIUM ON THIOACETAMIDE-INDUCED HEPATOTOXICITY IN *WISTAR* RATS

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Introduction: Hepatotoxicity is a liver injury caused by various factors such as drug usage, alcohol intake, malnutrition, viral infections, and others. Previous studies demonstrated the anti-inflammatory, antioxidant, and anti-apoptotic effects of meldonium (M). Therefore, we aimed to examine the effects of meldonium on thioacetamide (TAA)-induced hepatotoxicity in rats.

Methods: Female *Wistar* rats were randomly divided into three experimental groups. TAA and TAA+M groups were biweekly intraperitoneally (i.p.) injected with TAA (150 mg/kg) for 16 weeks. The control group received i.p. injections of saline. Simultaneously, the TAA+M group ingested meldonium (300 mg/kg) with drinking water. Blood samples were collected to measure AST and ALP serum activity. Using Western blot and RT-qPCR, we analyzed the hepatic expression of inflammatory markers, anti- and pro-apoptotic proteins, and oxidative stress parameters.

Results: TAA alone did not induce mortality, but co-administration with meldonium increased the mortality by 42%. As expected, TAA increased ALP serum activity, hepatic BAX/Bcl-2 ratio, and NFkβ, IL-6, TNFα, HMGB1, and CAT levels. Surprisingly, compared to the TAA group, meldonium did not alter ALP serum activity, hepatic BAX/Bcl-2 ratio, and NFkβ, IL-6, TNFα, HMGB1, and CAT levels. However, we observed a significantly increased AST serum activity in the TAA+M but not in the TAA group. Hepatic NOX4 and NRF2 levels remained unchanged in both treated groups.

Conclusion: Contrary to our expectations, meldonium not only failed to prevent hepatotoxicity but also significantly increased mortality. Despite the previously shown beneficial effects of meldonium, its potential application in other pathological conditions requires further investigation.

Key words: Meldonium; Thioacetamide; Hepatotoxicity; Inflammation; Oxidative stress

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RECONSTITUTION OF NON-CARRIER, HETEROZYGOUS AND HOMOZYGOUS PROTHROMBIN BELGRADE MUTATION CARRIER PLASMA USING RECOMBINANT PROTEINS

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Introduction: The prothrombin Belgrade variant (c.1787G>A, p.Arg596Gln) is a rare mutation found in Serbia, Japan, China, America, India and leads to antithrombin resistance. Prothrombin Belgrade mutation influences thrombin-antithrombin interactions and leads to impaired inactivation of mutated thrombin. Also, it affects sodium binding site in thrombin, which is important for switching from fast thrombin configuration (coagulant properties) to slow configuration (anticoagulant properties). It has only been found in a heterozygous state, which could mean that homozygous carriers are incompatible with life. By using prothrombin (FII) deficient plasma, we could reconstitute plasma of wild type, heterozygous and homozygous carrier, which could give more insight into the mechanism of this mutation.

Methods: Recombinant wild type and mutated prothrombin were generated by transient transfection in HEK293T cell line. Western blot analysis was performed to test the efficiency of transfection. Human Prothrombin ELISA (Nordic BioSite, Sweden) was used in order to measure recombinant prothrombin concentration. Overall Hemostasis Potential (OHP) assay was performed to assess recombinant protein activity. Recombinant wild type and mutated prothrombin were added to FII deficient plasma (Siemens, Germany) in order to create reconstituted plasma, in the final concentration of 0.1 mg/mL, as it is approximately the level of prothrombin in human plasma.

Results: Reconstituted plasma samples that correspond to non-carrier, heterozygous carrier, and homozygous mutation carrier plasma were reconstructed. Recombinant proteins tested by OHP assay were functional.

Conclusion: Reconstituted plasma samples allow us to examine the mechanism of prothrombin Belgrade mutation in various assays and in homozygous form as well.

Keywords: Transfection; Recombinant protein; Prothrombin; Mutation; Prothrombin Belgrade

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EXPRESSION PROFILES OF LONG NON-CODING RNA GAS5 AND MICRORNA-222 IN YOUNGER AML PATIENTS

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Introduction: Acute myeloid leukemia (AML) is a heterogeneous malignant disease, that accounts for 80% of all acute leukemias in adults. Imprecise risk stratification and lack of personalized treatment creates a constant need to find new prognostic markers and targets for innovative therapeutics. Recently, this search has pointed towards non-coding RNAs (ncRNA). Numerous studies have shown dysregulation of lncRNA *GAS5* in cancers, but it was poorly investigated in AML. Since *GAS5* acts like a molecular sponge for miR-222, co-expression profiles of these non-coding RNAs could be novel prognostic markers in AML.

Methods: *GAS5* expression levels were analysed in 94 AML patients and 14 healthy controls using Real-Time PCR and miR-222 expression levels were analysed in a subgroup of 39 patients with normal karyotype (AML-NK). ROC curve analyses were used to find a cut-off value between $GAS5^{high}$ and $GAS5^{low}$, while the median value was used for distinguishing between miR-222^{high} and miR-222^{low}.

Results: We showed that *GAS5* expression in AML patients was lower compared to healthy controls. Lower *GAS5* expression on diagnosis was related to an adverse prognosis. The disease-free survival and the overall survival were lower in the $GAS5^{low}$ group but survival analysis failed to confirm this finding. In the AML-NK group patients had higher expression of miR-222 compared to healthy controls. A synergistic effect of $GAS5^{low}$/miR-222^{high} status on disease prognosis was not established.

Conclusion: Our findings indicate the potential prognostic significance of *GAS5* expression and the need for further investigation of these two non-coding RNAs and their potential roles in leukemogenesis.

Key words: AML; *GAS5*; miR-222

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E-CIGARETTE LIQUID AND CONDENSATE LEADS TO IMPAIRED EMBRYONIC DEVELOPMENT OF ZEBRAFISH

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Introduction: E-cigarettes are advertised as safer alternative to tradicional cigarettes. However, they contain chemicals that can exhibit toxic effects on the organism. Notably, effects of e-cigarettes on *in utero* development are not well studied. We wanted to compare potential toxic effects of e-cigarette liquid and vapor condensate on development of zebrafish embryos.
Methods: Six hour old zebrafish embryos were exposed to different concentrations of e-cigarette liquid or vapour condensate – 0.1% and 1%. Untreated embryos were used as control. Each treatment and control were set up in triplicate, with at least 20 embryos per treatment. The effects on survival, hatching and developmental malformations were monitored using light microscopy, at 3 timepoints - 24, 48 and 72 hours post fertilization (hpf).
Results: No noticeable differences between control and treated groups were observed 24 hpf. Hatched larvae (35%) treated with 0.1% condensate had scoliosis and malformations- yolk sac and pericardial edema at 48 hpf. In groups treated with 1% of condensate or liquid, hatching was delayed and did not start 48 hpf. At 72 hpf timepoint, in wells with 1% condensate, less than 30% of larvae hatched in total, which was comparable to wells with e-cigarette liquid (25%). Malformations that were observed in all treatements are hemagglutionation, pericardial or yolk sac edema, and scoliosis. In groups with 0.1% condensate these maformations were observed in lower number of embryos, but the percentage of hatched larvae was higher (approximately 80%) at 72 hpf.
Conclusions: Chronic exposure to e-cigarette vapor condensate and liquid leads to severe disorders of zebrafish embryonic development.

Key words: e-cigarettes; toxicology; developmental malformations; zebrafish

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POLYSTYRENE NANOPARTICLES NEGATIVELY INFLUENCE TROPHOBLAST CELL FUNCTION

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Introduction: Plastics represents a predominant environmental pollutant. Waste plastic degrades into micro and nanoplastics, which can be found in food and drinking water. These particles are easily ingested, potentially affecting physiological processes. One of the most commonly used plastics is polystyrene. Extravillous trophoblast cells (EVT) of the placenta play a major role during placentation enabling progress of healthy pregnancy. Matrix metalloproteinases (MMP)-2 and -9 are key mediators of EVT invasion into maternal decidua.

Methods: In this study we wanted to investigate the impact of polystyrene nanoparticles (PN) of 40nm ($1.5x10^{11}$ and $1.5x\ 10^{12}$ p/ml) and 200 nm ($2.2x10^{7}$ and $2.2x\ 10^{8}$ p/ml) diameter on EVT cell line HTR-8/SVneo *in vitro*. PN internalization into cells was observed by fluorescent microscopy. Cell viability was determined by MTT assay, and cell migration by "Wound healing" scratch assay. Expression of MMPs was determined at mRNA and protein levels by qPCR and gelatin zymography, respectively. The genotoxic potential was assessed by alkaline comet assay.

Results: There was an apparent cellular internalization of 40nm NPs. HTR-8/SVneo cell viability was significantly reduced after 72h of treatment with higher concentration of 40nm NPs (83% of control). Cell migration was inhibited by both concentrations of 40nm NPs (74% and 57% of control), as were MMP-2 protein levels (51% and 14% of control). Moreover, both 40 and 200nm PN showed a substantial genotoxic effect.

Conclusion: It can be concluded that PN have a substantial negative effect on EVT function *in vitro*, potentially implicating them in aberrant placentation process *iv vivo*.

Key words: nanoplastics; trophoblast; cell migration; MMPs

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DYNAMICS OF CTG REPEAT EXPANSION IN BLOOD OF MYOTONIC DYSTROPHY TYPE 1 PATIENTS OVER TIME

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Introduction: Myotonic dystrophy type 1 (DM1) is the most common adult muscular dystrophy caused by CTG repeat expansion in *DMPK* gene. DM1 mutation shows tissue-specific instability with continuous increase in repeat number, contributing to disease progression. We aimed at studying CTG expansion size dynamics over time.

Methods: A group of 40 patients with two blood samples available in an interval >5 years, without repeat interruptions and congenital form, was selected from the Serbian DM registry. Single-molecule small-pool PCR was used, with 30–60 pg of DNA and >150 alleles sized per sample per time point. The allele frequency distributions were compared for each patient using the Wilcoxon–Mann–Whitney test and described by kurtosis.

Results: In 39 patients significant difference in distribution over >5 years was found (p for individual patient ≤0.026). Of these, 26 patients demonstrated a unidirectional shift in distribution towards larger expansion, with an average modal allele increase of 160 repeats. Changes from bimodal to unimodal distribution and vice versa was observed in 9 patients, all with modal allele size <1000 repats. Additionally, 4 patients with the age at onset <25 and modal allele >1000 repeats showed a wide allele distribution at both time points (kurtosis <3).

Conclusion: Although our longitudinal analysis confirmed that DM1 mutation continues to expand throughout patients' lifetime, individual differences in the mutation rate are observed. The introduction of additional parameters for a comprehensive description of the changes in allele distribution is needed for better understanding DM1 mutation dynamics.

Key words: Myotonic dystrophy type 1 (DM1); CTG repeat expansion; Somatic instability; Longitudinal analysis

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AUTOPHAGY RECEPTOR P62 REGULATES SARS-COV-2-INDUCED INFLAMMATION IN COVID-19

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Introduction: Since the interaction between autophagy and virus-induced inflammation is complex, we investigated the interplay between autophagy and inflammation in COVID-19 patients and THP-1 cells expressing SARS-Cov2 proteins NSP5 and ORF3a.

Methods: Autophagy markers in blood from 19 control subjects and 26 COVID-19 patients at hospital admission and one week later were measured by ELISA, while cytokine levels were examined by flow cytometric bead immunoassay. The level of p62 in cells and its concentration in cell culture supernatants was measured by immunoblot/ELISA. The mRNA levels of proinflammatory cytokines were measured by RT-qPCR.

Results: IFN-α, TNF, IL-6, IL-8, IL-17, IL-33, and IFN-γ were elevated in COVID-19 patients at both time points, whereas IL-10 and IL-1β were elevated at admission and one week later, respectively. Autophagy markers LC3 and ATG5 were unchanged in COVID-19. The concentration of autophagic cargo receptor p62 was significantly lower and positively correlated with TNF, IL-10, IL-17, and IL-33 at hospital admission, returning to normal levels after one week. The expression of SARS-CoV-2 proteins NSP5 or ORF3a in THP-1 cells caused an autophagy-independent decrease/autophagy-inhibition-dependent increase of intracellular and secreted p62. This was associated with an NSP5-mediated decrease in TNF/IL-10 mRNA and an ORF3a-mediated increase in TNF/IL-1β/IL-6/IL-10/IL-33 mRNA levels. A genetic knockdown of p62 mimicked the immunosuppressive effect of NSP5, while a p62 increase in autophagy-deficient cells mirrored the immunostimulatory action of ORF3a.

Conclusion: The autophagy receptor p62 is reduced in acute COVID-19, and the balance between autophagy-independent decrease and autophagy blockade-dependent increase of p62 levels could affect SARS-CoV-induced inflammation.

Key words: inflammation; COVID19; p62; NSP; ORF3a

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SALIVARY CARCINOEMBRYONIC ANTIGEN AS A BIOMARKER OF SJÖGREN`S SYNDROME

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Introduction: Sjögren's syndrome is a lymphoproliferative disease with autoimmune features, characterized by a progressive hypofunction and inflammation of salivary and lachrymal glands. Diagnosis of syndrome is problematic, because it often relies on nonspecific signs and symptoms with no specific biomarker(s). Since saliva directly reflects salivary gland inflammation, this body fluid appeared as useful tool for new biomarker research. Saliva also contains a number of glycosylated proteins, including heavily glycosylated carcinoembryonic antigen (CEA), described as inflammatory protein. This study aimed to investigate CEA as a potential salivary biomarker in Sjogren's syndrome patients**.**

Methods: This study was carried out on a group of healthy volunteers (n=21), patients with osteoarthritis (49), rheumatoid arthritis (45) and Sjögren's syndrome (n=44). The salivary protein concentration was determined by using BCA protein assay kit (ThermoScientific), while the levels of salivary CEA were measured using immunoradiometric assay IRMA CEA (INEP).

Results: The salivas from healthy subjects revealed CEA concentrations with median value of 238 µg/L. Salivary CEA in patients with osteoarthritis, rheumatoid arthritis and Sjögren's syndrome had the following median values: 346 µg/L, 490 µg/L and 1298 µg/L, respectively. Analysis of the obtained results indicated statistically significant increase in salivary CEA in patients with Sjögren's syndrome in comparison to healthy subjects ($p<0.05$), osteoarthritis ($p<0.01$) and rheumatoid arthritis ($p<0.05$).

Conclusion: The obtained results indicate that salivary CEA could be a potentially useful diagnostic and follow-up Sjögren syndrome biomarker. Due to many indicated roles of CEA family members, CEA presence could be functionally relevant in the pathogenesis of disease.

Key words: Sjögren syndrome; saliva; biomarker; CEA

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MICROBES AS TRIGGERS OF RHEUMATOID ARTHRITIS: REVISITING THE IMPACT OF MOLECULAR MIMICRY THROUGH IMMUNOINFORMATICS

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Introduction: Autoimmune diseases, affecting ~5% worldwide population, are connected to infection through molecular mimicry – the concept of shared specificity between foreign and self-peptides, driving cross-activation of autoreactive lymphocytes. Studying molecular mimicry has traditionally been limited to clinically important microbes and canonical autoantigens. Contrarily, evolutionary conserved autoantigens (systems-level acting) should preferentially be mimicked by phylogenetically distant organisms. Also, any human-associated microorganism, pathogen/commensal, coding for disease-related mimotopes, might trigger autoimmunity in genetically susceptible individuals, which we explore through a customized immunoinformatics protocol on the example of rheumatoid arthritis (RA).

Methods: A comprehensive specificity analysis of all the experimentally validated T-epitopes in RA (IEDB) was performed against bacterial, fungal, and viral proteomes. Candidate RA-mimotopes were filtered based on the assignment of pathogenicity/commensalism for originating species (NCBI Taxonomy Browser) and binding affinity prediction to RA-related MHC molecules (TepiTool). The robustness of RA-triggering capacity for proposed microbes was inferred by *de novo* T-epitope prediction in homologous antigens through targeted MHC restriction analysis.

Results: A much larger repertoire of RA-triggering microbes was proposed, providing novel insights for the underestimated role of Fungi and bacterial commensals in RA-etiology. Endoplasmic reticulum chaperone BiP emerged as the most likely mimicked RA-autoantigen, according to initial hypothesis. Despite restrictive filtering, majority of previously known RA-triggers were identified, corroborating the applied search methodology.

Conclusion: The presented protocol enables exploring *en gro* molecular mimicry as a pathogenetic mechanism for autoimmune pathologies with a pre-defined set of T-epitopes, whereby the identification of most potent/likely mimicked autoantigens might have possible implications for the design of novel autoantigen-specific, tolerance-inducing therapeutics.

Key words: molecular mimicry; immunoinformatics; rheumatoid arthritis; heat shock proteins; fungi

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VITAMIN B COMPLEX SUPPRESSES NEUROINFLAMMATION IN ACTIVATED MICROGLIA: *IN VITRO* AND *IN SILICO* APPROACH

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Introduction: Vitamin B deficiency is associated with cognitive dysfunction and various neurological diseases. Neuroinflammatory processes play a key role in the development of central nervous system (CNS) pathology. These processes are initially caused by an altered immune response, mediated by various peripheral immune cells, as well as resident CNS immune cells, microglia cells.

Methods: The anti-inflammatory effects of the vitamin B complex (VBC – B_1, B_2, B_3, B_5, B_6, and B_{12}) on the function and phenotype of lipopolysaccharide (LPS)-stimulated BV2 microglia cells were examined *in vitro*. Additionally, VBC-treated microglia supernatants were evaluated on SH-SY5Y cells to investigate the effects on neurons' viability. The potential anti-inflammatory mechanisms of VBC were examined by molecular docking studies to determine the binding affinity of the each VBC component to Toll-like receptor 4 (TLR4) signalling pathway proteins and inducible nitric oxide synthase.

Results: VBC treatment reduced the inflammatory mediators secreted by LPS-stimulated microglia, diminished their neurotoxic effects against neurons, and induced changes in phenotype profile toward M2 microglia type. As demonstrated by molecular docking, different B vitamins have the potential to inhibit proteins within the LPS-induced BV2 inflammatory pathways, mediated by LBP, CD14, TLR4/MD2, as well as iNOS as a significant marker of microglial activation.

Conclusion: Vitamin B complex exhibit a prominent synergistic effect and have the potential to be used as an additional therapy in reducing neuroinflammation and subsequent neurodegeneration.

Key words: Microglia; Neuroinflammation; Vitamin B complex; Neuroprotection; Molecular docking

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ASSESSMENT OF ASSOCIATION BETWEEN GENETIC VARIANTS IN microRNA GENES *hsa-miR-146* AND *has-miR-27a* AND MALE INFERTILITY IN NORTH MACEDONIAN POPULATION

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Introduction: Previous studies show that aberrant expression of microRNAs (miRNAs) impairs spermatogenesis, most often resulting in infertility. Furthermore, an association between idiopathic male infertility and the presence of a single nucleotide variant (SNV) at the miRNAs' binding site in its targeted mRNAs has been reported. Nevertheless, the link between SNVs in the miRNA genes and male infertility has not yet been investigated. The aim of this study was to assess the association between genetic variants rs2910164 and rs895819 and male infertility in North Macedonian population.

Methods: The study used peripheral blood samples obtained from the patients with idiopathic infertility while the control group comprised volunteers derived from general population. Genomic DNA was isolated using magnetic bead technology following the manufacturers' protocol. Genotyping of rs2910164 in miR-146a gene and rs895819 in miR-27a gene was performed using Taqman SNV Genotyping Assay. Statistical analysis of SNV association as well as Hardy-Weinberg equilibrium were done using SNPStats software.

Results: The genotyping of genetic variants rs2910164 and rs895819 was successful for 126 control subjects and 158 patients. The comparison of genotype frequencies in patients and controls yielded no evidence of association between these genetic variants and male infertility.

Conclusion: The main limitation of this study is a relatively small sample size. Therefore, to make further conclusions about the association between SNVs rs2910164 and rs895819 and male infertility, the number of patients selected needs to be enlarged and studies in other populations are required.

Key words: association study; microRNA; miR-146; miR-27-a; male infertility

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NUDT15 AS POTENTIAL MARKER FOR PHARMACOGENETIC-GUIDED 6-MERCAPTOPURINE THERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN SERBIA

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Introduction: The *NUDT15* is new pharmacogene of importance for 6-mercaptopurine therapy, given to children with acute lymphoblastic leukemia (ALL). The association of side effects in children with variants in *NUDT15* are well established in Asian populations, yet the relevance of this pharmacogene in European populations remains largely unexplored. The aim of this study was to identify pharmacogenetic variants in coding and neighbouring regions of *NUDT15* gene and analyse if the expression levels of *NUDT15* can predict the occurence of side effects of 6-mercaptopurine during the maintenance therapy in children with ALL of Serbian origin.

Methods: The genotyping of coding and neighbouring regions of *NUDT15* gene was performed using PCR and Sanger sequencing based technology in 48 children with ALL. *NUDT15* expression was analyzed in mononuclear cells of 24 ALL patients at diagnosis and 6 healthy controls by qRT-PCR, and association with surogate markers was assessed using adequate statistical methodology.

Results: The genotypig revealed the presence of 5 variants in *NUDT15* (NUDT15(NM_018283.4):c.36A>C, NUDT15(NM_018283.4):c.158+117C>T, NUDT15(NM_018283.4):c.158+174G>A, NUDT15(NM_018283.4):c.159-91G>A, NUDT15(NM_018283.4):c.*7G>A), none of them with effects on the expression or the function of NUDT15 protein. There was no statistically significant association between the expression of *NUDT15* at diagnosis and the surogate markers of side effects (number of episodes of leukopenia (p=0.821), number of weeks without therapy (p=0.507), number of weeks with lower dose (p=0.434), average doses (p=0.374)) of 6-mercaptopurine during the maintenance therapy.

Conclusion: Presently, *NUDT15* cannot be used as a pharmacogene in predicting the toxicity of 6-mercaptopurine terapy in children with ALL in Serbia.

Key words: *NUDT15*; 6-mercaptopurine; pharmacogene; acute lymphoblastic leukemia

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GENERATION OF EXPANDED CTG REPEAT PLASMIDS IN E. COLI FOR MYOTONIC DYSTROPHY TYPE 1 MODEL SYSTEMS

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Introduction: Expansions of short repetitive DNA regions are known as the cause of various neurodegenerative diseases including Huntington's disease, myotonic dystrophies, Fragile X syndrome and many others. Myotonic dystrophy type 1 is a rare neuromuscular disorder in which the molecular pathogenesis involves the expansion of CTG repeats in the 3' untranslated region of the DMPK gene. A common challenge in research is the difficulty of cloning trinucleotide repeats, which often becomes a significant bottleneck. Aim of our study is to generate a model for studying repeat instability and disease pathogenesis.

Methods: We performed *in vitro* ligation of constructs derived from a plasmid vector peGFP-N1 maintained in *E. coli*. Restriction enzymes type IIs were used for cloning. Constructs' sequences were validated by Sanger sequencing.

Results: We chose to model DM1 patients with 35 to 200 CTG repeats. The first construct was generated through the annealing of oligonucleotides containing 10 CTG repeats. Subsequently, we *in vitro* ligated constructs and, obtained sequences with 18, 34, 66, and 130 CTG repeats, respectively, according to 2n-2 formula. The maximum repeat length that could be validated using Sanger sequencing was 130 CTG repeats.

Conclusion: Our study successfully generated plasmids with expanded CTG repeats using traditional cloning methods. However, given the technical challenges associated with this approach, our next step is taking a cell-free approach for generation of larger repeat lengths. These models provide valuable tools for studying repeat instability and unraveling the molecular mechanisms of myotonic dystrophy type 1, potentially leading to new therapeutic targets.

Key words: cloning; myotonic dystrophy type 1; repeats expansions; RNA toxicity

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UNVEILING THERAPEUTIC POTENTIAL OF BACTERIOPHAGE TREATMENT IN ACINETOBACTER BAUMANNII-INFECTED ZEBRAFISH EMBRYO MODEL

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Introduction: There is an urgent demand for the development of new therapeutic approaches to combat multidrug-resistant *Acinetobacter baumannii*, and bacteriophages appear to be a highly promising solution. Phages are suitable to precisely target the infection-causing bacteria without disrupting the beneficial microbiota. The zebrafish (*Danio rerio*) embryo model represents an insightful animal model for preclinical studying of various infectious diseases and for discovery of novel safe and effective antimicrobial drugs.

Methods: Systemic bacterial infection was established by microinjection of 2000 cells of nosocomial carbapenem-resistant *A. baumannii* strain 6077/12 into the bloodstream of 48 hour old zebrafish embryos. Infected embryos were treated by parenteral administration of 4 different doses (10, 50, 100, 500 PFU) of bacteriophage vB_AbaM_ISTD at 6 hours after infection (hpi). Efficacy of treatment was evaluated according to embryo survival, morphological malformations and bacterial burden (CFU) over a 3-day period.

Results: *A. baumannii*-infected embryos treated with bacteriophage resulted with 100% survival rate, while 70% of untreated embryos survived to 24 hpi and none to the end of the experiment. Viable bacterial cell count and embryo morphology observations indicated that the administered phage effectively reduced *A. baumanii* infection *in vivo*. The most effective dose was 500 PFU, decreasing the bacterial load by 3.09 log units during 24 hpi, while lower bacteriophage doses (10, 50 and 100 PFU) produced less prominent, but also significant bacterial reduction of 2.10, 2.19 and 2.67 log units, respectively.

Conclusion: Parentheral administration of phage ISTD demonstrated potent therapeutic activity against *A. baumannii* infection in every investigated dose.

Key words: bacteriophage; Acinetobacter; zebrafish; therapy; antimicrobial

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GENERATION OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM PATIENTS WITH 22Q11.2 DELETION SYNDROME AS A TOOL FOR STUDYING NEURODEVELOPMENTAL DISORDERS

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Introduction: Neurodevelopmental disorders (NDDs), such as autism spectrum disorders (ASD), intellectual disability (ID), schizophrenia, and bipolar disorder, are caused by the alterations in early brain development. They affect approximately 4% of the European population and represent a high socio-economic impact and financial burden. Treatments of NDDs are focused on symptoms since molecular mechanisms underlying NDDs are still unknown. One of the syndromes with a high risk for NDDs is 22q11.2 Deletion Syndrome (22q11.2DS) caused by microdeletion 22q11.2. 22q11.2 microdeletion is the most common microdeletion in humans; it is one of the strongest known risk factors for development of psychiatric illness and the highest known genetic risk for schizophrenia (approximately, 25% of patients with 22q11.2DS develop schizophrenia compared to 1% in the general population).

Methods: Genomic and clinical findings in 35 patients with 22q11.2DS were analyzed and peripheral blood mononuclear cells of patients with 22q11.2DS and healthy controls were reprogrammed.

Results: The majority of patients have 3 Mb deletion and nine of them have inherited 22q11.2 microdeletion from parents. Twenty-one different clinical presentations are revealed in the cohort with developmental delay detected in about 50% of patients. iPSCs were generated from four patients with 22q11.2 microdeletion and five healthy controls.

Conclusion: Cohort of patients with 22q11.2DS is form and iPSCs were generated which enable research of molecular mechanisms underlying NDDs.

Key words: 22q11.2 Deletion Syndrome; neurodevelopmental disorders; iPSCs

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THROMBOPHILIAS AND COMPLICATIONS IN PREGNANCY AND INFERTILITY

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Introduction: Thrombophilia is inherited/acquired blood clotting disorder associated with arterial/venous thrombosis. Inherited forms include deficiencies of antithrombin, protein S/C, and polymorphisms of procoagulant factor V/II. Thrombophilia affects pregnancy outcomes and implantation vascularization.

Methods: To assess thrombophilias in women with infertility, including acquired and inherited factors, a study examined 275 women. Among them, 255 had secondary infertility (196 spontaneous miscarriages in the first trimester, 29 in the second trimester, and 30 with unsuccessful IVF outcomes), 20 women had no pregnancies. To determine the frequency of acquired (LAC, ACA) and inherited thrombophilias (AT III, PS, PC, hyperhomocysteinemia, polymorphisms of FV, FII, MTHFR, PAI) by tests performed on serum or plasma and analysis mutations: FV 1691 G>A, FII 20210G>A, MTHFR 677 C>T, PAI-1 -675 4G/5G using DNA by amplifying the target genes through real-time PCR.

Results: The same frequency of carriers of A alleles (15%) was observed in the group of women with primary infertility for FII and in those with a positive family history for FV. In women with spontaneous miscarriages in the first trimester, the frequency of carriers of the 4G allele for PAI was 81%, and in those with hyperhomocysteinemia, the frequency of the MTHFR T allele was 85%.

Conclusion: Inherited thrombophilia impacts the process of implantation and placental vascularization.

Key words: pregnancy; infertility; thrombophilias

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MOLECULAR BASIS OF THALASSEMIA SYNDROMES IN SERBIA: AN UPDATE

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Introduction: Thalassemia syndromes are heterogeneous group of hereditary anemias characterized by defects in the synthesis of hemoglobin (Hb) polypeptide chains. These disorders comprise thalassemias and thalassemic hemoglobin variants which are predominantly caused by mutations in a- and b-globin genes (*HBA* and *HBB* genes). Clinical manifestations of thalassemia syndromes range from asymptomatic thalassemia minor to severe anemia in thalassemia major cases. The aim of this study was to update our previous findings on frequency of thalassemia mutations which result from a 13-year-old systematic survey in Serbia.

Methods: Two hundred and fourteen patients from 149 unrelated families presented with hematological parameters indicative of thalassemia syndromes were studied. Detection of α- and β-globin gene mutations was performed using PCR and direct sequencing.

Results: Two Hb variants and twelve different β-thalassemia mutations, including two mutations previously not reported in Serbian population, were detected. Hb variant Lepore Boston-Washington was the most common cause of thalassemia, with frequency of 24.3%, followed by *HBB*:c.316-106C>G mutation detected in 18.1% of families. The third most frequent cause of β-thalassemia were *HBB*:c.118C>T and *HBB*:c.93-21G>A mutations with 16.6% incidence each. Together, these four variants account for over 75% of all mutated β-globin alleles. In addition, five families affected with α-thalassemia were detected.

Conclusion: Despite the increase in cohort size by 50% between this and our previous studies, the frequency of mutations affecting *HBB* gene remained unchanged. Results presented in this study will update Serbian national mutation database and contribute to better understanding of geographic history of South European and Balkan populations.

Key words: thalassemia syndromes; Hb variants; β-thalassemia mutations; Serbia

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BIOINFORMATICAL FINE-MAPPING OF *TNFRSF11A* LOCUS IDENTIFIED RS4574025 AND RS4369774 TO BE POTENTIALLY CAUSAL VARIANTS FOR MYASTHENIA GRAVIS

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Introduction: Myasthenia gravis (MG) is a polygenic autoimmune disease mediated by antibodies against postsynaptic components of the neuromuscular junction, particularly the acetylcholine receptor (AChR). One of the consistent results from a few genome-wide association studies (GWAS) is an association of AChR-MG with the *TNFRSF11A*, a gene with a role in the immune response. Since the GWAS identifies single nucleotide variants (SNVs) serving as proxies for large genomic regions containing unmeasured SNVs, this *in silico* study aimed to identify potentially causal *TNFRSF11A* variants for validation in Serbian patients.

Methods: Bayesian multivariate variable selection was applied through bioinformatical fine-mapping of *TNFRSF11A* locus, using summary statistics from a GWAS of European ancestry and publicly available Linkage disequilibrium (LD) data from 1000 Genomes Project. FINEMAP software was used for the initial mapping of the AChR-MG causal variants, followed by PAINTOR software that also integrates functional genomic annotation data, in this case obtained from RegulomeDB, to improve accuracy.

Results: Multiple independent signals within *TNFRSF11A* locus were identified, out of which SNVs rs4574025 and rs4369774 showed probability >75% for being causal in models with 1 or 2 causal variants per locus, when LD scores corresponding to Utah residents with Northern and Western European ancestry were used. RegulomeDB annotation of these SNVs indicated enrichment within transcription factor-binding sites and expression quantitative trait loci.

Conclusion: Our results point to *TNFRSF11A* rs4574025 and rs4369774 as potentially causal AChR-MG variants. Ongoing genotyping of these SNVs in a case-control study with >1000 individuals tests their association with AChR-MG in the Serbian population.

Key words: myasthenia gravis; GWAS, *TNFRSF11A,* bioinformatics; fine-mapping

Acknowledgment: The study was supported by the Ministry of science, technological development and innovations, Grant no. 451-03-47/2023-01/200178.

FADS2 GENE VARIANT rs174593 IS ASSOCIATED WITH MULTIPLE SCLEROSIS

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Introduction: The hallmark pathogenic mechanisms of multiple sclerosis (MS) are proposed to be associated with long chain polyunsaturated fatty acids(LC-PUFA)-mediated neuroinflammation, through LC-PUFA-derived pro- and anti-inflammatory eicosanoids. Variants in genes coding for fatty acid desaturases (FADS), the key enzymes in LC-PUFA biosynthesis from essential fatty acids, are associated with changes in circulating LC-PUFA levels. The aim of this study was to investigate the *FADS2* intronic variants, rs174576 (C/A), rs174593 (T/C) and rs174616 (G/A), in association with MS.

Methods: The study involved 124 patients with relapsing-remitting form of MS and 83 healthy control subjects. The *FADS2* gene variants were detected using TaqMan® SNP genotyping assays. Analysis of allele and genotype distributions in patients and controls was done by using the chi-square test.

Results: According to the model of dominant effect of allele, genotypes containing the alternative, C, allele of *FADS2* rs174593 variant were significantly less frequent in MS patients than in controls (MS: TT=57,26%, TC+CC=42,74%; controls: TT=42,17%, TC+CC=57,83%; p=0,03). In addition, the frequency of rs174593 C allele was significantly lower in patients, compared to controls (MS: T=0,76, C=0,24; controls: T=0,67, C=0,33; p=0,04). The frequency distributions of rs174576 and rs174616 alleles and genotypes were not significantly different between the study groups (p>0,05).

Conclusion: The obtained results supply a rationale for further investigation of the association of *FADS2* rs174593 with circulating LC-PUFA levels, in the context of MS. The genotype-LC-PUFA phenotype association could provide guidelines for personalized LC-PUFA supplementation, to potentially ameliorate the disease course and improve the effectiveness of therapy.

Key words: gene; variant; *FADS2*; multiple sclerosis

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MOLECULAR BIOMARKERS AS A PROGNOSTIC TOOL FOR CLINICAL COURSES OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN RATS IMMUNIZED WITH SPINAL CORD HOMOGENATE

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Introduction: Experimental autoimmune encephalomyelitis (EAE) in inbred rodents commonly shows different clinical courses, so that the diseased animals can be clustered into four groups: mild, moderate, severe, and lethal. Our aim was to determine biomolecular markers in the preclinical phase of EAE that allow the prediction of clinical course.

Methods: Female Dark Agouti rats were immunized with spinal cord homogenate without adjuvant and examined for four weeks for clinical signs of EAE. Cells and sera from blood collected on days 0, 3, and 7 after immunization were processed for detection of proinflammatory cytokines (IL-1, IL-6, TNF-α, and IFN-γ) by "real-time" RT-PCR and ELISA, respectively.

Results: Induction of EAE resulted in the downregulation of *ifng* and *tnfa* in the preclinical phase of disease, whereas *il1* and *il6* expression levels were unaffected. However, there was no correlation between the relative expression of *ifng* or *tnfa* and the cumulative clinical score (sum of daily clinical scores), suggesting that they are not predictive markers of EAE severity. Our preliminary results that suggest a negative correlation between *il1* expression level before EAE induction and cumulative score require further justification.

Conclusion: The proinflammatory cytokines investigated so far in our study cannot be considered as good biomarkers of EAE severity. However, the downregulation of *ifng* and *tnfa* in the blood cells during the asymptomatic phase of EAE suggests that they enter the central nervous system early from the bloodstream, which argues for the study of chemokine and/or chemokine receptors expression as potential biomarkers for the clinical courses of EAE.

Keywords: biomarkers; cytokines; experimental autoimmune encephalomyelitis; multiple sclerosis; neuroinflammation

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THE ROLE OF MIR-34 FAMILY MEMBERS ON THE MUCOCILIARY PROCESS IN THE CELLULAR RESPIRATORY MODEL SYSTEM

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Introduction: Primary ciliary dyskinesia is a rare and heterogeneous disorder primarily affecting the respiratory organs, with impaired mucociliary clearance being a common characteristic. Recently, the importance of the miR34/449 family in ciliogenesis in animal models has been described. This study aimed to establish a model system to study respiratory diseases and assess for the first time the role of the miR-34 family on the mucociliary process in humans.

Methods: We cultured the primary Normal Human Bronchial Epithelial (NHBE) cells in the air-liquid interface system, enabling the differentiation of multiciliated cells (MCCs) and goblet cells (GCs). During the differentiation process, transient overexpression of miR-34a/b/c members was conducted. The model system and treatments were validated through confocal microscopy (β-tubulin, MUC5B, MUC5AC antibodies) and qRT-PCR of miRNAs, specifically ciliogenesis markers (*NOTCH1*, *MCIDAS*, *GEMC1*, *CCNO*, *RFX3*), and differentiated cell markers (*FOXJ1* and *TFF3*).

Results: Expression levels of ciliogenesis and differentiated cells markers and detection of cilia and mucins at confocal microscopy confirmed the successful establishment of cellular model system. During the initial differentiation stage, an overexpression of miR34a/b/c changed the expression profile of ciliogenesis and differentiated cell markers.

Conclusion: The established model system provides a valuable platform for exploring innovative treatment approaches for lung diseases. These findings suggest that overexpression of miR34a/b/c has impact on mucociliary process by reducing the duration required for the process of ciliogenesis. Furthermore, the expression levels of differentiated cell markers suggest increased number of MCCs and decreased number of GCs, indicating the role of miR34a/b/c in enhancing mucociliary clearance.

Key words: Primary ciliary dyskinesia; Normal Human Bronchial Epithelial cell; miR-34 family; Mucociliary clearance

Acknowledgements: This work was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia (451-03-47/2023-01/200042).

COMPUTATIONAL PIPELINE FOR TANDEM REPEAT ANALYSIS IN LONG READ SEQUENCING DATA

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Introduction: The advent of novel long-read sequencing technologies has facilitated the characterization of challenging STR expansions. We have developed a computational pipeline capable of accurately quantifying tandem repeats on a per-read basis, while also detecting interruptions. To assess its performance, we applied the pipeline to sequencing data from plasmid constructs containing known CPG tandem expansions.

Methods: The pipeline involves inputting basecalled reads, mapped to the reference sequence. In each read, the pipeline detects the repeat number and presence of interruptions. Reads are categorized based on expansion size, and a consensus sequence is generated for each category. A repeat profile is created for each category, providing an overview of allele size distribution and interruption presence. To evaluate the pipeline, plasmid constructs were subjected to Sanger sequencing and fragment analysis to establish a ground truth.

Results: The read distributions obtained through the pipeline aligned with the plasmid data derived from laboratory experiments. The pipeline accurately predicted repeat numbers and identified interruptions in different repeat regions. Repeat counting accuracy was over 97% for $(CTG)_{12}$, $(CTG)_{51}$ and a plasmid with complex structure. Moreover, repeat profile generated by the pipeline matches the profile obtained from fragment analysis.

Conclusion: We have successfully demonstrated a robust and reproducible computational pipeline for analyzing long-read tandem repeat sequencing data. While initially developed for detecting tandem CTG repeat expansions, the pipeline can be easily adapted for analyzing similar motifs. Furthermore, our pipeline has the potential to accurately describe complex repeated regions and potentially replace time-consuming procedures like Southern blot.

Key words: long-read sequencing, short tandem repeats, computational pipeline, bioinformatics

Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia, Grant number #7754217, Understanding repeat expansion dynamics and phenotype variability in myotonic dystrophy type 1 through human studies, nanopore sequencing and cell models – READ-DM1

INVESTIGATION OF THE ROLE OF THE GLUCOSE-6-PHOSPHATE TRANSLOCASE IN THE ACTIVATION OF AUTOPHAGY AND GLYCOGEN-SELECTIVE AUTOPHAGY IN GLYCOGEN STORAGE DISEASE TYPE IB PATIENTS

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Introduction: Glycogen storage disease type Ib (GSD-Ib) is characterized by a deficiency of glucose-6-phosphate translocase (G6PT) encoded by the *SLC37A4* gene, affecting glucose homeostasis and disrupting autophagy. Recent findings suggest that G6PT may also play a role in autophagy and glycogen-selective autophagy (glycophagy) activation independent of its transport function. To investigate this hypothesis, two groups of GSD-Ib patients carrying variants with different effects on G6PT transport activity and stability (p.Asn27Lys and p.Leu348Valfs*53), were compared to the control group of subjects.

Methods: The relative expression levels of *SLC37A4* gene, autophagy (*mTOR*, *ULK1*, *PRKAG1*), and glycophagy markers (*GABARAPL1*, *GAA*, *STBD1*) were assessed in mononuclear cells of GSD Ib patients (four carrying p.Asn27Lys and four carrying p.Leu348Valfs*53 variant) compared to control group using RT-qPCR. Statistical analysis was performed using one-way ANOVA followed by a post-hoc t-test.

Results: The p.Asn27Lys group exhibited 1.5-2.5 times higher expression of *SLC37A4* and autophagy markers, while the p.Leu348Valfs*53 group showed downregulation by approximately 50% compared to the control group. Glycophagy markers were increased twofold in both patient groups, except for *GAA*, which had similar expression levels as the control group.

Conclusion: Individuals carrying the p.Asn27Lys variant display the presence of *SLC37A4* transcript in their cells, which correlates with autophagy activation. Conversely, in patients with the p.Leu348Valfs*53 variant *SLC37A4* is downregulated, indicating compromised autophagy activation. These findings support the role of G6PT in autophagy activation, independent of its transport activity. Furthermore, the elevated expression of glycophagy markers observed in both patient groups can be attributed to the accumulated glycogen.

Key words: glycogen storage disease type Ib; glucose-6-phosphate translocase; autophagy; glycogen-selective autophagy

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MOLECULAR BASIS OF PHENYLKETONURIA IN SERBIA: AN UPDATE

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Introduction: Phenylketonuria (PKU) is the most frequent inborn disorder of amino acid metabolism caused by variants in human phenylalanine hydroxylase gene (*PAH*).

Methods: In this study (an update for the time period of 10 years, with patients from our previous studies included) a total of 109 PKU patients from Serbia were analyzed. They were classified into three phenotypic categories in accordance with pre-treatment plasma phenylalanine level: classic PKU, mild PKU and mild hyperphenylalaninemia. For genetic analyses, we combined Sanger sequencing, MLPA and next generation sequencing to identify disease-causing variants in *PAH* gene, which were further classified using ACMG classification. Additionally, we used *in silico* and/or eukaryotic expression studies to assess the effect of novel genetic variants identified in our patients.

Results: Disease-causing variants were identified in 217 of 218 alleles, reaching detection rate of 99.5%. We detected a total of 32 different variants, of which 29 previously described and three novel ones: p.Gln226Lys, p.Pro244His and p.Pro416Leu. *In silico* and/or eukaryotic expression studies confirmed pathogenic effect of all novel genetic variants. The most frequent variant was p.Leu48Ser (31.2%), followed by p.Arg408Trp (13.8%), p.Ile306Val (9.2%). p.Glu390Gly (5%), p.Pro281Leu (4.6%), and p.Arg261Gln (3.2%). All detected disease-causing variants were classified as pathogenic using ACMG classification.

Conclusion: Our study brings the updated spectrum of molecular genetic data, variant classification and detailed phenotypic characteristics for PKU patients from Serbia. Therefore, our study contributes to better understanding of molecular landscape of PKU in Europe and to general knowledge on genotype–phenotype correlation in PKU.

Key words: phenylketonuria; phenylalanine hydroxylase; variant; genotype-phenotype correlation

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COMPARATIVE TRANSCRIPTOMIC ANALYSIS IMPLIES INNATE IMMUNE RESPONSE AND CELL CYCLE DISREGULATION IN PATIENT-DERIVED DM1 CELL MODELS

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Introduction: Myotonic dystrophy type 1 (DM1) is a rare neuromuscular disorder caused by a CTG expansion in the 3' UTR of *DMPK*. Expanded CUG repeats form hairpin RNA structures of different lengths which provide toxicity to mutant RNA. According to the best-studied mechanism, mutant RNA sequesters MBNL proteins, leading to aberrant splicing and overall transcriptome perturbation. To explore whether the innate immune response triggered by mutant RNA plays a role in DM1 molecular pathogenesis, we analyzed transcriptomes of patient-derived DM1 cell models.

Methods: We retrieved 3 publicly available RNA-seq datasets of patients DM1 myoblasts cell cultures and preformed preprocessing and alignment using FASTQC, Cutadapt, Bowtie2 and Subread. Differential gene expression was performed using DESeq2, and gene set enrichment analysis performed using GSEA software.

Results: The average number of upregulated genes was 2326, while the number of downregulated genes was 1945. DM1 upregulated genes were significantly enriched in immune system pathways (FDR ≤ 0.05). Moreover, genes were significantly enriched in pathways associated with cell cycle and extracellular matrix dysregulation (FDR ≤ 0.05). Interestingly, we found significant upregulation of genes important for RNA editing.

Conclusion: Our analysis revealed that transcriptomes in three different patient-derived DM1 myoblast cell models show significant immune response activation and extracellular matrix and cell cycle dysregulation. Upregulation of genes associated with RNA-editing may indicate a cellular counter response to innate immunity related pathways overactivation. These results call for detailed research about immune response and cell cycle regulation in the context of DM1.

Key words: myotonic dystrophy type 1; CTG expansion; RNA-seq; RNA toxicity; innate immunity

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A SEQUENCING ERROR OR THE PRESENCE OF HETEROPLASMY?

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Introduction: Our aim was to differentiate between potential causes of difficulty in analyzing the chromatogram, specifically determining whether it is attributed to an error or the presence of heteroplasmy.

Methods: Sequencing of the mtDNA control region was performed in Macrogen Europe and in the Center for Human and Molecular Genetics.

Results: Recent analysis of the control region of mtDNA from *Salmo* cf. *trutta* L. species revealed the presence of dual peaks in the majority of samples at site 111. Of the 42 samples sequenced in Macrogen, only two samples were not heterozygous for T and G at this specific position. In most samples of *Salmo trutta* (20/21) belonging to the Danubian haplogroup, the nucleotide at position 111 was G, while in the *marmoratus* haplogroup it was present in 10/21 samples. This could be a novel haplotype for the Danubian brown trout haplogroup characterized by the presence of the G nucleotide at this position. Interestingly, previous orders from Macrogen for the same species from the Zadlaščica River in Slovenia also showed some cases of double peaks at the same position. In contrast, some samples from the same river sequenced at the Center for Human and Molecular Genetics exhibited only a single peak for the T-nucleotide at position 111.

Conclusion: These results could indicate either a sequencing error or the presence of heteroplasmy with a novel haplotype. Further investigation is needed to determine the exact cause and significance of these dual peaks observed in the majority of samples at site 111.

Key words: dual peaks; mtDNA; Control region; *Salmo marmoratus*; SNP

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*CYP2C19*2* POLYMORPHISM AND NON GENETIC RISK FACTORS IN ACUTE CORONARY SYNDROME PATIENTS TREATED WITH CLOPIDOGREL

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Introduction: Clopidogrel is still a widely used drug in the treatment of acute coronary syndrome (ACS). Given that it is a prodrug that requires biotransformation through P450 enzymes, it is essential to recognize the genetic influence on the effectiveness of the therapeutic response. The aim of the study is to determine the effect of *CYP2C19*2* genetic polymorphism in the therapeutic response of patients.

Methods: A total of 196 patients diagnosed with ACS who were treated with clopidogrel for at least one year were recruited. Subjects were monitored in the Clinical Center of Montenegro. Data were collected by reviewing the anamnesis and talking to the patient. Genotyping for *CYP2C19*2* polymorphism was carried out by real-time PCR method. We analyzed risk factors: age, BMI, smoking, alcohol consumption, as well as the influence of comorbidities and additional therapy.

Results: Out of a total of 196 subjects, 167 of them had no adverse cardiovascular events during the follow-up period, while in 29 subjects the therapy did not prove to be effective. No statistical significance was found between the studied genotype and the effectiveness of clopidogrel (p=0.840), as well as BMI (p=0.410), smoking (p=0.887), alcohol consumption (p=0.607) and the effectiveness of the drug. In our research, we found a statistically significant decrease in drug effectiveness in patients with atrial fibrillation (p=0.04).

Conclusion: No correlation was found between the examined genotype and ineffective response to the drug. The association found between atrial fibrillation as a comorbidity in ACS patients with an ineffective therapeutic response indicates the need for a more personalized medical approach in these patients.

Key words: ACS, clopidogrel, *CYP2C19*2* polymorphism

Acknowledgements: This study was supported by the Ethics Committee of the Clinical Center of Montenegro.

SINGLE-MOLECULE RNA ANALYSIS THROUGH NANOPORE SENSING IDENTIFIES ALTERNATIVE TRANSCRIPTION TERMINATION SITES

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Introduction: Transcription has emerged as a valuable tool in both research and industry. However, current RNA characterization technologies suffer from amplification and enzymatic biases. Here, we introduce a strategy to study transcription at the single-molecule level by sizing RNA with RNA nanotechnology and nanopores.

Methods: Linear and circular plasmid DNA (3.1 kb) containing T7 promoter, CTG repeats, OriC and no terminator sequence were *in vitro* transcribed. Purified RNA was hybridized with DNA oligonucleotides (some of which were labeled) to produce RNA-DNA duplex molecules (RNA IDs) which were then characterized using solid-state nanopore sensing.

Results: Transcription of linear DNA revealed two distinct types of RNA: full length transcripts (mean length of 3.19 ± 0.27 kb), as well as shorter transcripts (1.75 ± 0.17 kb) indicating the prematurely terminated transcription within the OriC. The translocation time and the charge deficit were shown to be reliable indicators of transcript length. Transcription of circular plasmids resulted in RNA IDs originating from one up to five transcription cycles. The number of transcripts was inversely related to their length. Premature transcription termination was also observed during multiple transcription cycles, each time within the OriC.

Conclusion: We demonstrate the utilization of RNA IDs to analyse complex transcript populations at the single-molecule level. In addition, direct labeling of RNA molecules enables the visualization of sequence-specific biomarkers. This approach holds significant potential for various downstream applications, including therapeutic RNAs, messenger RNA vaccines, and the production of RNAs and proteins both *in vitro* and *in vivo*.

Key words: nanopores; RNA nanotechnology; T7 RNA polymerase; transcription

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DETECTION OF GENOMIC INSTABILITY IN MALIGNANT BRAIN TUMORS

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Introduction: Astrocytoma and glioblastoma are the most agressive type of brain tumor. Glioblastoma *IDH* wild-type is a primary tumor which develops *de novo*, while Astrocytoma *IDH* mutant progresses from lower grade tumors. They are characterized by high heterogeneity and resistance to therapy which develop as a consequence of accumulation of mutations that lead to genomic instability.

Methods: We analysed genomic instability in 66 patients with malign brain tumors using arbitrarily primed PCR as DNA profiling method. Comparing DNA profiles of tumor and normal (blood) tissues, we detected quantitative and qualitative differences. Quantitative differences are represented by different band intensities and correspond to chromosomal instability (CIN). Qualitative changes seen as band shifts represent microsatellite instability (MIN). We correlated frequencies of genomic instability with tumor gradus and histophatological data.

Results: In patients with Glioblastoma *IDH* wild-type, percentages of high total genomic instability, MIN and CIN were 65%, 32% and 57%, respectfully. In patients with Astrocytoma *IDH* mutant, percentages of high total genomic instability, MIN and CIN for gradus 3 were 45%, 36% and 72%, respectfully while they were 40%, 40% and 40%, for gradus 4. In patients with NOS (not otherwise specified glioblastoma) percentages are 50%, 50% and 70%, respectfully.

Conclusion: Our results show that Glioblastoma *IDH* wild-type and Astrocytoma *IDH* mutant gradus 3 have higher genomic instability, while it is lower in Astrocytoma *IDH* mutant gradus 4. These results are in line with evolutionary theory of origin of cancer. Genomic instability in NOS tumors could be used as a prognostic marker.

Key words: Astrocytoma; Glioblastoma; genomic instability; DNA profiling

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DIAGNOSTIC TESTING FOR SARS-COV-2 BY REAL TIME RT-PCR

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged at the end of 2019 and caused COVID-19 pandemic. This coronavirus disease pandemic demonstrated the importance of diagnostic testing in disease outbreak monitoring and control. So, reliable and accurate testing for SARS-CoV-2 was the principal prerequisite for preventing the spread of COVID-19.

Methods: Real Time RT-PCR (RT-qPCR) unquestionably represent the most reliable, rapid and sensitive method for detection of SARS-CoV-2 RNA. However, there are numerous different assays, protocols, reagents, instruments and result analysis methods in use without certified standards, standardized RNA extraction and reporting procedures. Therefore, in practice, the reliability of RT-qPCR results depends on a number of parameters that include sample collection and processing, method of RNA extraction, choice of assay, efficiency of assay, choice of instrument, analysis method as well as operator intervention.

Results: Here we present comparative analyses of the efficiency and sensitivity of 10 different amplification assays, as well as the relevance of manual RNA extractions compared to automatic one. Our results revealed that manual viral RNA extraction should be a method of choice for high sensitivity. In addition, amplification assays targeting three SARS-CoV-2 genes are much more efficient from those targeting one.

Conclusion: Unfortunately, RT-qPCR is almost exclusively used as qualitative diagnostic test for SARS-CoV-2. We think that the ideal testing regimen would involve not just qualitative detection of SARS-CoV-2 but reliable and meaningful quantitative reporting of viral load.

Key words: SARS-CoV-2; RT-qPCR; viral load

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Abstracts

Session MOLECULAR BIOTECHNOLOGY

Session dedicated to the memory of Professor Đorđe Fira (1959-2022),
Full Professor of Biochemistry at the University of Belgrade-Faculty of Biology

BACTERIOPHAGE-BASED TECHNOLOGY: BACK TO THE PAST TO MOVE FORWARD INTO THE FUTURE

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The escalating global challenge of antibiotic-resistant bacterial infections has spurred a paradigm shift in medical research towards innovative solutions. Bacteriophages, viruses that infect and lyse bacteria, were initially recognized for their therapeutic potential nearly a century ago, but their significance waned with the advent of antibiotics. Nowadays, bacteriophage-based technology has reemerged as a potent strategy for addressing the crisis of antimicrobial drug resistance.

Modern biotechnology, including genomics, bioinformatics, and synthetic biology, is empowering researchers to explore the intricate interactions between bacteriophages and bacteria. By leveraging this knowledge, scientists are designing bacteriophages with exquisite specificity, enabling the targeted elimination of multidrug-resistant bacterial strains. This targeted approach holds promise in minimizing disruption to the human microbiome while effectively tackling bacterial infections. Moreover, bacteriophages are developed as innovative tools for the rapid and specific detection of bacterial infections. Phage-based biosensors, utilizing the dynamic interactions between bacteriophages and their bacterial hosts, offer sensitive and cost-effective platforms for pathogen detection. Their high specificity and speed make them valuable assets in point-of-care diagnostics, enhancing early detection and timely intervention.

Here, the advantages and challenges associated with bacteriophage therapy, underscoring its potential precision, adaptability, and ability to counter bacterial resistance mechanisms as well as phage biosensing for detecting bacterial infections are addressed. Regulatory considerations and the necessity for robust clinical validation are also discussed, highlighting the importance of comprehensive evaluation to ensure the safety and efficacy of bacteriophage-based interventions.

DEVELOPMENT OF BIOSIMILARS AND TRANSFER OF KNOW-HOW TO INDUSTRY: ICGEB APPROACH

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Biologics are one of the fastest growing segments of the pharmaceutical industry. However, the cost of them has often been prohibitive, thereby limiting their use, particularly in developing countries. The expiration of the patents for many biologics has ushered in an era of products that are designed to be 'similar' to an originator product. Worldwide, the development of biosimilars has contributed to reduce the cost of biologics compared to the originators by up to 50%. Our vision at ICGEB is that biologics, with sustainable technologies and local productions, should be available and affordable to each person that needs treatment. The ICGEB team established technology transfer packages for the production of 14 biosimilars, such as filgrastim, erythropoietin, growth hormone and insulins, covering the most comprehensive breadth of activities in bioprocessing such as upstream, downstream operations and QC analysis. Our team is engaged in multiple trainings and transfer of know-how to industry. Availability of this kind of scientific support in the ICGEB represents a significant incentive for the development of biosimilars locally and internationally.

Key words: Biologics; biosimilars; technology; transferring know-how; training

MICROBIAL TRENDS IN SUSTAINABLE BIOTECHNOLOGY

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Introduction: An advance in microbiome technology and computational biology enabled the evaluation of community composition, potential function and activity of the selected microbial strains as co-formulants in the phytobiome. Our goal is to formulate a series of environmentally friendly bio-based mixtures that are not too technologically complex and have commercialization potential that would significantly increase the sustainability and yield of a variety of crops in different soil types.

Methods: Bacterial isolates from the endosphere of different crops were selected for their plant growth promoting activity, biocontrol capacities, and environmental competitiveness. Differently designed products were used in the treatment of seeds of different plant varieties and crops, evaluating disease severity reduction, crop yield, and community composition using next-generation sequencing techniques.

Results: We have developed a mixture of compost and biochar as an organo-mineral component, which is enriched by biological components of selected bacteria and algae that act simultaneously as fertilizer and pesticide. Advances in microbiome technology and computational biology enabled an assessment of community composition, potential function, and activity of the selected strains as co-formulants in the phytobiome. Results indicate that appropriate levels of selected endophytes are maintained in seedlings without affecting the "core" microbiota and that plant disease severity is reduced.

Conclusion: We strongly believe that these smart platform products will enable plant disease control and efficient fertilization, eliminate the negative effects of pest resistance, pollution, toxicity and soil degradation caused by the use of conventional chemicals, and enhance the use of plant byproducts and beneficial microbes in sustainable agriculture.

Key words: smart biofertilizers; beneficial bacteria; biocontrol; microbiome; sustainable agriculture

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ANTIMICROBIAL PEPTIDES AS PROMISING ALTERNATIVE FOR TREATMENT OF PATHOGENS

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Uncontrolled use of antibiotics and chemical additives, both in human and veterinary medicine, has resulted in the emergence of bacterial strains that no longer respond to antimicrobial therapy. In parallel, consumers demand for natural, preservative-free, safe yet mildly processed foods with an extended shelf life. All these circumstances have created a need for development of new classes of antimicrobial agents that are active against pathogen which cause nosocomial infections and tend to adopt to resistance. Antimicrobial peptides (AMPs) represent one of promising alternative as therapeutic compound against pathogens. These peptides are made up of around 12 to 50 amino acids, found and isolated from all classes of living organisms. AMPs are produced as secondary metabolites, part of non-specific innate immune response, and mostly ribosomally synthetized. Among these peptides, some are involved in the inhibition of various microorganisms, such as bacteria, fungi, enveloped viruses. According to The Antimicrobial Peptide Database more than 3000 peptides have been described, many of them with very promising therapeutic properties. They have amphipathic structures, cationic nature, and low probability of microbial resistance. Considering their broad spectrum of activity, AMPs have been the base for the production of chemical analogs, which have been tested *in vitro* and *in vivo* for their antimicrobial activity. The present work reviews the most investigated peptides and accounts for their potential use as alternatives in the food and pharmaceutical industry. The focus is on research aspects aiming at understanding the mechanism of action of these peptides at extreme environments of various systems.

Key words: antimicrobial resistance; antimicrobial peptides; food and pharmaceutical industry

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BACTERIA–PLANT INTERPLAY ENABLES DIFFERENT RESPONSES TO COMPLEX ENVIRONMENTAL CONDITIONS

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Higher average annual temperatures and droughts caused by climate change, as well as soil degradation due to the overuse of artificial fertilizers and pesticides, are limiting global food production and posing a serious threat to the future of humanity. The plant microbiome, together with the soil microorganisms that colonize the rhizosphere, the root, and the entire plant, is considered one of the key factors in the plant's rapid adaptation to environmental stress. Consequently, this interaction leads to the formation of the holobiont, a structure that influences the stability, adaptability, and evolution of the organisms it contains. Bidirectional communication between plants and microorganisms is a hallmark of their development, growth, and survival under harsh environmental conditions. Here is an overview of our work to understand the mechanisms involved in this interplay and the fine-tuning of the responses achieved. Specifically, we are looking at i) the influence of plant genotype on drought as an abiotic stressor - what do we want in breeding experiments and what do we have? ii) drought tolerant bacteria as valuable components of future biofertilizers - evaluating their efficiency as plant growth promoting (PGP) strains for the same and different plant species in alleviating drought stress; iii) how to overcome discrepancies between metabarcoding and culture-dependent outcomes to obtain the most diverse bacterial isolates with desirable traits. Ultimately, our research aims to explore the molecular mechanisms underlying this relationship and the cues that lead them to different responses.

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MICROBES AND MICROBIAL ENZYMES FOR DEGRADATION OF (BIO)PLASTICS

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Introduction: Plastic films, containers, and fibers are almost ubiquitous, making our life better, easier, and safer. However, the uncontrollable disposal of plastic waste has raised global concern. Plastic pollution is not a recent issue; it originated decades ago with the advent of industrial plastic production. While recycling, incineration, and other methods exist for managing plastic waste, unfortunately, landfilling remains the most prevalent "solution" adopted by many countries.

In response to the pressing issue of plastic pollution, a new scientific field has emerged, dedicated to employing innovative green methodologies inspired by nature's mechanisms. This approach centers around the discovery and identification of microorganisms with the ability to harness the carbon derived from plastic waste for their growth and survival. In the context of this research, we aim to accomplish two main objectives: isolating enzymes expressed by diverse microbial strains and exploring the potential of well-known hydrolytic enzymes in breaking down synthetic and biosourced polymers.

Methods: To optimize and improve biodegradation yields, our approach combines enzymatic and microbial plastic degradation with polymer treatment techniques. These techniques are designed to modify the structure of polymers, making them more accessible for hydrolysis and assimilation by microorganisms. After polymer hydrolysis, our concept emphasizes the recovery and utilization of the released compounds, which can be further converted into valuable bio-products through fermentation.

Results: Number of new enzymes, microorganisms and microbial communities has been isolated and characterized with the potential to degrade both single and mixed plastic substrates.

Conclusion: By adopting this multidisciplinary approach, we aim to establish a sustainable pathway for the efficient management of plastic waste. Through the transformation of polymers into high-added value products such as bioplastics, biopigments and biosurfactants, we contribute to a circular economy plan and mitigate the environmental impact associated with plastic waste.

Key words: microbial degradation; plastics; circular economy; bioplastics

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BACTERIAL NANOCELLULOSE – NEW BEGINNING FOR END-OF-LIFE PLASTICS

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Introduction: Fossil-based polymers continue to be widely used despite their negative environmental impact. Bioplastics, such as polylactic acid (PLA), offer a promising alternative as they are derived from renewable resources and provide more environmentally friendly end-of-life options. However, marketing PLA as simply biodegradable can be misleading, as the current PLA degradation strategy contributes to microplastics pollution, thus posing even greater threat. This research focuses on the upcycling of PLA degradation products into valuable biomaterial - bacterial nanocellulose.

Methods: PLA samples were pretreated using ultraviolet and ultrasonic waves, individually and in combination, to enhances susceptibility to bacterial degradation. Pretreated PLA was subjected to enzymatic degradation under mild conditions, using various enzyme combinations. The resulting biodegradation products served as a growth medium for nanocellulose producing bacteria *Komagataeibacter medellinensis* ID13488. Obtained nanocellulose was characterized using SEM, FTIR, AFM, and XRD.

Results: The combined PLA pretreatment using ultraviolet and ultrasonic waves, followed by enzymatic degradation with savinase, demonstrated the highest degree of PLA degradation in this study. Furthermore, *K. medellinensis* ID13488 efficiently utilized the biodegradation products, producing nanocellulose with yields and performance comparable to those obtained through standard cultivation using glucose as a carbon source.

Conclusion: This study highlights the potential of combined pretreatment and enzymatic degradation for efficient PLA degradation and sustainable bacterial nanocellulose production. The findings suggest promising avenues for utilizing PLA biodegradation products in the production of other valuable biomaterials. Further research is needed to optimize the pretreatment and degradation processes, facilitating the wider application of biodegradable materials and promoting sustainability.

Key words: PLA; pretreatment; biodegradation; savinase; bacterial nanocellulose

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THE ROLE OF THE GUT BACTERIA DURING HOST AGING

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Introduction: Microbial community established in the gut has been recognized as an important factor which influence host aging. Bacteria from the gut co-evolved with the host resulting in mutually beneficial interactions essential for host's wellbeing. This complex crosstalk reflects mainly through the interaction between bacterial macromolecules (e.g., exopolysaccharides) and the host receptors leading to the activation of various cellular pathways. Here, we explore the potential of different lactobacilli, commonly used as probiotics, to activate longevity signalling in *Caenorhabditis elegans*.

Methods: Evaluation of *C. elegans* lifespan and aging parameters (locomotion rate and pharyngeal pumping) were performed by feeding N2 wild-type worms with different *Lactobacillus* species. Worms fed with selected strains were subjected to RNAseq analysis, qPCR and Western blot to evaluate activation of autophagy, immunity, antioxidative response and mitochondrial function. Activation of autophagy was confirmed in DA2123 GFP-labelled LGG-1 transgenic strain and JIN1375 *hlh-30* (tm1978) mutant, while immunity activation was evaluated by using KU25 *pmk-1* (km25) mutant and through nematode killing assays.

Results: Selected strains of lactobacilli promoted health and lifespan of worms through activation of TFEB/HLH-30 dependent autophagy and p38 MAPK/PMK-1 dependent immune response which provided resistance of worms exposed to pathogens. Moreover, RNAseq analysis identified core gene signature associate with exopolysaccharide-induced longevity highlighting involvement of *fmo-2*, *gsto-1*, *nlp-29*, and *clec-47* genes in increased lifespan of the worms.

Conclusion: Analyzed lactobacilli showed potential to promote healthy aging and could be further investigated in order to better understand application of lactobacilli as pro-longevity probiotics.

Key words: *Lactobacillus*; *Caenorhabditis elegans*; autophagy; immunity; aging

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REPERTOIRE AND ABUNDANCE OF TYPE III SECRETION SYSTEM EFFECTORS SHAPE THE VIRULENCE CAPACITY OF *PSEUDOMONAS SYRINGAE* PATHOGENIC ON SUGAR BEET

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Introduction: *Pseudomonas syringae* is a member of the sugar beet pathobiome and relies on the secretion of immunosuppressive proteins (effectors) by type III secretion system (T3SS), which influence host-pathogen interactions to establish infection. This study was focused on identifying and quantifying T3SS effectors to link the repertoire of secreted effectors and disease outcomes.

Methods: We performed a machine learning-based predictive analysis to identify T3SS effectors in genomes of *P. syringae* strains with different pathogenic capabilities. To test T3SS pili formation and secretion of effectors, we performed visualization of T3SS pili, secretion of HrpA pilus protein, and label-free quantitative proteomic analysis (LC-MS/MS) of the secretome.

Results: Genome-based analysis of the T3SS effector repertoire of high-virulence *P. syringae* strains revealed a broader repertoire (26 effector genes) compared with the low- virulence strain (16 effector genes). We detected pili formation (7.3 ± 0.8 nm in diameter) exclusively under secretion conditions and confirmed the secretion of the HrpA pilus protein (11 kDa). Most of the T3SS effectors were secreted in the greatest relative amounts by the strains with low pathogenicity and, to a lesser extent, by the strains with intermediate and high virulence. Particular effectors, HopAU1, HopAW1, HopAH1, and AvrRpm1, showed a markedly different secretion pattern and were detected in the high amount by the strains with intermediate and high pathogenicity.

Conclusion: Secreted T3SS effectors allow us to distinguish different virulence strategies and highlight fine-tuning of effector secretion and a broad effector repertoire as crucial mechanisms of T3SS-mediated disease development.

Key words: *Pseudomonas syringae*; T3SS; effectors; virulence; sugar beet

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DRYING WITHOUT DYING: REVEALING THE ROLE OF LATE EMBRYOGENESIS ABUNDANT PROTEINS DURING DESICCATION IN *RAMONDA SERBICA*

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Introduction: Resurrection plants (such as *Ramonda serbica*) can survive a long desiccation period and fully resume their metabolism upon watering. The hallmark of desiccation tolerance (DT) is the accumulation of protective, intrinsically disordered proteins (IDPs), called late embryogenesis abundant proteins (LEAPs). Although their high structural plasticity allows them to interact with various partners, no specific cellular targets of LEAPs have been identified so far.

Methods: To identify LEAPs involved in DT, differential transcriptome and proteome analyses of hydrated and desiccated *R. serbica* leaves were performed. The identified LEAPs were structurally characterised and classified. To evaluate their structural properties *in vitro* and their potential functions *in vivo*, the representative RsLEA proteins, were produced in *Escherichia coli* using recombinant DNA technology.

Results: Members of the LEA4 protein family represent the majority of desiccation-inducible LEAPs. Even 17 proteins belonging to the LEA4 protein family group were induced by desiccation. They show high disorder propensity (82 %), and at the same time, a high tendency to form α-helices (>80%). Although recombinant DNA technology has traditionally been used to overexpress and purify various globular proteins, the production of IDPs is challenging due to their high susceptibility to proteolytic cleavage and aggregation. Nevertheless, the representative LEAPs containing hexa-His tags immunoglobulin G-binding protein and a proteolytic TEV site were produced, purified and cleaved by TEV protease.

Conclusion: The combination of *in silico* and *in vitro* results will be crucial for the identification of endogenous partners of LEAPs, providing further insight into their role in DT.

Key words: late embryogenesis abundant proteins; desiccation tolerance; recombinant DNA technology; intrinsically disordered proteins

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BIOCTA: NOVEL APPROACH TO BIOCONTROL OF RECENTLY DESCRIBED PLANT TUMOROGENIC *RHIZOBIUM* SPP. USING AUTOCHTHONOUS MICROBIAL SOLUTIONS

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Introduction: A novel group of *Rhizobium* spp. strains belonging to the "tumorigenes" clade has recently been described on blackberry in Serbia and Germany and on rhododendron in Germany. The BIOCTA project aimed to characterize efficient plant-associated bacterial strains for biocontrol of crown gall, thus providing an environmentally friendly alternative to pesticides that would contribute to the development of sustainable agriculture.

Methods: Antagonistic potential of 37 biocontrol strains against two *R. tumorigenes* strains 932 and 1078 and *Rhizobium sp.* strain rho-6.2 was evaluated *in vitro* using the "well diffusion" method, as well as *in vivo* on tomato plants, using two inoculation strategies (co-inoculation and preventive). DNA metabarcoding approach was used to analyze the phytobiome of treated and non-treated tomato plants.

Results: Based on the determined *in vitro* antagonistic potential, seven strains – *Bacillus* spp. (*B. amyloliquefaciens* ID084 and GT28.3, *B. velezensis* X5-2, and *B. subtilis* GD1), *Pseudomonas* sp. (R-6.10 and R11-20) and *Agrobacterium rosae* rho-6.1 were selected for further *in vivo* experiments. Of all tested strains/treatments, two *Pseudomonas* strains were the most efficient, showing up to 92.86% efficacy in suppressing tumors caused by *Rhizobium* sp. strain rho-6.2 when applied in a co-inoculation strategy. Based on the DNA metabarcoding analysis, genera *Pseudolabrys* and *Asanoa* prevailed in the co-inoculation strategy, while *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* prevailed in positive control.

Conclusion: Crown gall tumors have shown to be a valuable source of antagonistic isolates. *Pseudomonas* strains R-6.10 and R11-20 could be proposed for the efferent control of crown gall caused by newly described *Rhizobium* spp. strains in nurseries.

Key words: crown gall; *Rhizobium*; biocontrol; DNA metabarcoding

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SHORT-TERM EFFECT OF *BREVIBACILLUS LATEROSPORUS* SUPPLEMENTED DIET ON WORKER HONEY BEE MICROBIOME

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Introduction: *Brevibacillus laterosporus* is a promising microbiological agent that can be used to prevent and control destructive diseases affecting honey bee colonies. In the present study, the short-term effect of the *B. laterosporus* BGSP11 bee diet on microbiota and mycobiota was investigated.

Methods: The honey bee diet was supplemented with spores of *B. laterosporus* BGSP11 at a concentration of 1×10^8 CFU/mL in sucrose solution. Metabarcoding analysis of the bee microbial community profile was performed based on 16S RNA (bacteriobiota) and Internally Transcribes Spacer (ITS) region (mycobiota) obtained using MiSeq Illumina sequencing. The QIIME2 v2021.4 pipeline was used to analyze the obtained amplicon data library.

Results: The results show that the BGSP11 bee diet slightly altered the bee microbiota and did not lead to potentially harmful changes in the bacterial microbiota. Moreover, it can potentially induce positive changes, mainly reflected in the reduction of opportunistic bacteria. On the other hand, the treatment had a greater effect on mycobiota. However, the changes in the bee mycobiome caused by the treatment cannot be considered a priori as beneficial or harmful, since the interaction between the bee and its mycobiome is not sufficiently studied. The observed positive changes in the bee mycobiome are mainly reflected in the reduction of phytopathogenic fungi that may affect the organoleptic and techno-functional properties of honey.

Conclusion: This pilot study suggests that the introduction of BGSP11 in beekeeping practice as a biological agent could be considered due to no harmful effects observed on the microbiota of bees.

Key words: *Apis melifera*; *Brevibacillus laterosporus*; microbiome; bacteria; fungi

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IMMOBILIZED NT2/D1 CELLS IN ALGINATE FIBERS: A PROMISING 3D MODEL SYSTEM FOR INVESTIGATING HUMAN NEUROGENESIS AND SCREENING THE EFFECT OF DRUGS AND BIOACTIVE COMPOUNDS

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Introduction: The NT2/D1 embryonal carcinoma cell line represents a well-established *in vitro* model of human neurogenesis. It's widely used for studying neurodevelopmental processes, neurotoxicity, and neurodegenerative disorders. The utilization of alginate fibers as a 3D cell culture system offers a biocompatible and structurally supportive environment for neural differentiation and maturation of cells, making it a suitable tool for investigating neurodevelopmental processes.

Methods: In this study, we evaluated the alginate microfibers as a 3D model system for *in vitro* neural differentiation of NT2/D1 cells. We described the immobilization of NT2/D1 cells in alginate microfibers and the effect of propagation in this 3D model on morphological features, viability, and proliferation of immobilized cells. We also assessed the RA-induced initiation of neural differentiation of NT2/D1 cells in alginate microfibers by comparison with the initiation of neural differentiation in adherent 2D cell culture.

Results: Our results showed that immobilized NT2/D1 acquired morphological features characteristic of cells propagated in 3D model systems and retain viability, proliferative capacity, and ability to attach to adherent surfaces. In addition, immobilized NT2/D1 cells preserved neural differentiation capacity. Upon RA induction we detected a marked decrease in the expression of specific pluripotency-maintaining markers, *SOX2*, *OCT4,* and *NANOG*. Consecutively, the expression of early neural markers, *SOX3*, *PAX6,* and *miR219* was significantly increased.

Conclusion: Neural differentiation of NT2/D1 cells immobilized within alginate fibers represents a highly promising 3D model system for studying human neurogenesis and offers a valuable platform for screening the effect of drugs and bioactive compounds on human neural differentiation.

Key words: NT2/D1 cell line; neural differentiation; alginate fibers; 3D model system

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CHARACTERIZATION OF *BACILLUS* SP. BIOSOL021 IN TERMS OF LIPOPEPTIDES PRODUCTION

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Introduction: The aim of this study was to evaluate *Bacillus* sp. BioSol021 lipopeptide production capability as many *Bacillus* strains exhibit potential to produce various types of lipopeptides with broad spectrum of antimicrobial activity against human and plant pathogens.

Methods: *Bacillus* sp. BioSol021 biocontrol strain was isolated from the rhizosphere of common bean. After genomic DNA extraction, PCR in combination with gel electrophoresis visualization was applied to investigate genetic basis for lipopeptides production by using specific primer pairs to detect genes for production of surfactin (*srfAA*), fengycin (*fenD*) and iturin (*ituA* and *ituD*). Production of lipopeptides was investigated using HPLC-MS (high performance liquid chromatography – mass spectrometry) in the supernatant of the *Bacillus* sp. BioSol021 cultivation broth obtained after 96 h-cultivation at 28 °C with external agitation (150 rpm) using nutrient broth medium.

Results: PCR and horizontal gel electrophoresis have confirmed genetic basis of the strain *Bacillus* sp. BioSol021 for production of surfactin, fengycin and iturin by detecting the DNA fragments of 201 bp, 269 bp, 647 bp and 1207 bp, respectively. The presence of synthesized lipopeptides was also confirmed by the HPLC-MS, with *m/z* values of 1022, 1036, 1044 and 1058 for surfactins, 1046.5, 1060.6, 1074.6 and 1088.6 for protonated forms of iturins and 1449.8, 1461.8, 1463.8, 1475.8, 1477.8, 1491.8 and 1505.8 for protonated forms of fengycins.

Conclusion: The obtained results show promising perspective of *Bacillus* sp. BioSol021 in production of lipopeptide-based biocontrol agents and represent basis for further research, optimization and development of lipopeptides production at a larger scale.

Key words: surfactin; fengycin; iturin; PCR; HPLC-MS

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DETECTION OF THE TYPE 3 SECRETION SYSTEM IN PLANT BENEFICIAL *PSEUDOMONAS* ISOLATES FROM SUGAR BEET

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Introduction: The type 3 secretion system (T3SS) injects bacterial effector proteins directly into the host cytoplasm to modulate cellular functions. *Pseudomonas syringae* is a pathogen of several plant species that uses T3SS to suppress plant immunity. Recently, T3SS has also been identified in non-pathogenic bacteria. The aim of this study was to investigate whether T3SS is present in plant-beneficial *Pseudomonas*.

Methods: The conserved *sctRST* genes of the T3SS operon were detected by PCR in a collection of plant-beneficial *Pseudomonas* strains isolated from various plants (pepper, plum, ramonda, sugar beet, tomato). Expression of the system was tested by qPCR and the tobacco hypersensitivity reaction (HR). Plant growth-promoting activity was tested on two sugar beet genotypes, Heston (Maribo, Denmark) and Eduarda (KWS, Germany).

Results: T3SS was detected in 26% of the isolates. T3SS-positive isolates cluster into two phylogenetic groups and belong to 9 different species. The system is expressed in all isolates and several induce HR. The most (61%) of T3SS-positive isolates originate from sugar beet and promote its growth. The best growth stimulation was observed in *P. grimontii* OL141 isolated from the phyllosphere of sugar beet. This strain increased leaf mass, root length, and shoot length in both sugar beet genotypes.

Conclusion: T3SS is present in plant-beneficial *Pseudomonas*, and in our collection the most T3SS-positive isolates are from sugar beet. Future studies will investigate whether the system is directly linked to growth stimulation by generating T3SS deletion mutants and exploring the T3SS effector repertoire in our strain collection.

Key words: T3SS; *Pseudomonas*; sugar beet; plant growth promotion

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DETERMINATION OF HYDROGEN CYANIDE PRODUCING STRAINS AS POTENTIAL BIOCONTROL AGENTS

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Introduction: Hydrogen cyanide (HCN) is a volatile secondary metabolite synthesized by some bacteria, and this ability enables their activity against various pathogens. The aim of this study was to identify HCN-producing bacteria and investigate their biocontrol potential.

Methods: Three HCN-producing strains were detected in a collection of bell pepper plant isolates using a semi-quantitative assay with picric acid. The presence of *hcnABC* operon genes was confirmed by PCR. The biological control potential of the HCN-producing strains was tested against three fungal (*Fusarium oxysporum, Rhizoctonia solani, Verticillium dahliae*) and eight bacterial (genera *Xanthomonas, Pseudomonas* and *Clavibacter*) pathogens of bell pepper plants in a split-section Petri dish experiment. The potential nematocidal activity was demonstrated by using the *Caenorhabditis elegans* AU37 strain, with temperature-sensitive sterility and enhanced sensitivity to pathogens.

Results: Detailed characterization of 300 isolates from our collection revealed that we have three different HCN-producing strains identified as *Bacillus subtilis, Pseudomonas moraviensis*, and *P. putida*, with *P. putida* A32 being the most potent. This strain is used for the deletion of the *hcnB* gene to confirm HCN as a biocontrol agent.

Conclusion: The HCN-producing strains showed biocontrol potential against bacteria, fungi, and nematodes. It is concluded that the biological control activity is the result of a volatile metabolite diffusing through the air. Our future experiments will confirm the role of HCN in biological control by generating an HCN deletion mutant.

Key words: plant pathogens; hydrogen cyanide (HCN); biological control

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OVERVIEW OF MUTATION EVENTS OBSERVED AT RM Y-STR LOCI IN FATHER-SON PAIRS FROM SERBIA

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Introduction: During its transmission from father to son, the male-specific region of Y chromosome escapes recombination with X chromosome and mutation events are rare. In order to distinguish close male relatives in crime cases, a range of panels containing highly polymorphic and rapidly mutating loci have been developed. Most of these markers are located within the region with repetitive structure on Y chromosome long arm which is prone to microdeletions or duplications.

Methods: Our study included DNA samples of 246 genetically confirmed father-son pairs from Serbia. They were genotyped for 30 rapidly mutating (RM) Y-STR markers contained in RMplex and analyzed using the 3500 Genetic Analyzer (Thermo Fisher Scientific).

Results: A total of 140 mutation events were observed (72 repeat losses and 58 gains), among them 4 two-step mutations, 3 three-step mutations and 2 double-allele mutations. Moreover, there were a number of microvariants found in this study. Additionally, we revealed new duplications within father-son pairs at loci DYF1000, DYS1012, DYS1005, DYS1007, DYR88, DYF399S1 DYS449, DYF1002 and null allele at DYS1005. Finally, we discovered cases with additional and missing alleles in both fathers and sons, indicating that mutations occurred in a common ancestral Y-chromosome.

Conclusion: We reported analyses of mutations for 30 RM Y-STR markers in father-son pairs with occurrences of some new duplication/deletion patterns. These unusual mutations may lead to misinterpretation of mixed Y-STR profiles, so it is of fundamental importance in forensic genetics to describe as many variants as possible, and also to explore the underlying mechanisms.

Key words: RM Y-STRs; duplications/deletions patterns; father-son pairs

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GENETIC ANALYSIS OF MITOCHONDRIAL DNA OF DONKEYS FROM THE SPECIAL NATURE RESERVE "ZASAVICA"

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Introduction: The Balkan donkey (*Equus asinus*) is a breed distributed across the Balkan Peninsula and classified as an endangered species in Serbia. It can be found in the Special Nature Reserve „Zasavica", on the Stara Planina mountain and in Kovilj village. Another group with distinct morphological features is called Banat donkeys. Based on mitochondrial DNA (mtDNA) control region variability, 19 mtDNA haplotypes, grouped in two lineages, clade 1 and clade 2, have been found in population of donkeys in Serbia. Clade 2 was found to be prevalent in Banat donkeys. The aim of this study was to evaluate the genetic structure of the population from "Zasavica" based on mtDNA.

Methods: Blood samples were collected from 40 animals. DNA was extracted and quantified. Control region of mtDNA was amplified and sequenced using the 3130 Genetic Analyzer (*Applied Biosystems*). Further analyses were carried out using Sequencing Analysis Software v6.0 program (*Thermo Fisher Scientific*).

Results: We distinguished 9 haplotypes grouped into two clades. 24 individuals were presented with haplotype h12, and together with 6 more belonged to clade 2 (30 out of 40 donkeys). We were able to assign 38 samples to 9 previously published haplotypes in Serbia. Almost all donkeys were confirmed to belong to the population of "Zasavica", except for one with a haplotype found in the population of Kovilj.

Conclusion: The most common haplotype in our sample was h12, and clade 2 was predominant, which is in concordance with previously published data for individuals with morphological features of Banat donkeys.

Key words: mtDNA; donkeys; haplotype; clade

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VALIDATION OF LAMP ASSAY FOR *KLEBSIELLA AEROGENES* DETECTION IN THREE VEGETABLE SPECIES

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Introduction: *Klebsiella aerogenes* is a foodborne pathogen that contaminates fruits and vegetables. Detecting it in a timely manner is essential to prevent its transmission through contaminated food sources. In this regard, the LAMP method proved to be a robust, highly sensitive and highly specific method for real-time pathogen detection, and thus method of choice for detection of *K. aerogenes.* In order to check efficiency of LAMP methodology with fast DNA extraction we conducted LAMP-based *K. aerogenes* detection in vegetables spiked with bacteria.

Methods: We selected three distinct vegetable types (carrot, cucumber, and lettuce) that were artificially contaminated (spiked) with a bacterial suspension containing 1.5 x 10^9 CFU/mL of *K. aerogenes*. DNA extraction from the samples was performed using two approaches: Chelex approach for fast extraction, and Plant/Fungi DNA Isolation Kit (Norgen Biotek Corp., Canada) for spin-column extraction. The total DNA extracted from both methods was subsequently diluted to a concentration of 1 ng/μL and used for the LAMP assay.

Results: The results indicated successful detection of *K. aerogenes* by LAMP using total DNA from both extraction approaches. DNA isolated with a commercial kit reaches the exponential phase of the reaction approximately five minutes earlier compared to DNA obtained by the Chelex method. This can be related to the fact that fast DNA extracts often contain nucleic acid amplification inhibitors.

Conclusion: The presented research highlights the remarkable potential of the LAMP methodology that can be efficiently combined with fast DNA extraction methods for in-field detection of pathogens.

Key words: LAMP; *Klebsiella aerogenes*; foodborne pathogens; molecular diagnostics

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TESTING THE EFFECT OF HEMOLYMPH FROM SELECTED INSECT SPECIES ON CELL VIABILITY

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Introduction: Insects have long been an important nutrient source for human consumption worldwide. Given that they are protein-rich, especially their hemolymph, insects represent one of the most promising sustainable solutions to the global problem of protein deficiency due to the growing world population. However, insect proteomes are generally understudied, which is one of the obstacles for insects to be accepted as future food. Therefore, it is very important that insect proteins, as well as hemolymph as their primary source, be well characterized. The first step would be to elaborate their biological functions and potential biotoxicity, so here the effect of hemolymph from *Tenebrio molitor* and *Zophobas morio* larvae on MRC-5 cell viability was investigated.

Methods: Hemolymph was collected from *T. molitor* and *Z. morio* larvae, hemocytes were removed and the concentration of total hemolymph proteins was determined by Bradford assay. Next, MRC-5 cells were treated with different hemolymph concentrations. Finally, the effect of hemolymph on the viability of MRC-5 cells after 48h was examined by MTT assay.

Results: Obtained results showed that *T. molitor* hemolymph had no negative effect on MRC-5 cell viability after 48h regardless of concentration, while only the highest concentration of *Z. morio* hemolymph slightly decreased cell viability after 48h.

Conclusion: Given that all tested concentrations of *T. molitor* hemolymph, as well as most concentrations of *Z. morio* hemolymph, had no cytotoxic effect on MRC-5 cells as a model of normometabolic human cells, they could be used in further research as a source of potential alternative proteins.

Key words: insects; hemolymph; cell viability; alternative proteins; future food

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A NOVEL YTNP LACTONASE REDUCES THE EXPRESSION OF *P. AERUGINOSA* MMA83 QUORUM SENSING AND VIRULENCE FACTORS GENE EXPRESSION

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Introduction: Quorum quenching (QQ) is the enzymatic degradation of cell-to-cell signaling molecules. In this study, the potential of the novel YtnP lactonase, the quorum quenching enzyme derived from *S. maltophilia*, to reduce *P. aeruginosa* quorum sensing and virulence factor gene expression was investigated.

Methods: MMA83 culture (adjusted to $1.5x10^5$ CFU/ml) was treated with recombinant YtnP lactonase (final concentration 50 µg/ml) at 37°C for 12 hours under aeration. RNA isolation of the treated and untreated MMA83 culture was performed using the RNeasy Mini Kit (Qiagen, Germany) according to the protocol. Quantitative reverse transcription-polymerase chain reaction (RT-qPCR), was used to analyze the effect of YtnP lactonase on the relative mRNA levels of the LasI/LasR, RhiI/RhiR, and PQS signaling network genes of *P. aeruginosa* MMA83 and virulence factor genes. The *rpsL* was used as an endogenous control to normalize obtained data following the $2^{-\Delta\Delta Ct}$ method.

Results: The QS genes belonging to three QS networks – LasI/LasR, RhiI/RhiR, and PQS of *P. aeruginosa* MMA83 treated with YtnP lactonase were significantly downregulated. The RT -qPCR results show that treatment with YtnP-lactonase decreased the relative mRNA levels of genes involved in the production of elastase (*lasB* approximately 2-fold), alginate (*algK* approximately 2.2-fold), pyocyanin (*phzM* approximately 3.5-fold), pyoverdin (*pvdS* approximately 2-fold), and rhamnolipid (*rhlC* approximately 4-fold). These results suggest that YtnP lactonase exerts an antivirulence effect at the transcription level.

Conclusion: YtnP lactonase, a quorum quenching (QQ) enzyme, has the potential to be used as an innovative enzyme-based antivirulence therapeutic to combat infections caused by *P. aeruginosa*.

Key words: *Stenotrophomonas maltophilia*; lactonases, antivirulence therapy; *Pseudomonas aeruginosa*; quorum sensing

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STUDY ON THE ANTIOXIDANT ACTIVITY AND THE CONTENT OF POLYPHENOLS AND TANNINS OF DIFFERENT PARTS OF THE SPECIES *PRIMULA VERIS*

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Introduction: *Primula veris* (cowslip) represent a valuable species known for its benefits for cardio-respiratory and neurological disorders. The present study aimed to evaluate the antioxidant activity and the total content of polyphenols and tannins of different plant organs of cowslip individuals collected from a low pollution area from Brașov County, Romania.

Methods: Frozen and dried root, leaves and flowers of *P. veris*, collected in May 2023, were extracted in 70% ethanol for 24 h at room temperature. The *in vitro* antioxidant activity, the total content of polyphenols and tannins were determined spectrophotometrically using the FRAP assay, Folin-Ciocalteu method and the vanillin assay, respectively. The results were analysed using R 4.2.0 software.

Results: The total phenolic content and antioxidant activity were higher in frozen samples, while tannin content was higher in dried samples. The extract of the frozen flowers showed the highest level of both investigated compounds (2567.029±1.811 mg catechin 100^{-1} g, respectively 2807.560±0.030 mg gallic acid 100^{-1} g), but also the highest antioxidant activity (2785.176±20.539 mg ascorbic acid equivalents 100^{-1} g). The Kruskal-Wallis test indicated a considerable difference ($p = 0.04953$) of the mean antioxidant activity values between frozen and dried samples. The Dunn's test showed that the antioxidant activity of dry samples was significantly lower than that of frozen samples ($p=0.0248$).

Conclusion: *Primula veris* is an important plant from a medicinal point of view, showing a high level of antioxidant compounds and activity efficiently preserved in frozen samples compared to the dried ones, that gives it a good applicative potential.

Key words: *Primula veris*; polyphenols; tannins; antioxidant activity

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INFLUENCE OF AMINO ACID SUBSTITUTION ON THE ANTIMICROBIAL ACTIVITY OF BACTERIOCIN LACTOLISTERIN BU

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Introduction: Lactolisterin BU (LBU) is a potent bacteriocin derived from *Lactococcus lactis* subsp. *lactis* bv. diacetylactis BGBU1-4. It exhibits antimicrobial properties against Gram-positive food spoilage and foodborne pathogens. This research aimed to explore the impact of amino acid substitution in LBU on its antimicrobial activity by utilizing *in silico* prediction of LBU's secondary structure and amino acid substitutions.

Methods: The secondary structure of LBU was predicted using Phyre2 software. Five variants of LBU were selected and chemically synthesized, along with unaltered LBU and BHT-B, serving as controls. Peptides were twofold diluted in distilled water, resulting in final concentrations ranging from 1000 µg/ml to 0.5 µg/ml. An agar spot test, employing 5 µl of the dilution, was conducted on three indicator strains: *Lactococcus lactis* BGMN1-596, *Listeria monocytogenes* ATCC19111, and *Staphylococcus aureus* ATCC25923. The presence of inhibition zones was analyzed after overnight incubation at 37°C (*S. aureus*) and 30°C (*L. lactis* and *L. monocytogenes*).

Results: Phyre2 analysis unveiled the presence of two α-helices in LBU's structure. The majority of LBU variants displayed altered antimicrobial activity, with some changes being genus specific, potentially attributable to variances in cell wall composition. Some variants completely lost their activity, underscoring the significance of native amino acids or their physicochemical properties in the corresponding positions within LBU's structure. Furthermore, it was confirmed that chemically synthesized LBU effectively retains its antimicrobial activity.

Conclusion: Changes in amino acid composition give insight on structure-function relationship of LBU.

Key words: peptides; antimicrobials; LAB, lactolisterin BU; amino acids

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SHORT CHAIN FATTY ACID PRODUCING *FAECALIMONAS* SP. NGB245 ISOLATED FROM HUMAN GUT MODULATES NEUROSIGNALING IN *CAENORHABDITIS ELEGANS*

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Introduction: Gut-brain axis has been identified as an important target for prevention of neurodegenerative and psychiatric disease. To date, specific microbial strains resident in the intestinal ecosystem have been described to modulate several behaviour-related functions in the host. *Faecalimonas* sp. is anaerobic bacteria affiliated with the family *Lachnospiraceae*, which represents a highly prevalent beneficial bacteria in the human gut and have potential to be used as next generation probiotic.

Methods: *Faecalimonas* sp. NGB245 was isolated from human fecal material by pre-inoculation in BACTEC media followed by serial dilutions spreading on Columbia Blood Agar supplemented with cysteine and sodium thioglycolate in Whitley Anaerobic Workstation. Production of short chain fatty acid (SCFA) was detected after bacterial growth in Columbia broth supplemented with cellobiose by HPLC. Host response was followed on *Caenorhabditis elegans* model by evaluated expression of the genes involved in neurosignaling by qPCR.

Results: We showed that *Faecalimonas* sp. NGB245 exhibits high capacity of production of SCFA including acetate (12,17 mM), propionate (3,02 mM) and butyrate (10,33 mM). Moreover, *C. elegans* fed with *Faecalimonas* sp. NGB245 showed higher expression of the genes involved in neurotransmitter synthesis (*tph-1*, *cat-2*), neurotransmitter release (*unc-64*, *snb-1*, *snt-1*), neurotransmitter receptor (*npr-1*) and different classes of neuropeptides (*flp-18*, *flp-21*, *nlp-28*, *nlp-29*) in comparison to worms fed with *Escherichia coli* OP50, as a standard laboratory food.

Conclusion: The obtained results imply that *Faecalimonas* sp. NGB245 isolate could be considered as next generation probiotic to be used in prevention and treatment of neurodegenerative and psychiatric diseases.

Key words: *Faecalimonas* sp; next generation probiotics; SCFA; *Caenorhabditis elegans*; neuropeptides

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Y-STR ANALYSIS OF SKELETAL REMAINS FROM THE PERIOD OF MEDIEVAL BOSNIA

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Introduction: Numerous archaeological sites on the territory of modern Bosnia and Herzegovina are witness to active and continuous life from the Middle Ages. The aim of this research was to determine the Y-haplotypes and predict Y-haplogroups, as well as the frequency of Y-haplogroups in the medieval population sample.

Material and Methods: 42 male samples from 12 archaeological sites were collected from the period of medieval Bosnia. DNA was extracted from bones and teeth by phenol-chloroform extraction, and Y-STR analysis was performed with the PowerPlex® Y23 System. Based on the obtained haplotypes, Y-haplogroups were predicted using two methods available in online software. χ^2 test level of statistical significance $p<0.05$.

Results: Based on the obtained haplotypes, the most frequently detected haplogroups were: I2a, R1a, R1b, J2a. The most frequent haplogroup in the medieval Bosnian population was I2a, as well as in the recent B&H population. The European haplogroup E1B1b also detected in the recent B&H population with a percentage of 10%, was not detected in the medieval population. It is assumed that the obtained results are a consequence of the insufficient number of successfully amplified Y-STR profiles, the small number of samples from the Middle Ages, as well as the possible stochastic effect. χ^2 test based on the frequency of haplogroups was performed. The results indicate the absence of significant differences between the haplogroups in the two time periods.

Conclusion: Based on the obtained Y-haplotypes, Y-haplogroups were detected for the first time, and their frequency in a sample of the medieval Bosnian population was determined.

Keywords: Ancient DNA; Y-STR markers; Medieval Bosnia Archaeology

Session MOLECULAR MECHANISMS OF CELL FUNCTIONS

PRONOUNCED SEQUENCE SPECIFICITY OF THE TET ENZYME CATALYTIC DOMAIN GUIDES ITS CELLULAR FUNCTION

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TET (ten-eleven translocation) enzymes catalyze the oxidation of 5-methylcytosine bases in DNA, thus driving active and passive DNA demethylation. Here, we report that the catalytic domain of mammalian TET enzymes favor CGs embedded within basic helix-loop-helix and basic leucine zipper domain transcription factor–binding sites, with up to 250-fold preference in vitro. Crystal structures and molecular dynamics calculations show that sequence preference is caused by intrasubstrate interactions and CG flanking sequence indirectly affecting enzyme conformation. TET sequence preferences are physiologically relevant as they explain the rates of DNA demethylation in TET-rescue experiments in culture and in vivo within the zygote and germ line. Most and least favorable TET motifs represent DNA sites that are bound by methylation-sensitive immediate-early transcription factors and octamer-binding transcription factor 4 (OCT4), respectively, illuminating TET function in transcriptional responses and pluripotency support.

THE CIRCADIAN SYSTEM – COORDINATING PHYSIOLOGY AND BEHAVIOR

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The mammalian circadian system, which is comprised of multiple cellular clocks located in the organs and tissues, orchestrates their regulation in a hierarchical manner throughout the 24 hours of the day. At the top of the hierarchy are the suprachiasmatic nuclei (SCN), which synchronize subordinate organ and tissue clocks using electrical, endocrine and metabolic signaling pathways that impact the molecular mechanisms of cellular clocks. The interplay between the central neural and peripheral tissue clocks is not fully understood and remains a major challenge in determining how neurological and metabolic homeostasis is achieved across the sleep-wake cycle. Disturbances in the communication between the plethora of body clocks can de-synchronize the circadian system, which is believed to contribute to changes in behavior and as a consequence may lead to the development of diseases such as obesity and neuropsychiatric disorders. This presentation will highlight the role of the circadian system in the regulation of physiology and its impact on behavior.

GUT MICROBIOME AS MEDIATOR OF CHEMICAL EXPOSOME - HOST METABOLISM CROSSTALK

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The origins of many non-communicable diseases occur often as a result of complex interactions of environmental and genetic factors. Particularly, growing evidence suggests that exposure to endocrine disrupting chemicals such as per- and polyfluoroalkyl substances (PFAS) interfere with host metabolism. In a cohort of 264 Danes (121 men and 143 women, aged 56.6 ± 7.3 years, BMI 29.7 ± 6.0 kg/m2), we measured serum bile acids (BAs), PFAS and additional twenty-seven environmental toxicants as well as gut microbiome composition by shotgun metagenomic sequencing. We found that blood concentrations of widespread environmental toxicants such as PFAS associate with measures of body fat accumulation and insulin resistance in a sexually dimorphic manner. These associations may be mediated by gut microbiome-synthesized secondary BAs. These findings were substantiated by the outcome of the murine exposure study. In another study, we investigated potential role of exposure to PFAS in the developmental origin of metabolic disease. Human fetal livers from elective termination of pregnancies between 11-19 weeks of gestation (n = 78) were analyzed by both targeted and untargeted metabolomic analyses of lipids, polar metabolites, BAs and PFAS. Several amino acids, fatty acids and sugar derivatives in fetal livers were inversely associated with PFAS exposure, while the BA glycolithocholic acid was markedly positively associated with all quantified PFAS. Furthermore, 7α-hydroxy-4-cholesten-3-one (C4), a marker of BA synthesis rate, was strongly positively associated with PFAS levels and was detectable as early as gestational week 12. Identification of metabolic perturbations in the human fetus associated with PFAS exposure demonstrates that environmental exposure and its potential harmful impacts start in utero. These effects of PFAS in the fetus, particularly with respect to lipid and BA metabolism, might be responsible for reported adverse health effects during early life.

THE COST OF CIRCADIAN CLOCK DISORDER ON TESTICULAR FUNCTION

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A circadian rhythm is an internalized timing system that synchronizes all physiological processes with the 24-hour changes in the environment. As physiological activities occur at specific times, disturbances in circadian rhythms can have detrimental effects on overall health, with testicular function being particularly vulnerable. The increasing prevalence of lifestyles that disrupt circadian rhythms, coupled with the rise in male idiopathic infertility, highlights the need for a comprehensive understanding of the impact of circadian rhythm disruption on fertility regulation.

Recently, our animal model studies have provided insights into the consequences of circadian disturbances on testicular function. These disturbances lead to desynchronization between the central brain circadian pacemaker and peripheral clocks within the reproductive axis including testicular somatic and germ cells, impacting hormonal signaling pathways and impairing Leydig cell steroidogenesis. This results in reduced testosterone production and compromised testosterone-dependent functions, including spermatogenesis. Moreover, circadian disruptions negatively affect spermatozoa motility and impair the acrosome reaction.

The underlying mechanisms connecting circadian clock disruption to testicular dysfunction involve dysregulation in the expression of key clock genes, as well as genes involved in steroidogenesis, mitochondrial network control, and biogenesis. These changes disrupt energetic homeostasis in testosterone -producing Leydig cells and germ cells, contributing to the deterioration of testicular function, ultimately compromising male fertility.

However, there are still numerous gaps in understanding of the mechanisms through which the circadian system impacts testicular physiology. The need to expand knowledge in this area is particularly urgent, given the ever-evolving work schedules and lifestyle choices that individuals are faced with.

Key words: circadian clock; testis; Leydig cells; spermatozoa; mitochondria

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MOLECULAR MECHANISMS OF FREE FATTY ACID RECEPTOR 2-MEDIATED EFFECTS ON GUT INNATE LYMPHOID CELLS

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Intestinal immune cells have been increasingly appreciated as important players in the initiation, propagation and regulation of the autoimmunity directed against the central nervous system (CNS) and pancreatic beta islets, as observed in multiple sclerosis and type 1 diabetes (T1D), respectively. We are particularly interested in the role of intestinal innate lymphoid cells type 3 (ILC3) in autoimmunity. It is our hypothesis that ILC3 play the major regulatory role in the intestine, and that they are able to potentiate regulatory T cells (Treg) and tolerogenic dendritic cells (tolDC), and to inhibit autoreactive effector T helper (Th) cells: Th1 and Th17. We are exploring possibilities to potentiate regulatory properties of intestinal ILC3 cells as a mean to treat autoimmunity. To this end, we use two models of multiple sclerosis: MOG35-55-induced EAE in C57BL/6 mice and spinal cord homogenateinduced EAE in DA rats, and two models of T1D: multiple low dose streptozotocin-induced T1D in C57BL/6 mice and spontaneous T1D in NOD mice. Immune cells from the intestinal lamina propria, Peyers patches, or gut epithelial monolayer are isolated and detailed phenotypic and functional analysis of ILC3, Treg, tolDC, Th1, and Th17 cells, but also of other immune cells of interest, is performed. Finally, different pharmacological agents are applied to mice and rats orally, as the route of choice for delivery to intestinal immune cells. Molecular targets on ILC3 that we are exploring are receptors for short chain fatty acids, aryl hydrocarbons, microbe-associated molecular patterns, and bile acids. Our research contributes both to the basic knowledge on the role of ILC3 in the immune system, and to the discovery of novel therapeutic options for autoimmune and chronic inflammatory diseases. Here, molecular mechanisms of free fatty acid receptor 2-mediated effects on gut ILC will be presented.

Key words: innate lymphoid cells; intestine; autoimmunity; free fatty acid receptor 2

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EPIGENETIC EDITING AS A POTENTIAL THERAPEUTIC TOOL FOR THE TREATMENT OF NONCOMMUNICABLE DISEASES

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Epigenetic editing has a powerful potential to direct reprogramming of cellular phenotype that can be used in disease modeling. The reprogramming of cells from different origin into insulin-producing cells could provide a solution for restoring functional beta cell mass in diabetic patients. We used CRISPR/dCas9-based epigenetic tool for targeted hypermethylation of *Arx* promoter and its subsequent suppression in mouse pancreatic α cell line. By epigenetic silencing of *Arx* we successfully triggered a direct, transient switch of pancreatic α- to insulin-producing cells obtaining approximately 1% of transiently transfected cells which were able to produce 35% more insulin than Mock transfected α cells.
As a future perspective we intend to address the potential use of epigenetic editing tool as a pre-therapeutic approach in triple-negative breast cancers (TNBCs) with unknown mutational signature of *BRCA1*. The *BRCA1* methylation (BRCAness) as a predictor for response to therapeutics such as PARPi would allow direct TNBC treatment without previous screening for *BRCA1* mutations. The main objective will be to induce BRCAness by suppressing *BRCA1* expression in TNBC cells via targeted DNA methylation of *BRCA1* promoter using the synthetic epigenetic editing tool. This approach would enable the faster decision toward the use of newest medicaments to increase cells' apoptosis and cancer cell diminishment.

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INTEGRATION OF METABOLOMICS AND TRANSCRIPTOMICS DATA REVEALS THE MOLECULAR BACKGROUND OF THE IRIDOID DIVERSITY WITHIN THE GENUS *NEPETA* (FAM. LAMIACEAE)

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Introduction: Recent intensive research on the genus *Nepeta* has resulted in increasingly accumulated information regarding the biosynthetic pathways and chemical evolution of iridoids; however, several intriguing aspects remain yet to be resolved. Our objective was to deeply investigate the molecular background of the diversity of iridoid compounds within the genus *Nepeta*, as well as regulative mechanisms of their biosynthesis.

Methods: Leaves of greenhouse-grown plants were analysed for the composition of iridoid aglycones (IAs) and iridoid glucosides (IGs) adopting non-targeted (UHPLC/LTQ Orbitrap MS^n, GC/MS) and targeted (UHPLC/DAD/(±)HESI-MS^2) metabolomics approaches. Following RNA-Seq, transcriptomes of phylodiverse *Nepeta* taxa were searched for the presence/absence of iridoid-related biosynthetic genes. Co-expression patterns (qPCR) of biosynthetic genes and transcription factors were determined following plants' exposure to various environmental stresses.

Results: The comparison of metabolite composition among phylodiverse *Nepeta* taxa provided a systematic understanding of qualitative and quantitative composition of iridoids in leaves of the selected *Nepeta* taxa. Mining of RNA-Seq data in search for iridoid biosynthesis-related genes pointed to significant differences between taxa producing both IAs and IGs and those producing only IGs. Comparative metabolomics and gene co-expression analysis provided new information about mechanisms of regulation of iridoid biosynthesis.

Conclusion: Integration of data from several techniques analysed by different methods resulted in identifying key genes involved in the regulation of metabolic flux through the iridoid biosynthetic pathway and offered an explanation why some *Nepeta* taxa produce only IGs, while others produce both IAs and IGs. The obtained results pointed to new gene targets for improving iridoid production through biotechnology-based approaches.

Key words: *Nepeta*; iridoids; metabolomics; transcriptomics; gene co-expression analysis

Acknowledgements: The research is financed by the Science Fund of the Republic of Serbia, Grant No. 7749433, project „Omics-guided disentangling of the iridoid diversity in the genus *Nepeta* L. for *in planta* metabolic engineering towards sustainable exploitation- NEPETOME", and is also supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-47/2023-01/200007.

ACTIVATION OF COAGULATION FACTORS AND PROTHROMBOTIC PROPERTIES OF ENDOTHELIUM IN HEMATOLOGICAL MALIGNANCIES

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Introduction: Patients with hematological malignancies have an increased risk of thrombotic complications, ranging from 3-5% in patients with lymphoma and acute myeloid leukemia (AML). The presented study observed the onset of thrombus formation to predict risk factors for thrombosis in lymphoid and myeloid malignancies.

Methods: Coagulation factors, inflammatory signaling pathways and adhesion molecules have been observed in patients with Hodgkin lymphoma (HL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and AML. Their mononuclear cells (MNC) trans-endothelial migration through human microvascular endothelial cells (HMEC-1) monolayer is observed by Boyden chamber.

Results: Thrombin was in positive correlation with tumor necrosis factor alpha (TNF-α) in HL, while with P-selectin ($p<0.001$), tumor growth factor-beta (TGF-β) and factor VIII ($p<0.05$) in DLBCL and AML. Trans-endothelial migration of MNC was increased by TNF-α ($p<0.001$) in DLBCL regardless of previous thrombosis. Regarding coagulation, factor VIII was increased in HL and AML ($p<0.05$), while tissue factor in non-Hodgkin lymphomas (DLBCL and FL, $p<0.05$). Tissue factor was in positive correlation with adhesion molecule P-selectin and factor VIII ($p<0.05$). P-selectin was increased in non-Hodgkin lymphomas ($p<0.0001$), while TGF-β only in FL ($p<0.001$). Fibrinolytic activity was decreased in plasma of patients with HL, DLBCL, and FL ($p<0.05$), but largely in AML ($p<0.01$) as measured by tissue-type plasminogen activator. Inflammatory NF-κB signaling has been activated in HL and DLBCL, while p38 signaling only in HL.

Conclusion: Coagulation factors and inflammation are increased in hematological malignancies along with the interaction of the endothelium and circulating cells that predispose to thrombus formation.

Key words: hematological malignancies; thrombus; coagulation factors; adhesion molecules.

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DNA METHYLATION IN AGE PREDICTION: A FORENSIC PERSPECTIVE OF EPIGENETIC CLOCK

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Aging is a universal process associated with impaired functioning, leading to increased morbidity and mortality rate. Various hallmarks of aging were defined, with epigenetic alterations among them. It is well-known that DNA methylation (DNAm) changes correlate with age, in general, leading to global hypomethylation, but also local hypermethylation of cytosine in the CpG-rich regions. Genome-wide studies revealed a high number of age-dependent DNAm positions, and various mathematical models, using, mostly, selected age-dependent DNAm markers, were created for DNAm-based age prediction. Those models, used for „epigenetic age" estimation, were named „epigenetic clocks".

The purpose of the „epigenetic clock" depends on the selection of age-related DNAm markers. „Biological epigenetic cloks" developed for the estimation of „biological age" compare the difference between calculated „epigenetic age" and real „chronological age", actually measuring alterations in functionally important DNA methylation patterns resulting in acceleration or deceleration of aging. On the other hand, the aim of the „forensic epigenetic clock" is to predict the „chronological age" with maximal accuracy, so age-dependent DNAm markers are selected to be the least sensitive to genetic and environmental factors, but associated with the age itself.

Forensic analyses are generally constrained with low quality and quantity, and often the unknown origin of available biological material, mixtures of different cell types and tissues, and material of individuals of varying age and gender, pointing out the factors that should be considered when creating „forensic epigenetic clock", both for marker selection and methodology used, which makes its creation rather challenging.

UNDERSTANDING MOLECULAR PATHWAYS OF ADIPOCYTE DIFFERENTIATION: CLOSER INSIGHT INTO LIPOMA STORY

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Introduction: Lipomas are benign adipose tissue tumors of still unknown etiology and pathogenesis. Lipoma-derived stem cells (LDSCs) are a potential tool in tissue engineering due to their reported similarity to adipose-derived stem cells (ADSCs). However, their involvement in lipoma's formation hint at differences in molecular signature. In order to understand the molecular pathways involved in lipoma formation we analyzed both the adipogenic potential of LDCSs *vs*. ADSCs and the expression of adipogenesis-related gene markers in tissue samples.

Methods: LDSCs and ADSCs were isolated by enzymatic digestion of lipoma and subcutaneous adipose tissue samples, cultured and expanded in standard cell culture conditions. Mesenchymal stem cell phenotype was analyzed by gene expression and flow cytometric analysis of stem cell markers. After adipogenesis induced by 21-day cultivation in adipogenic differentiation media, adipocyte phenotype was investigated by microscopic, cytochemical and immunocytochemical, gene and protein expression analyses. Gene expression of the same adipogenesis-related markers was analyzed in tissue samples as well.

Results: Adipogenesis-related genes' expression pattern and presence of more mature adipocytes in ADSCs than in LDSCs culture after 21 days of adipogenic differentiation was noticed. Differences in expression levels of analyzed genes were also noticed in examined tissue samples.

Conclusion: Differentiation capacity of LDSCs was significantly lower compared to ADSCs. Differences in gene expression levels observed in both isolated cells and tissue samples revealed the potential molecular pathways involved in lipoma formation and giving us closer insight into lipoma story.

Key words: lipoma; adipose tissue; mesenchymal stem cells; gene expression; adipogenesis

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VENOMS – THE SOURCE OF DRUGS WITH ANTICANCER POTENTIAL

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Introduction: Through evolution, venoms had acquired very important roles concerning the survival of the species that produce them. Based on that role, animal venoms can be divided into predatory and defensive, which also determines differences in their protein composition. The use of venoms in medicine is widespread today, but no venom component has yet deserved attention for anticancer testing in a clinical study. Considering the increasing incidence of cancer in the human population and current failures in cancer therapy, mainly associated with drug non-selectivity and cancer cell resistance, there is a need to find new drugs with good antitumor potential. Our current research was focused on one defensive (from *Apis mellifera* L.), and predatory venom (from *Vipera ammodytes* L.), as well as their constituents (melittin and LAAO).

Methods: The antitumor properties of venoms and their constituents were evaluated by analysis of its cytotoxicity, ability to induce apoptosis (AO/EB double staining), monitoring the hallmarks of apoptosis, redox status, and biotransformation on gene (qPCR) and protein levels (immunostaining and colorimetric methods), on colon cancer cells (HCT-116, SW-480 and HT-29).

Results: Regarding the achieved anticancer properties of venoms and their components related to their potential to modulate apoptosis, redox homeostasis, drug metabolism, and export via membrane transporters, our results emphasize their importance as a source of anticancer substances. The difference in selectivity and apoptotic vs necrotic activity were observed among total venoms and their individual constituents.

Conclusion: Although animal venom was avoided due to its toxic nature, they are a valuable source of many components with great antitumor potential.

Key words: colon cancer; Honeybee; Nose-horned viper; resistance; venom

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PURINERGIC SIGNALING IN THE CENTRAL NERVOUS SYSTEM IN HEALTH AND DISEASE: FOCUS ON ECTONUCLEOTIDASES

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Introduction: Sequential hydrolysis of extracellular ATP catalyzed by ectonucleotidases (CD39, CD73) is the main pathway for adenosine formation. Together with purinergic receptors (P2XR, P2YR, AR) they form the purinergic signaling system, which is essential for the regulation of cellular functions and in pathological conditions of the central nervous system, especially those with an inflammatory component. We hypothesized that components of the purinergic system can assign functional states of glial cells.

Methods: The distribution of ecto-5`-nucleotidase (eN/CD73) and adenosine receptors was studied in rat hippocampus from early postnatal to late adulthood. The activities and localization of NTPDase1/CD39, NTPDase2, and eN/CD73, the expression/localization of purinoreceptors and proinflammatory mediators associated with reactive glial cells were analyzed in the rat model of trimethyltin-induced hippocampal degeneration leading to Alzheimer-like behavioral and neurological dysfunction.

Results: NTPDase1 is localized in microglia and synaptic membranes, NTPDase2 in fibrous astrocytes and synaptic terminals. eN activity and expression increase during aging in synapse-rich hippocampal layers. A_1R expression is decreased, whereas $A_{2A}R$ is increased in pyramidal cell layers. Glial upregulation of $A_{2A}R$, typical of both advanced age and chronic neurodegeneration, did not occur. During neurodegeneration, upregulation of NTPDase1 occurred in all $Iba1^+$-cells in the injured area, whereas eN was induced in amoeboid $Iba1^+$-cells within the degenerated neuronal layers. NTPDase2 decreased shortly after intoxication and retracted from synaptic boutons. Reactive astrocytes expressed $A_1R/A_{2A}R$, $P2Y_1R$, and induced proinflammatory molecules.

Conclusion: Increased set of purinergic system components on activated microglia ($CD39/CD73/P2X_7R$) and astrocytes ($A_1R/A_{2A}R/P2Y_1R$), and loss of homeostatic glial and neuronal purinergic pathways ($NTPDase2/P2Y_{12}R/A_1R$) may shift the purinergic signaling balance toward excitotoxicity and inflammation, promoting the progression of pathological events.

Key words: NTPDase1/CD39; eN/CD73; hippocampal neurodegeneration; purinergic receptors; astrocyte-derived inflammation

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ELECTRONIC CIGARETTE VAPOUR CONDENSATE AFFECTS MITOCHONDRIAL POTENTIAL IN BEAS2B CELLS

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Introduction: Cigarette smoke exposure is a known risk factor for development of lung diseases and electronic cigarettes (e-cigarettes) were introduced as a popular and safer alternative to combustible tobacco products. Increasing number of studies are reporting their adverse biological effects both in vivo and in vitro. Aim of this study was to evaluate the effect of e-cigarettes on mitochondrial function in lung bronchial epithelial cells.

Methods: Electronic cigarette vapor condensate (ECC) was generated using an e-cigarette device on a suction trap cooled in a dry ice/ethanol bath. We used unflavoured and flavoured e-cigarette liquids with and without nicotine. Human bronchial epithelial BEAS2B cells were seeded in 96well plates and treated with 2% e-cigarette vapour condensate for 24h. Mitochondrial membrane potential was measured using 50nM TMRE (Tetramethyl rhodamine ethyl ester) and cells were visualized on ImageXpress® Pico Automated Cell Imaging System (Molecular Devices, San Jose, CA, USA) with a 10x objective.

Results: We found a significant reduction of TMRE fluorescence in treated cells compared to the control. Imaging of treated cells also revealed changes in cell morphology and the presence of mitochondria in TNT-like structures.

Conclusion: Mitochondrial dysfunction has been associated with various pathological conditions including lung diseases such as asthma, COPD and lung cancer. Due to their relative novelty, the role of electronic cigarette use in development of chronic lung diseases is still relatively unknown. Our findings contribute to the growing list of studies pointing to their adverse biological effects and imply their involvement in processes contributing to chronic lung diseases.

Key words: electronic cigarettes; mitochondria; lung disease

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E-CIGARETTE VAPOR CONDENSATE AFFECTS MITOCHONDRIAL FUNCTION IN A549 CELLS

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Introduction: E-cigarettes are becoming increasingly popular, but their potentially harmful effects are not well studied. Although they are used to aid smoking cessation, there is evidence that the chemicals they contain can affect lung cells, and the effects may also be important for lung cancer cells. Our objective was to investigate the effects of e-cigarette vapor condensate on mitochondrial function of A549 cells.

Methods: We used the Agilent Seahorse Mito Stress Test, according to the manufacturer's protocol to evaluate the mitochondrial function of A549 cells under the treatment with different concentrations of e-cigarette vapor condensates. Vapor condensates were prepared from e-cigarette liquid base (PG/VG), base with nicotine (PG/VG+N), with flavoring (PG/VG+F), or with nicotine and flavoring (PG /VG+F+N). The cells were exposed to 2% or 3% PG /VG, PG /VG+F, PG/VG+N, and PG/VG+F+N for 24 hours before testing.

Results: All treatments with 2% vapor condensates affected mitochondrial function of A549 cells. Basal and maximal respiration were decreased, indicating mitochondrial dysfunction. Higher concentrations of vapor condensate (3%) significantly increased proton leak and decreased mitochondrial coupling efficiency, indicating mitochondrial damage. However, the increased spare respiratory capacity, in 3% vapor condensate treated cells, may indicate activation of a compensatory response in mitochondria.

Conclusion: Our results suggest that e-cigarette vapor condensate may have deleterious effects on mitochondria. Further analysis of mitochondrial function and morphology would further elucidate the effects of e-cigarette vapor condensate.

Keywords: e-cigarette; lung cancer; mitochondria; mito stress

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SPATIAL PROFILE OF ANKRD1A ACTIVATION DURING REGENERATION OF ZEBRAFISH HEART

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Introduction: In contrast to humans, zebrafish have a remarkable ability to regenerate injured heart through a complex and highly orchestrated process involving all cardiac structures. The major source of new myocardial cells are resident cardiomyocytes, which dedifferentiate and reinitiate proliferation, invading the area of injury to replace the lost myocardium. The response of the myocardium and coronary vasculature is preceded by activation of epi- and endocardium, which form active scaffolds to guide regeneration. The aim of this study was to identify cardiac structures in which *ankrd1a* gene is activated during zebrafish heart regeneration.

Methods: We crossed several zebrafish reporter lines: *TgBAC*(*ankrd1a*:*EGFP*) (to identify cells expressing *ankrd1a*), *Tg*(*myl7*:*nls-dsRedExpress*) (for labeling cardiomyocyte nuclei) and *Tg*(*kdrl*:*RAS-mCherry*) (for labeling endocardial/endothelial cells). Zebrafish hearts were cryoinjured and left to regenerate for 3 and 7 days. Dedifferentiating cardiomyocytes and epicardial cells were immunostained with anti-MYH7 and anti-caveolin1 antibody, respectively. Cells labeled with transgenes and immunostaining were visualized on tissue cryosections by fluorescent microscopy.

Results: Zebrafish *ankrd1a* was activated in the injury border zone cardiomyocytes, located between the injured and remote myocardium. Its expression preceded that of a dedifferentiation marker, MYH7. The *TgBAC(ankrd1a:EGFP)* transgene was not detected in epicardial or endocardial cells of regenerating zebrafish heart.

Conclusion: Activation of *ankrd1a* during regeneration of zebrafish heart is restricted to borderzone cardiomyocytes, implicating this gene in dedifferentiation and proliferation of cardiomyocytes. The absence of *ankrd1a* expression in epicardium and endocardium indicates that this gene does not contribute to the regeneration process occuring in these layers of the heart.

Keywords: zebrafish; heart; regeneration; *ankrd1a*

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PLASTIC RESPONSE OF *IRIS PUMILA* SMALL HEAT SHOCK PROTEIN HSP17.6 TO EXPERIMENTAL WARMING *IN SITU*

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Introduction: Global warming profoundly impacts ecological communities as higher temperatures affects growth and metabolism of individuals, altering their survival and reproductive success. To anticipate plant response to future temperature increase, two natural populations of *Iris pumila* inhabiting sun-exposed environment were subjected to an open-top chamber (OTC) experiment and investigated for the amount of Hsp17.6.

Methods: One half of all randomly selected circle-shaped clones of *I. pumila* were experimentally warmed, by 1-2°C, using clear-sided OTC, while the other half was exposed to ambient temperature conditions. In spring and summer, over a two-year period, ramets of each clone growing inside and outside of OTC were analyzed for the Hsp17.6 content.

Results: Immunoblot analysis revealed the presence of two Hsp17.6 isoforms, whose quantities were greater in ramets growing inside the OTCs than in those growing outside. The mean response profiles of both protein isoforms were parallel over time and the total amount of Hsp17.6 reached its maximum in the summer. A repeated-measures profile analysis revealed significant treatment and season effect for both Hsp17.6 isoforms, whereas year effect was significant only for the higher molecular weight isoform. Furthermore, profile analysis of the between-population effects showed that the mean response profiles, for both isoforms, differ between populations.

Conclusion: A small temperature increase can alter both the level and shape of the mean response profiles of Hsp17.6 in *I. pumila*, suggesting the species' capability to acclimate to increasing temperatures by plastic response of small heat shock proteins, the plants' key molecular chaperones associated with enhanced thermotolerance.

Key words: global warming; open-top chamber (OTC); plastic response; Hsp17.6; *Iris pumila* L.

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THE EFFECT OF UVB RADIATION ON THE EXPRESSION OF *SOX2* AND *SOX9* GENES IN HUMAN KERATINOCYTES *IN VITRO*

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Introduction: Prolonged exposure to sunlight, has a harmful effect on skin cells encompassing reduced viability, morphological changes, and altered gene expression. The two most prevalent types of skin cancer, squamous cell carcinoma (cSCC) and basal cell carcinoma (BCC), arise from malignant transformation of keratinocytes. UV radiation, among other factors, serves as the primary cause of these tumors. Previous data has shown that changes in different *SOX* genes expression in these cancer types correlates with disease progression, suggesting their role as oncogenes/tumor suppressors. The presented work is focused on examining the impact of UVB radiation on the expression of *SOX2* and *SOX9* genes in HaCaT cells derived from human keratinocytes.

Methods: Using a custom-made UV solar simulator for the irradiation of HaCaT cells with 150 mJ/cm^2 or 300 mJ/cm^2, we analyzed *SOX2* and *SOX9* gene expression. In order to determine the protective effects of quercetin, anti-inflammatory bioflavonoid, we treated irradiated HaCaT with quercetin, and analyzed *SOX* gene expression.

Results: Our results indicate that UVB radiation induces a dose dependent decrease of *SOX2* expression while expression of *SOX9* was increased at the dose of 150 mJ/cm^2 in HaCaT. Treatment of cells with quercetin increased the expression of both *SOX2* and *SOX9* genes in HaCaT cells following UVB radiation at both doses compared to irradiated cells.

Conclusions: Further research is needed to understand the molecular mechanisms and significance of *SOX2* and *SOX9* in UVB-induced cellular responses, in the context of nonmelanoma cancers with potential implications for targeted therapeutic strategies for nonmelanoma cancers.

Keywords: UVB; *SOX2; SOX9;* HaCaT cells.

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METABOLIC DISTURBANCES IN ANIMAL MODEL OF POLYCYSTIC OVARY SYNDROME: IMPACT OF EARLY POSTNATAL OVERFEEDING

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Introduction: Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects women's fertility and metabolic health throughout their life time. Insulin resistance and obesity, in conjunction with excess androgens, are undeniably involved in its development. We aimed to elucidate how hyperandrogenemia and prepubertal adiposity contribute to the development of metabolic disturbances in rat model of PCOS**.**

Methods: The animal model of PCOS induced by 5a-dihydrotestosterone (DHT) was additionally challenged by litter size reduction (LSR) during suckling period, to ensure overfeeding and development of prepubertal adiposity. Systemic parameters of insulin sensitivity, along with markers of energy sensing, insulin signaling, and lipid metabolism were analyzed in visceral adipose tissue (VAT) and skeletal muscle.

Results: The combination of treatments led to hyperinsulinemia and impaired systemic insulin sensitivity. This was not accompanied with altered insulin signaling in the VAT, in spite of observed adipocytes hypertrophy probably due to activation of AMPK and restrained lipogenesis in this tissue. On the other hand, insulin signaling in skeletal muscle was impaired, which resulted in increased muscle fatty acid uptake and oxidation after combined treatment. The switch to fatty acids oxidation subsequently led to oxidative stress and inflammation, which was followed by adaptive activation of AMPK and increased expression of its targets involved in antioxidant protection and mitochondrial biogenesis.

Conclusion: Our results suggest that prepubertal weight gain predisposes to insulin resistance development in androgen-excess PCOS. The protective activation of AMPK in VAT and muscle makes it a potential therapeutic target for insulin-resistant PCOS patients.

Key words: polycystic ovary syndrome; early postnatal overfeeding; insulin resistance; adipose tissue; skeletal muscle

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ISORHAMNETIN – ANTITUMOR POTENTIAL AND IMPACT ON DRUG RESISTANCE IN COLORECTAL CARCINOMA CELL LINES

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Introduction: Colorectal cancer is the second-leading cause of death in the world. The main problem in the treatment of this type of cancer is innate or acquired cell resistance to conventional drugs. Therefore, a large number of research is focused on discovering new substances or strategies in antitumor therapy to overcome drug resistance. Considering that, the aim of this study was to evaluate isorhamnetin, a plant flavonol, as an antitumor agent, as well as its ability to modulate the development of drug resistance.

Methods: The antitumor potential of isorhamnetin was evaluated in HCT-116, SW-480, HT-29, and DLD-1 colorectal carcinoma cells through the determination of cytotoxic (MTT assay), and pro-apoptotic activity (acridine orange/ethidium bromide microscopic method). The effect of isorhamnetin on the expression of genes, whose protein products were related to drug biotransformation and development of cancer cell resistance were monitored by qPCR and immunocytochemistry.

Results: The obtained results show that isorhamnetin induces dose and time-dependent cytotoxic and proapoptotic activity in all investigated cell lines. Isorhamnetin induced equally good cytotoxic activity as 5-fluorouracil – a standard cytostatic in colorectal cancer therapy. Additionally, isorhamnetin changed the gene and protein expression of targeted ATP-binding cassette (ABC) transporters (*ABCB1*, *ABCC1*, *ABCC2*, *ABCC5*, *ABCC11*, and *ABCG2*), as well as biotransformation-related genes (*CYP1A1*, *CYP1B1*, and *GSTP1*), involved in the development of drug resistance.

Conclusion: Based on obtained results, isorhamnetin shows pronounced cytotoxic and proapoptotic activity on colorectal cancer cells and a significant impact on drug resistance parameters, which makes it favorable for further studies in antitumor therapy.

Key words: antitumor therapy; colorectal cancer; drug resistance; isorhamnetin

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CHANGE IN ANTIOXIDATIVE STATUS DURING THE AGING OF HONEY BEES (*APIS MELLIFERA* L.)

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Introduction: Aging is a prominent scientific topic, and social insects like honey bees are excellent model organisms for studying the mechanisms underlying aging because of their phenotypic plasticity. One of the theories that explain aging is an increase in oxidative stress, which arises from an imbalance between free radical production and the body's ability to neutralize their harmful effects. The aim of this study was to investigate age-related changes in activity and gene expression of the antioxidant enzymes superoxide dismutase (Sod) and catalase (Cat), which play an important role in detoxifying free radicals, in summer bees.

Methods: Honey bees were continually collected for 5 weeks during summer of 2022. Six experimental groups were formed, control group (S0) representing newly hatched bees, and groups S1-5 representing bees of different ages. The relative expression of *Sod1*, *Sod2*, and *Cat* genes was analyzed in the head and abdomen by qPCR. The activity of Sod and Cat enzymes in the head and abdomen was determined spectrophotometrically.

Results: Cat activity increased in both the abdomen and head with aging, while Sod decreased in the abdomen and increased in the head in the S5 group. *Cat* was upregulated in the abdomen and head; the *Sod1* and *Sod2* were upregulated in the abdomen, while only *Sod2* was downregulated in the head compared to newly hatched bees.

Conclusion: The pattern of age-related changes in the activity of antioxidant enzymes and their gene expression differs for various antioxidant enzymes; therefore, additional research is needed to understand these mechanisms.

Key words: aging; oxidative stress; genes; enzymes; social insects

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MODULATION OF HEPATIC LIPID METABOLISM IN OBESITY-RESISTANT MICE ON A HIGH-FAT DIET

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Introduction: High-fat diet primarily leads to obesity but it can also lead to obesity resistant (OR) phenotype with various metabolic complications. Liver plays central role in modulating lipid metabolism in response to dyslipidemia induced by adipose tissue hypertrophy. The aim of this study was to define key regulatory points *that adjust lipid metabolism in the liver of OR mice on high-fat diet (HFD).*

Methods: Male C57BL/6J mice were divided into two groups: control group on normal diet (10 kcal% fat, D12450J, Research Diets, USA) and HFD group (60 kcal% fat, D12492, Research Diets, USA). After 14 weeks, mice on HFD were classified as obese or OR based on 30% difference in body weight gain compared with controls. Liver sections were analyzed histologically, while alterations in hepatic lipid metabolism were assessed by qPCR and Western blot.

Results: Although HFD restricted hepatic de novo lipogenesis, increased influx of free fatty acids (FFA) led to accumulation of lipid droplets in the liver of obese mice. In OR mice, liver morphology was restored, as was expression of insulin sensitive sterol regulatory element-binding protein 1c (SREBP-1c). Level of FFA transporter CD36 was reduced, whereas higher expression of diacylglycerol acyltransferase 2 limited lipotoxicity in OR compared with obese mice. FFA β-oxidation remained unchanged in both HFD groups.

Conclusion: Lower FFA input and reduced lipid storage and lipotoxicity in the liver of OR mice suggest that dyslipidemic complications associated with obesity could be ameliorated by targeted modulation of expression of FFA transporters and regulators of lipid droplet formation.

Key words: obesity, obesity resistance, lipid metabolism, liver

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THE EFFECT OF GALECTIN-8 ON TROPHOBLAST ADHESIVE PROPERTIES

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Introduction: Galectins have been shown to play an important role in placentation. The presence of galectin-1, -3 and -8 has been demonstrated in extravillous trophoblasts (EVTs), where galectins-1 and -3 were found to be important regulators for the functional properties of trophoblasts *in vitro*. We aim to investigate the effect of galectin-8 on trophoblast function, namely cell adhesion to collagen and its effect on integrins, which are one of the mediators of trophoblastic adhesion and invasion.

Methods: We used the recombinant human galectin-8 and HTR-8/SVneo trophoblast cell line. Experiments performed were cell adhesion on collagen in the presence of galectin-8 with/without lactose, CELISA and qPCR to study the effect of galectin-8 on integrins α_1 and β_1, and Comet assay to assess the genotoxicity of galectin-8.

Results: Galectin-8 had a stimulating effect at 100 and 200ng/ml compared to the control on cell adhesion to collagen of human trophoblast *in vitro*. The percentage increase was 122.8% and 124.2% respectively with p-value < 0.01 for both concentrations. The presence of lactose reversed to some degree the effect of galectin-8 to 107.2% and 119.5% respectively compared to the control. No significant effect of galectin-8 was observed on integrins α_1 and β_1 at the mRNA and protein level. Galectin-8 has been shown not to be genotoxic to HTR-8/SVneo cells using the Comet assay.

Conclusion: The results showed that galectin-8 is an important molecule for the adhesion of EVT *in vitro*, as well as that its influence is realized partially by lectin activity.

Key words: galectin-8; extravillous trophoblast; cell adhesion; collagen; integrins

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EXPRESSION OF MARKERS OF NEURAL FUNCTION IN UNDIFFERENTIATED AND RETINOIC ACID-DIFFERENTIATED SH-SY5Y CELLS

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Introduction: Human neuroblastoma SH-SY5Y cell line, both undifferentiated and differentiated cultures, is extensively used as experimental model in neuroscience and toxicology research. However, there is a lack of understanding related to expression of various markers of neural function in undifferentiated and differentiated SH-SY5Y cells, and their suitability for investigation of particular cellular features and mechanisms relevant for neurotoxicity studies.

Methods: For the differentiation of SH-SY5Y cells, retinoic acid (RA) was added to the culture medium in concentration of 20 nM for 7 days. Expression of various genes, encoding proteins involved in neurotransmitter signaling pathways, exocytosis of neurotransmitters, neuron excitability, neuronal development and maturation was investigated by RQ-PCR analysis in undifferentiated and RA-differentiated SH-SY5Y cells. The levels of gene expression normalized to endogenous control were compared.

Results: Both SH-SY5Y cultures express the selected genes, but for some of them the expression was higher in RA-differentiated SH-SY5Y cells. The difference in expression was especially pronounced for genes encoding subunits for serotonin (*HTR1E*), dopamine (*DRD2*) and GABA (*GABBR1*) receptors, enzymes involved in neurotransmitters removal (*ACHE, MAOA, ABAT*), voltage-gated Na^+ (*SCN4B*, *SCN1A*) and Ca^{2+} (*CACNA2D1*) channel subunits, and elements involved in neuronal development and maturation (*TUBB3, NEFL, SYN1, PNPLA6*).

Conclusion: Analyzed markers of neural function can be investigated in both undifferentiated and RA-differentiated SH-SY5Y cells. Comprehensive data on their applicability in neurotoxicity studies will be provided after analyses of the responsiveness of these genes to xenobiotics.

Key words: gene expression; neurotoxicity; SH-SY5Y

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IMBALANCE IN REDOX HOMEOSTASIS INDUCED BY ORLISTAT IN BREAST CANCER CELLS

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Introduction: Orlistat (Xenical™) is an approved drug for treating obesity, but recent studies suggested its significant potential as an antitumor drug in breast cancer treatment. Redox status and its imbalances in breast cancer cells are associated with aggressive phenotype of these cells. Modulating oxidative stress by antitumor drugs may be a promising strategy to control breast cancer progression.

Methods: The concentration of redox status parameters was measured by colorimetric assays. The concentration of superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and glutathione (GSH) was measured in control and breast cancer cells (MDA-MB-231 and MDA-MB-468) treated with Orlistat (1-50 μg/ml) after 72 h.

Results: Based on the results, concentration of $O_2^{\cdot-}$ and H_2O_2, as the most important reactive oxygen species (ROS), was increased in breast cancer cells after Orlistat treatment. The increased concentration of MDA, as indicator of lipid damage, was also observed in treated cells. The level of GSH, as parameter of antioxidant defense, was significantly increased. According to presented results, Orlistat acts as a strong pro-oxidative agent in breast cancer cells.

Conclusion: Cancer cells produce high levels of ROS and lipid peroxides that leads to oxidative stress. Disbalance in redox homeostasis makes them vulnerable to drugs that increase ROS and lipid peroxide levels. The use of pro-oxidant agents is an emerging strategy to selectively target only cancer cells. Since Orlistat shows a significant pro-oxidant effect in tested breast cancer cells, there is a need for future investigation of essential molecules that participate in the modulation of redox homeostasis.

Key words: anti-obesity drug; antitumor drugs; breast cancer; prooxidant effect

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CHANGES IN UP AND DOWN REGULATED GENE EXPRESSION AFTER TRANSIENT SUPPRESSION OF *ARX* GENE IN PANCREATIC ALPHATC1-6 CELL LINE

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Introduction: The Aristaless-related homeobox (*Arx*) gene plays a key role in the development and maintaining pancreatic alpha cell phenotype, and as such represents an excellent target for alpha cell identity change towards insulin-producing cells. Therefore, this cell switch and increase in beta cell mass could be of potential use in diabetes management.

Methods: On the fifth day after transient transfection with dCas9-Dnmt3a3L-KRAB construct and four gRNAs targeting *Arx* promoter, αTC1-6 were sorted to collect GFP-positive (transfected) cells (EpiC). The mRNA-seq libraries were pooled in equimolar amounts and sequenced in a single end-setting on the Illumina NextSeq 500 High output machine with 75 bases long reads. KEGG pathway overrepresentation analysis was performed using an application on all significantly up- or downregulated genes using default settings.

Results: Directed induction of DNA methylation on the *Arx* gene promoter reduces its expression and causes the up-regulation of 357 genes, while 266 genes were down-regulated in EpiC compared to Mock transfected cells. The KEGG pathways analysis of biological processes confirmed several biological pathways associated with genes differentially expressed in EpiC vs. Mock transfected cells at the 5th post-transfection day (pval ≤ 0.05). As the most significant, up-regulated pathways we found Type II diabetes, Insulin secretion, Longevity regulation pathways. As significant, down-regulated pathways pop-up Fatty acid metabolism and PPAR signaling pathway.

Conclusion: Reduction of *Arx* mRNA level is sufficient to initiate the transdifferentiation process of alpha cells into insulin-producing cells by triggering several biological pathways tight related to insulin secretion and function.

Key words: CRISPR/dCas9; alpha cells; methylation; Arx gene; epigenetic editing

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MITO-SPERM-SIGNATURE: A POSSIBLE NEW PROGNOSTIC/DIAGNOSTIC TOOL TO ASSESS MEN (IN/SUB)FERTILITY BY USING MITOCHONDRIAL DYNAMICS MARKERS IN SPERMATOZOA AND HORMONES IN SEMINAL PLASMA

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Introduction: Given the importance of mitochondria for spermatozoa homeostasis and the lack of accurate infertility tests, our study investigated the transcriptional/protein profiles of human spermatozoal mitochondrial dynamics markers and seminal plasma hormone levels in relation to spermatozoa functionality and spermiogram types.

Methods: The samples were collected from men participating in the national assisted-reproductive-technology-program. The study design and procedures were approved by the Ethical Committee. The normozoospermic (N), teratozoospermic (T), asthenoteratozoospermic (AT), and oligoasthenoteratozoospermic (OAT) spermatozoa were separated from seminal plasma and incubated with/without progesterone (an acrosome reaction inducer) to assess functionality. Hormonal, real-time PCR, and Western blot analyses were conducted.

Results: The levels of hormones (testosterone, cortisol, estradiol) in seminal plasma and transcriptional pattern of mitochondrial dynamics markers in spermatozoa displayed individual variations. The significant increases in the level of *TFAM*, *OPA1*, *PINK1* and *PRKN* transcripts and decreases of *PPARGC1B* and *DRP1* were observed in T-spermatozoa. *MFN1/MFN2* increased in AT-spermatozoa. In OAT-spermatozoa, significant increasement of *PPARGC1B*, *NRF2*, *MFN1/MFN2* and *OPA1* transcripts were detected, while *TFAM* transcript decreased. Although trends-of-changes in PGC1, NRF1/2 and MFN1/2 proteins-profiles were observed, they did not reach significance due to the limited number of samples. Some of the changes in the expressional-pattern could be associated with the hormonal-pattern to some extent, but more samples are required.

Conclusion: The above-mentioned markers could provide a solid foundation for the development of a Mito-Sperm-Signature - a potential new prognostic/diagnostic tool to assess male infertility, as semen quality and fertility are important fundamental markers of reproductive and overall health.

Key words: men (in/sub)infertility; human spermatozoa; mitochondrial dynamics markers; *MFN1/2; NRF1/2*

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INVESTIGATING MITOCHONDRIAL DYNAMICS IN SPERMATOZOA: IMPLICATIONS FOR MALE INFERTILITY

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Introduction: According to the World-Health-Organization, 8%-12% of couples worldwide are affected by infertility. This study aimed to investigate potential mechanisms underlying male (in/sub) fertility by examining molecular markers of mitochondrial dynamics (MitDs) in spermatozoa of animal models that mimic the situations in the human population.

Methods: The expressional profiles of MitDs markers and related signaling molecules were assessed using molecular biology techniques in spermatozoa isolated from the cauda epididymides. Spermatozoa were isolated from animals that were subjected to eight different *in vivo* treatments, such as acute and repeated psychophysical stress, ageing, ageing in combination with different pharmacological substances etc.

Results: The results revealed disturbances in circulating androgens in all experimental models. Results showed that acute stress with recovery periods decreased number of spermatozoa at ZT17 and ZT23, while functionality (response to acrosome-reaction-inducer progesterone) was decreased at ZT3 and ZT11 but recovered at ZT17 and ZT23. Transcriptional profiles of 91% (20/22) of tracked MitDs markers were disturbed after acute stress and during the recovery period. Also, repeated stress at different time points (ZT3, ZT11, and ZT23) disturbed the number and the functionality of spermatozoa as well as the transcriptional profiles of 22 MitDs markers. These changes dominantly increased in the transcriptional profiles of MitDs markers. On the other hand, acute and chronic treatment of aged rats with Sildenafil (Viagra®) increased spermatozoa number, while most of the tracked MitD markers were decreased.

Conclusion: Altered androgens affect MitDs markers, vital for mitochondrial network and sperm functionality, suggesting novel approach in male infertility treatments.

Key words: disturbed circulating testosterone, mitochondrial dynamics and functionality markers; spermatozoa number and functionality; stress; aging.

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AdeABC EFFLUX PUMP-MEDIATED RESISTANCE TO TIGECYCLINE IN *ACINETOBACTER BAUMANNII* ISOLATES FROM BALKAN HOSPITALS

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Introduction: Multidrug-resistant (MDR) *Acinetobacter baumannii* has been recognized as one of the most serious healthcare challenges worldwide. Although tigecycline represents one of the last resort therapies for MDR *A. baumannii*, resistance to this antibiotic has been reported and mostly is mediated by AdeABC efflux pump. The aim of our study was to investigate the molecular mechanism responsible for tigecycline resistance of thirty-seven *A. baumannii* isolates from Balkan medical settings (Serbia, Bosnia and Herzegovina and Montenegro) gathered in 2016 and 2022.

Methods: Minimal inhibitory concentration (MIC) values for tigecycline were determined using microdilution method according to EUCAST guidelines. Inhibition of the efflux of tigecycline was tested by the same method using a combination of antibiotic and efflux pump inhibitor (CCCP). Amino acid alternations within AdeS and AdeR proteins were detected by comparing to sequences of referent isolates ATCC19606 and ATCC17978. Expression of the *adeB* gene of selected isolates was monitored by RT-qPCR.

Results: All tested isolates were resistant to tigecycline and showed significant decrease in tigecycline MIC values in presence of CCCP (≥16-fold reduction) indicating that antibiotic efflux is responsible for tigecycline resistance. The analysis of two-component system AdeRS, regulatory system of RND efflux pump AdeABC, revealed that most of the isolates have G186V and N268H alternations in AdeS (n=32), while most common changes in AdeR were V120I and A136V (n=29). In addition, RT-qPCR showed that selected isolates upregulate expression of the *adeB* gene (from 1,13- to 3-fold).

Conclusion: This study revealed that AdeABC overexpression is the main mechanism of tigecycline resistance in *A. baumannii* isolated in Balkan hospitals.

Key words: *Acinetobacter baumannii*; tigecycline resistance; efflux pump; AdeABC; AdeRS

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DETERMINATION OF MUSCLE FIBER TYPES EXPRESSING ANKRD2

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Introduction: Ankyrin Repeat Domain 2 (ANKRD2) is expressed in skeletal muscle, where plays a role in muscle development, differentiation and adaptation to stress. Human skeletal muscle consists of three major fiber types: type 1 (slow-twitch, oxidative), type 2A (fast-twitch, oxidative) and type 2X (fast-twitch, glycolytic). ANKRD2 is reported to be primarily expressed in type 1 myofibers. However, recent findings on human single myofibers and our study of chicken muscles have shown that this protein may also be expressed in type 2A fibers. Hence, our objective was to examine whether ANKRD2 is present in human fast, type 2A muscle fibers using immunohistochemistry.

Methods: Samples of large leg muscles *soleus*, *gastrocnemius*, *vastus intermedius* and *vastus lateralis* were obtained from human cadaveric tissue. Serial cryosections were independently stained with anti-ANKRD2 and antibodies for different myosin heavy chain isoforms (6H1 for type 2X, BF35 for type 1 and 2A, anti-MHCs for type 1 and anti-MHCf for type 2A and 2X fibers). Immunostained tissues were analyzed by fluorescent microscopy.

Results: In addition to slow, type 1, ANKRD2 was found expressed in fast, type 2A myofibers, which both have oxidative metabolism. Further, we did not observe ANDRD2 expression in glycolytic, type 2X myiofibers. This pattern of ANKRD2 expression was consistent across all examined muscles.

Conclusion: Our results implicate that the regulatory mechanism of ANKRD2 expression in human skeletal muscle is associated with oxidative metabolism, rather than muscle contraction speed.

Key words: ANKRD2; muscle fibers types; protein expression; immunohistochemistry

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PROTHROMBIN INFLUENCES PROLIFERATION AND MIGRATION OF COLON CANCER *IN VITRO*

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Introduction: Thrombin, crucial member of the coagulation cascade, can influence growth and development of different types of cancer. Prothrombin, thrombin precursor, although predominantly secreted from the liver into the bloodstream, can also be expressed in the cancer cells. According to latest data prothrombin can bind *in vitro* to transmembrane receptors, which have previously been shown to be up-regulated in cancers and activate migration and invasion. Despite the significant amount of data on the effects of thrombin in cancer progression, there are little data of prothrombin´s effect. The aim of this study was to further examine the effects of prothrombin and thrombin in cancer cell lines.

Methods: Colon cancer cell lines (Caco2, SW480, SW620, HT29 and HCT116) were treated with prothrombin, thrombin and direct thrombin inhibitor, dabigatran, for 24h and 48h. To assess the effects of treatment on cell viability and proliferation MTT test was used, and wound healing assay was used for cell migration potential.

Results: Detected effects of treatment with prothrombin, thrombin and dabigatran varied between cell lines. Trend of lower cell viability, proliferation and migration was observed in cells treated with prothrombin in comparison to untreated controls.

Conclusion: Our results indicate that prothrombin, although considered an inactive zymogen, can exert an effect on colon cancer cells proliferation and migration *in vitro*.

Key words: prothrombin; thrombin; colorectal cancer; proliferation; migration

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Abstracts

MOLBIOS STUDENT SESSION

ANCHORING OF HYBRID TUBERCULOSIS ANTIGEN ON THE SURFACE OF *LACTIPLANTIBACILLUS PENTOSUS*

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Introduction: Many Lactic acid bacteria (LAB) are food-grade and inhabitants of the human gut. Food-grade *Lactiplantibacillus* species are tolerant to highly acidic environments, giving them the ability to survive and colonize the human gastrointestinal tract. In addition, some species interact with the human immune system and have adjuvant effects. Thus, *lactoplantibacilli* are promising candidates for the delivery of antigens to mucosal surfaces.

Methods: Two novel *Lactiplantibacillus pentosus* strains, KW1 and KW2, isolated from olives, were genetically modified to display antigen derived from *Mycobacterium tuberculosis* on their surfaces. The tuberculosis antigen was targeted to the bacterial surface using four different anchors derived from the genome of KW1 and KW2; (1) an N-terminal transmembrane anchor (NTTM) non-covalently attaching the antigen to the cell membrane, (2) a lipoprotein anchor covalently attaching the antigen to the cell membrane, (3) a LysM anchor non-covalently attaching the antigen to the cell wall, and (4) an anchor with a sortase motif (LPQTx) that leads to covalent attachment to the cell wall.

Results: The recombinant strains showed only a slight reduction in growth, except for strains harboring NTTM-anchored antigens. Western blot analysis confirmed antigen production for seven out of eight recombinant strains. Furthermore, flow cytometry analysis detected exposed antigens on the surface for all recombinants except for the KW2 LPxTG anchor. The strongest fluorescent shift was observed in *L. pentosus* KW1, especially with lipoprotein and LysM-anchored antigens.

Conclusion: The successful secretion and surface exposure of the tuberculosis antigen show that these recombinant bacteria are promising candidates for antigen delivery.

Key words: lactic acid bacteria; surface display; anchoring; tuberculosis; vaccine development

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ANTIPROLIFERATIVE AND PROAPOPTOTIC ACTIVITY OF ACETONE, ETHYLACETATE AND METHANOLIC EXTRAXTS FROM *CORNUS MAS* L. ON HCT-116 CELL LINE

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Introduction: *Cornus mas* L. (Cornaceae) is a wild or cultivated edible fruit species, shrub or small deciduous tree. Active components from fruit contribute to significant biological activity and therapeutic effects such as antioxidant, antimicrobial and antidiabetic. The aim of this study was to determinate the antiproliferative and proapoptotic activity of acetone, ethyl-acetate and methanolic extracts from *C. mas* on human colon cancer cell line HCT-116.

Methods: The cytotoxicity of the *C. mas* extracts (concentration range 0.1-500 ug/ml) was evaluated by MTT viability assay, while the type of induced cell death was determined by acridine orange/ethidium bromide double staining.

Results: The dose-response curves of HCT-116 cell viability indicate an inhibition of cell growth in all applied treatments. Among three different extracts, the ethyl-acetate (EA) extract induces significant cytotoxicity, with IC_{50} value of 85.52 ug/ml for 24 h and 29.61 ug/ml for 72 h. Followed analysis, cell staining and morphological observation under the fluorescent microscope showed that EA induces apoptosis, as a dominant type of cell death, without necrotic effects. Treatment induces some cell morphological changes characteristic for apoptosis, like a condensation and chromatin fragmentation, reduction of cell size, membrane blabbing, formation of apoptotic bodies, as well as specific fluorescent stain - green to orange fluorescence characteristic for early and late apoptosis.

Conclusion: Observed results indicate that *C. mas* is an available source of phytochemicals and deserves further examination. It can be used for design of drugs originating from nature, with properties in prevention and treatment of cancer.

Key words: Apoptosis; ethyl-acetate; HCT-116 cell line; *Cornus mas* L.; cytotoxicity

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ANALYSIS OF SPECTRAL PROPERTIES OF HELA CELLS IN DIFFERENT PHASES OF CELL CYCLE USING BRIGHT-FIELD TRANSMITTED LIGHT MICROSCOPY

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Introduction: There is a need for new microscopic observation techniques, especially without previous staining and fixation which are known to alter and damage cell properties. This will provide new kinds of data extracted directly from the filmed image. The project aims to develop a new cell visualization method by obtaining the cell spectra and linking them to the cell division phase.

Methods: Cell culture of HeLa line was cultivated and filmed in by using FUTURESCOPE, microscope developed by the Institute of Complex Systems, 2021 which can extract spectral properties from pixels. Algorithm for estimation of the visible reflectance spectra from an ordinary RGB camera: Program Spec-v2 (Institute of Complex Systems, Czech Republic) was used to reconstruct spectrum. Analyses in MATLAB (version R2021a) included calculation of distribution of clusters - groups of pixels with similar spectral properties: spatial parameter.

Results: Filmed cells were divided into several groups: Start of division, End of division, Interphase. Clusters of interest included in statistical analysis within each cell group are clusters number 2,4 (structures that were frequently repeated in measurements) and clusters number 5,7,9 (cell borders/membrane). Results for the clusters 2,4 show that there is a more noticeable difference between spatial distribution than in their spectral properties (change of location but not composition). Results for the clusters 5,7,9 show greater differences between cell groups in different stages of the cell cycle for both spectral and spatial differences.

Conclusion: It is shown that different phases in cell cycle have different spectral and spatial properties, especially in the group of clusters 5,7,9 which implicates noticeable shape changes during division. This represents a promising method for rapid distinction between cells in different cell cycle phases. However, it is necessary to provide more analyses in order to make precise connections between cell spectra and division state.

Key words: HeLa; cell division; spectrum; transmitted light-microscopy; FUTURESCOPE

MECHANISM OF ANTITUMOR ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING *FILIPENDULA ULMARIA* EXTRACTS

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Introduction: The unique physicochemical characteristics of silver nanoparticles, especially the increased surface activity area, make them a promising agent for biomedical applications. This study evaluates the potential antitumor activity of silver nanoparticles synthesized by using an aqueous extract of *Filipendula ulmaria* (FUAgNP) on chronic myeloid leukemia cell line (K562).

Methods: The cells were exposed to the treatment applied in five different concentrations (5 to 100 µg/mL), except for the test for migratory potential, where cells were treated in two concentrations (5 and 50 µg/mL), for 24 h. Antitumor effects were evaluated based on the influence of treatment on cell viability (MTT assay), oxidative stress parameters and migration potential (Transwell assay).

Results: The obtained results indicate an intense cytotoxic effect of investigated treatment on K562 cells, since FUAgNP significantly reduced the viability of K562 cells in a dose-depended manner compared to the control cells. Increased production of superoxide anions radical and nitrites was recorded after the application of nanoparticles, especially in cells exposed to higher concentrations (50 and 100 µg/mL). The concentration of reduced glutathione was also increased after treatment with FUAgNP compared to the control cells. Additionally, the migratory potential of K562 cells was significantly reduced.

Conclusion: Based on the demonstrated antitumor effect, confirmed by reduced viability and migratory potential of the tested cells, as well as disrupted redox stability of cells, these nanoparticles have potential for further research in order to improve the effectiveness of antitumor therapy.

Key words: Myeloid leukemia; silver nanoparticles; *Filipendula ulmaria*; oxidative stress; cell viability; migratory potential

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AGE-RELATED DIFFERENCES IN ANTIOXIDATIVE CAPACITY OF SUMMER AND WINTER HONEYBEES (*APIS MELLIFERA* L.)

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Introduction: According to the oxidative stress theory, aging is a consequence of oxidative damage accumulation that increases the risk of morbidity and mortality in an organism. Measurement of antioxidative capacity is commonly used for evaluation of the level and potential of oxidative stress in the aging process. Honeybees are often used as model organisms in aging studies due to their phenotypic plasticity regarding lifespan. The aim of this study was to measure the antioxidative capacity of summer honeybees, which have a short lifespan of only one month, and winter honeybees, which live up to six months. In addition to antioxidative capacity, we measured levels of reduced glutathione (GSH), and protein thiol groups (-SH) in both short- and long-living honeybees during their whole life.

Methods: Immediately after emerging, honeybees from three hives were marked and returned to hives. In summer, marked bees were collected every week during 5 weeks, and in winter, monthly during 4 months. In homogenates (10% w/v) of abdomen and head of honeybees were measured antioxidative capacity (FRAP assay) and GSH/–SH levels (Ellman's assay).

Results: The results showed that antioxidative capacity in abdomen and head decreased with ageing over both seasons. In abdomen, levels of GSH and –SH were lower in winter, when there was a trend of decrease with aging, but in head, no age- or season-related changes were measured for either parameter.

Conclusion: Because antioxidant capacity declines with age, increasing antioxidant capacity in honeybees, whether by diet or care, may benefit their health and longevity.

Key words: aging; season; FRAP assay; reduced glutathione; protein thiol groups

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SEASONAL AND AGE-RELATED VARIATIONS OF ACETYLCHOLINESTERASE AND GLUTATHIONE *S*-TRANSFERASE ACTIVITIES IN HONEY BEE (*APIS MELLIFERA* L.)

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Introduction: The honey bee (*Apis mellifera* L.) is an eusocial insect and the most important pollinator of many wild and cultivated plants. In temperate regions, bees present from autumn to early spring are called winter bees (long-lived), while bees present during the rest of the year are summer bees (short-lived). Summer bees are characterised by intensive foraging behaviour, while winter bees are less active and stay mainly in the hive during the cold winter months. The aim of the present study was to investigate the activity of two enzymes commonly used as biomarkers of exposure to environmental stressors, acetylcholinesterase (AChE) and glutathione *S*-transferase (GST), in summer and winter honey bees.

Methods: The newly hatched bees were marked on the thorax and returned to the hives. Bees were collected weekly during 5 weeks (summer bees) and monthly during 4 months (winter bees). Enzyme activities were measured in the homogenate (10% w/v) of abdomen of honey bees by spectrophotometric methods.

Results: AChE and GST activities were generally lower in winter than in summer. In summer bees, enzyme activities fluctuated during the lifespan, while in winter bees, activities of both enzymes were lower in winter months than in newly hatched bees.

Conclusion: The enzymes AChE and GST show seasonal differences, and the lower activity in winter bees is likely due to less exposure to environmental pollutants during overwintering. The fluctuations of AChE and GST in summer bees reflect a more dynamic life compared to winter bees and more exposure to environmental pollutants.

Key words: overwintering; enzyme activity; pollinators

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UTILIZING METABOLITES FROM *CURCUMA LONGA* FOR THE DEVELOPMENT OF PH-RESPONSIVE TEST STRIPS

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Introduction: Metabolites from *Curcuma longa* show pH-dependent color-changing properties. During this study, test strips were developed using *Curcuma longa* metabolites, which enable the rapid estimation of acidity/alkalinity in natural and artificial samples.

Methods: Commercially available *Curcuma longa* powder (5.00 g) was mixed with ethanol (45 mL) and subjected to 30 minutes of ultrasonic extraction. After 60 minutes of settling, the resulting suspension was filtered and supplemented with ethanol to reach a final volume of 50 mL. Circular pieces of filter paper were immersed in 15 mL of the colored filtrate in Petri dishes for 10 minutes. The impregnated pieces of filter paper were then dried at 65°C for 10 minutes and cut into desired rectangular shapes.

Results: Analysis of the prepared test strips' behavior was conducted across a pH range from 0 to 14, encompassing various solutions (HCl, NaOH, and buffered solutions) whose pH values were measured by a pH meter. The test strips exhibited a yellow-orange color at pH values below 8.5, while a brown color was observed at pH values of 8.5 and above.

Conclusion: The experimental data obtained in this investigation demonstrate significant agreement with the literature value for the first pK_a of curcumin (pK_{a1}=8.4), a compound displaying the distinctive orange color found in dry *Curcuma longa* powder, and possessing pH-dependent color-changing characteristics. Therefore, test strips prepared from an ethanolic extract of *Curcuma longa* powder constitute a promising tool for the routine assessment of acidity/alkalinity across various samples in molecular biology, (bio)chemistry, pharmacy, medicine, and related fields.

Key words: *Curcuma longa*, metabolites, test strips, acidity/alkalinity estimation

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DEVELOPMENT OF A COLORIMETRIC ASSAY FOR MEASURING THE ACTIVITY OF ALDO-KETO REDUCTASE 1C4

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Introduction: Aldo-keto reductases (AKRs) catalyze NAD(P)H-dependent reactions on a wide array of endogenous and exogenous substrates containing aldo or keto functional group, aiding in their biosynthesis, metabolism, and detoxification. AKR1C4 is predominantly found in hepatic tissue where it prevents the accumulation of steroid hormones. In certain types of cancers, this enzyme is overexpressed, hindering the effects of chemotherapeutics by metabolizing them. The aim of this research was to develop an easy, affordable, and reliable method for measuring AKR1C4 enzyme activity using nitrotetrazolium blue chloride (NBT)/phenazine methosulfate (PMS) system by measuring absorbance of the formazan product.

Methods: Chemically competent *E. coli* BL21 cells were transformed with pET28b(+)-AKR1C4 construct using a heat shock procedure. Protein expression was induced by adding 0.5 mM IPTG at 25°C. His-tagged AKR1C4 was purified using immobilized metal affinity and size-exclusion chromatography. Enzyme activity was monitored by measuring absorbance of resuspended formazan crystals at 540 nm. Based on structural properties, three potential substrates were tested: estradiol, 4-chloro-1-naphtol, and dihydrotestosterone.

Results: Human recombinant AKR1C4 was purified in catalytically active form. In the presence of PMS, NBT salt reacts with NADPH, which forms in the reaction catalyzed by AKR1C4, reducing it to purple formazan. Optimal enzyme concentration in the assay was determined to be 560 µg/mL and, based on the highest absorbance, the substrate that ensured the highest sensitivity was 4-chloro-1-naphtol.

Conclusion: Colorimetric assay for measuring the activity of AKR1C4 was successfully developed and optimized. It could be used to screen and identify potential AKR1C4 inhibitors as promising therapeutics.

Key words: aldo-keto reductase; 3α-hydroxysteroid dehydrogenase; enzyme assay; cancer; tetrazolium salt

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OPTIMIZATION OF PCR AMPLIFICATION OF *ALUC4* IN EVALUATING THE METHYLATION STATUS OF THE *MGMT* PROMOTER IN GLIOBLASTOMA

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Introduction: The methylation status of the promoter region of the gene for O^6-methylguanine-DNA methyltransferase (*MGMT*) is an important prognostic and predictive marker of glioblastoma (GB). The most common method of its evaluation is quantitative methylation-specific polymerase chain reaction (qMSP), which enables the quantification of the methylation level of *MGMT* based on the difference in the amount of *MGMT* and reference gene, simultaneously amplified in qMSP reactions. Several studies have shown that the *ALUC4* sequence represents one of the most reliable reference genes when performing qMSP on GB and glioma samples. The subject of this work was the examination of the optimal conditions for PCR amplification of the *ALUC4* gene in snap-frozen GB samples and after that the determination of the efficiency of amplification (E) of the reference gene in the previously optimized conditions.

Methods: Different sets of reactions were set up to optimize the annealing temperature and the $MgCl_2$ concentration in the PCR reaction. The amplification of the *ALUC4* product was performed in a series of diluted concentrations of bisulfite-converted DNA matrix isolated from snap-frozen GB tissue.

Results: Image J analysis of PCR products resolved on agarose gel electrophoresis revealed that the optimal annealing temperature was 60˚C, and the concentration of $MgCl_2$ was 1.5 mM. The obtained Ct values were used to construct a standard curve based on which the value of the amplification efficiency of the *ALUC4* product (E≈90%) was calculated. This efficiency value confirmed the optimal *ALUC4* qMSP amplification conditions.

Conclusion: The obtained results will provide a valid basis for conducting extensive MSP experiments evaluating the methylation status of the *MGMT* promoter region in GB samples.

Key words: glioblastoma; *MGMT*; *ALUC4;* qMSP

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EFFECTS OF CONTINUOUS LIGHT AND DARK ON PINEAL PHYSIOLOGY: DISRUPTION OF CLOCK GENES AND MELATONIN SYNTHESIS

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Introduction: The pineal gland is responsible for synchronizing the body`s circadian rhythms by producing melatonin in response to changes in the light-dark cycle. Our study aimed to evaluate how growing up in constant lighting conditions affects pineal physiology by monitoring the transcription of genes critical for the rhythmic endocrine activity of the pineal gland.

Methods: To achieve this, rats were exposed to different lighting conditions from P21 to P90, which included continuous light (LL), continuous dark (DD), and a 14:10 light-dark (LD, control) schedule.

Results: both LL and DD had an impact on the rats' physiology evidenced by the change in their bimodal voluntary activity pattern into free-running one. The serum melatonin and transcription of key enzymes involved in melatonin synthesis lost rhythmic characteristics detected in control group: *Hiomt* was reduced in both conditions, while *Nat* was flattened in LL. The transcription of clock genes critical for pineal rhythmicity was disrupted in both LL and DD conditions, with LL attenuating *Bmal1*, *Per1*, *Cry2*, *Nr1d1* and reversing *Per2*, *Cry1* and *Nr1d2* rhythm while DD attenuated *Per1* and reversed *Bmal1* and *Cry2* rhythmic pattern. Moreover, the circadian pattern of mitochondrial biogenesis markers was changed, especially in LL, with the reduced amplitude and messor of *Ppargc1a* and increased *Nrf1, Tfam, Cytc* indicating a possible impairment of mitochondria.

Conclusion: Our results suggest that growing up under constant lighting regime, particularly LL, poses a challenge to the pineal by altering the transcription of genes involved in regulating pineal function and, consequently, the body`s circadian rhythms.

Key words: pineal; melatonin; clock; mitochondria

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DEXTRAN COATED CERIUM OXIDE NANOPARTICLES INDUCE PRODUCTION OF REACTIVE OXIDATIVE SPECIES AND DECREASE MIGRATION IN A375 MELANOMA AND HELA CELLS

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Introduction: Nanoparticles have been widely investigated for cancer treatment due to physical and chemical properties enabling potentially better efficacy and reduced biotoxicity compared to conventional therapies. Cerium oxide nanoparticles (CONP) are promising anti-cancer agents since their unique characteristics favor prooxidant properties under acidic and hypoxic conditions, such as in the tumor environment, and antioxidant ones under physiological conditions. This study aimed to investigate the antitumor effects of dextran-coated CONP on A375 human melanoma and HeLa cervical carcinoma cells.

Methods: Cell viability and cell death were analyzed by sulforhodamine B (SRB) assay and flow cytometry. Dichlorodihydrofluorescein diacetate (DCF-DA) assay was applied to measure ROS production, and wound scratch assay to assess cell migration. Transferrin receptor (TfR1) and p62 protein expression was detected using Western blot.

Results: Dextran-coated CONP significantly reduced cell viability with IC_{50} of 175 and 150 mg/mL for A375 and HeLa cells, respectively. Detected ROS increase ranged from 65-67% for both cell lines followed by the increase of TfR1 protein expression (28-62%). Percent of late apoptotic and/or necrotic A375 and HeLa cells was increased by 35,7% and 29,7% after treatment. Significantly increased expression of autophagy marker p62 was observed in A375 cells after treatment. The migration of treated cells was almost completely stopped.

Conclusion: CONP showed a significant antitumor effect, inhibiting A375 and HeLa cell growth and metastatic potential. The growth inhibitory effect was caused by increased ROS production, followed by cell death induction, while decreased metastatic potential resulted from stopped cell migration.

Key words: CONP; cancer; cell migration; cell death; ROS

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ARE MITOFUSINS PROMISING MOLECULAR MARKERS OF STRESS-RELATED MEN INFERTILITY?

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Introduction: Animal models mimicking stress and different lifestyles have revealed significant differences in the expression patterns of spermatozoal mitochondrial fusion markers (MFN1, MFN2, OPA1) concerning psychophysical stress. In this study, we investigated the expression profiles of transcripts/proteins for MFN1/2 and OPA1 concerning human functionality of spermatozoa with different spermiogram types.

Methods: The samples were collected from men participating in the national assisted-reproductive-technology-program who completed a questionnaire related to their lifestyle (amount of stress, diurnal habits).The study design and experimental procedures were approved by the Ethical committee. The normozoospermic (N), teratozoospermic (T), asthenoteratozoospermic (AT) and oligoasthenoteratozoospermic (OAT) spermatozoa were separated from seminal plasma and incubated with/without progesterone (an acrosome-reaction-inducer) to assess functionality. The real-time PCR and Western blot analyses were conducted.

Results: The transcriptional pattern of *MFN1*, *MFN2* and *OPA1* in spermatozoa displayed individual variations. The significant increases in the *MFN1* and *MFN2* levels were observed in AT-spermatozoa and OAT-spermatozoa, while *OPA1* increased in T-spermatozoa and OAT-spermatozoa. Although trends-of-increases in MFN1/2 proteins profiles were observed, they did not reach significance due to the limited number of samples. Some of the changes were associated with stress, as 89% of the participants reported some type of stress (low, medium, high, frequent). It is important to note that the small number of samples (4-10 individuals/group) limits the preliminary nature of these results.

Conclusion: The increased expression of mitofusion markers suggests a shift in the mitochondrial-fusion/fission-balance towards fusion, indicating their potential as new markers of male (in/sub)fertility and as a basis for potential therapeutic approaches.

Key words: men (in/sub)infertility; human spermatozoa; *MFN1/*MFN1; *OPA1*; stress

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UNRAVELING CUTTING-EDGE MOLECULAR MARKERS OF (IN/SUB)FERTILITY IN MEN: CLOCK GENES

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Introduction: The increasing number of unexplained cases of infertile men in the peak of the reproductive period, coupled with the lack of accurate tests, has prompted World-Health-Organization to call for new markers. Consequently, our study aimed to investigate the transcriptional profiles of the main clock genes in relation to spermiogram-type and spermatozoa functionality.

Methods: Spermatozoa samples were collected from men participating in the national assisted-reproductive-technology program. The experimental procedures received approval from the Ethical Committee. Spermatozoa from individuals diagnosed with normozoospermia (N), teratozoospermia (T), asthenoteratozoospermia (AT), oligoasthenoteratozoospermia (OAT) were isolated from seminal plasma and incubated with/without progesterone (an acrosome-reaction-inducer) to assess functionality. Following RNA isolation and cDNA synthesis, real-time PCR analyses were conducted.

Results: The relative expression of the clock-genes-transcripts in spermatozoa exhibited individual variations within the same spermiogram-groups. The significant decreases of the *CLOCK* transcripts levels were detected in spermatozoa from T vs. N and AT vs. N and T. In the same samples, significant increases of *CRY1/CRY2* and *PER1/PER2* were revealed. Although trends-of-change in *BMAL1* transcriptional profiles were observed, they did not reach significance due to the limited number of samples. Some of the changes in transcriptional profiles were associated with varying spermatozoa functionality in samples with the same type of spermiogram. It is important to note that the small number of samples (4-10 individuals/group) limits the preliminary nature of these results.

Conclusion: The transcriptional profile of clock genes in human spermatozoa displays individual variations and distinct patterns, indicating their potential as new markers for (in/sub)fertility in men.

Key words: men (in/sub)infertility; human spermatozoa; *CLOCK*; *CRY1/CRY2*; *PER1/PER2*.

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ANTITUMOR EFFECTS OF ETHANOL *LYCIUM RUTHENICUM* EXTRACT ON HUMAN BREAST CANCER CELL LINE MDA-MB-231

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Introduction: According to data, cancer is the leading cause of death worldwide, and breast cancer is the most common type of cancer in women. The aim of this study was to investigate the effects of six different concentrations of ethanol extract of *L. ruthenicum* (EELR), popularly referred to as Goji berries, on MDA-MB-231 cell line.

Methods: The parameters of cell proliferation and oxidative/antioxidative status were measured spectrophotometrically after short-term treatments (24h), while MTT assay was also done for long-term treatment (72h). Migration potential was tested for two concentrations after 24h exposure, using Boyden chamber transwell migration assay.

Results: The acquired results suggest that on the lowest concentrations EELR exhibited antiproliferative effects, while cell viability was increased in dose-dependent manner after 24h treatment, whereas 72h treatment showed decreased cell viability. The results showed a decrease in superoxide anion radical and glutathione levels, while nitrite concentrations were increased compared to non-treated cells. The concentrations of total glutathione were reduced in comparison to control, but still exhibited an increase in dose-dependent manner. The migration index of MDA-MB-231 cells was reduced.

Conclusion: These data indicate that EELR exerts significant antiproliferative activity and reduces migratory capacity, suggesting a potential antitumor role of this extract. It also shows a potential antioxidant role.

Key words: Goji berry; breast cancer; oxidative stress; migration

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RECOMBINANT PRODUCTION OF NATIVE Λ-EXONUCLEASE IN DIFFERENT *E. COLI* STRAINS

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Introduction: Lambda exonuclease (λ-exo), isolated from lambda bacteriophage, plays a crucial role in DNA replication, repair and recombination. The enzyme specifically hydrolases double-stranded DNA (dsDNA) in a highly processive manner in 5′→3′ direction, yielding mononucleotides and single-stranded DNA (ssDNA). This efficient unidirectional degradation makes it an invaluable tool in various molecular biology techniques, including novel sequencing technologies. Hence, optimization of the expression conditions is a prerequisite to achieving high-level production of λ-exo.

Methods: The N-terminally His-tagged λ-exo fusion construct with thrombin cleavage site (AddGene #104531) was successfully transformed into five different *E. coli* strains (BL21(AI), Shuffle T7, C41(DE3), C43(DE3), and BL21(DE3)). Expression was tested under three temperature regimes (20 ˚C, 30 ˚C, and 37 ˚C) over time for enhanced soluble production. Crude extracts were analysed by SDS-PAGE for total protein expression, soluble and insoluble cytoplasmatic fractions. The exonuclease activity of the extracts was monitored via in-house developed fluorescence-based screening assay. Optimal conditions for high-yield production were determined by densitometric analysis using NIH ImageJ software. The soluble and active enzyme was produced on the large scale in a shaking flask culture under optimal conditions, and purified to homogeneity from the soluble lysate via metal affinity chromatography.

Results: We identified *E. coli* BL21(AI), SHuffle T7, and C41(DE3) as good producers of recombinant λ-exo and determined optimal conditions (30 ˚C, 6 h post-induction) for high-yield expression. The enzyme was eluted from Ni^{2+}-IDA-Sepharose 6B column in 300 mM imidazole and maintained its activity upon purification assessed by an in-house developed fluorescence-based screening assay.

Conclusion: This study provides a scalable cost-effective approach for soluble λ-exo production in selected *E. coli* strains. This expression system would be a helpful platform for development of high-yield production of λ-exo, easing its exploitation in biotechnology and other scientific frontiers. Additionally, we provided a valuable low-cost screening assay for monitoring exonuclease activity during each purification step.

Key words: λ-exonuclease; recombinant technology; optimisation; *E. coli*

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NRF2 STABILIZATION BY METHYLGLYOXAL IMPAIRS THE CONTROL OF *MYCOBACTERIUM TUBERCULOSIS* IN MACROPHAGES

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Introduction: Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (Mtb), an intracellular bacterium that primarily targets macrophages. Mtb can not only proliferate but also be controlled within macrophages. The comorbidity with diabetes mellitus (DM) can impair the Mtb control by macrophages. It has been reported that DM can increase the risk of active TB by two to four fold. However, the mechanism behind the TB-DM association is largely unknown. Methylglyoxal (MGO), a reactive byproduct of glycolysis, is enriched in diabetic patients. MGO mediates rapid non-enzymatic glycation of proteins, lipids, and DNA to promote formation of advanced glycation end products (AGEs), which eventually renders irreversible damage to these macromolecules, including their integrity of structure and function. Kelch-like ECH Associated Protein 1 (KEAP1) which contains reactive cysteine residues that collectively act as an electrophile sensor can respond to reactive species. Covalent modification of KEAP1, an adapter protein of E3 ubiquitin ligase, results in reduced ubiquitination and the accumulation of Nuclear factor-erythroid factor 2-related factor 2 (Nrf2). Aims of this research were to examine if MGO could impair Mtb control by macrophages and if KEAP1-Nrf2 pathway could play important role in Mtb control by macrophages.

Methods: The role of Nrf2, a transcription factor which regulates oxidative/xenobiotic and inflammatory responses, was studied in Mtb-infected bone marrow-derived macrophages using RNA silencing (to knock down Nrf2 expression), qPCR, flow cytometry, and immunocytochemistry. Treatments which were tested are Nrf2 silencing, 15mM MGO and combined treatment, with both Nrf2 siRNA and MGO. These treatments were tested in both uninfected and Mtb infected cells.

Results: The expression levels of antioxidative transcripts *Nqo1* and *Gclc,* which are known to be regulated by Nrf2, were increased upon MGO treatment and partly inhibited by Nrf2 silencing. MGO treatment also attenuated the induction of proinflammatory transcript *Il1b* by Mtb infection, while Nrf2 silencing increased transcriptional level of this transcript. At protein level, both infection and MGO could stabilize Nrf2 protein and the combination could further increase Nrf2 expression. After infection with GFP-expressing Mtb, higher percentage of infected cells and increased median fluorescence intensity was observed in the MGO-treated group. The effect of MGO treatment was completely blocked by Nrf2 silencing.

Conclusion: These findings suggest that Nrf2 stabilization by MGO might explain TB-DM association and provide a potential target for host-directed therapy.

Key words: Tuberculosis; diabetes mellitus; KEAP1-Nrf2 signal pathway; MGO; macrophages

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GSTM1 NULL AND COMBINED *GSTM1/GSTT1 NULL* GENOTYPES AS GENETIC MARKERS FOR THE DEVELOPMENT OF ALCOHOLIC LIVER CIRRHOSIS IN ALCOHOLICS IN SERBIA

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INTRODUCTION: Alcoholic liver cirrhosis (ALC) is the most common cause of death due to alcohol abuse worldwide and oxidative stress (OS) is a pathogenic factor in alcohol-related liver damage. Glutathione S-transferases (GSTs) play a significant role in protecting cells and tissues from OS. Our study aimed to determine the association of *GSTT1*0* and *GSTM1*0 (null)* genotypes with ALC susceptibility.

METHODS: In total, 114 patients with ALC and 262 sex and age-matched controls were clinically examined and genetically tested. Genotyping of the two genes was performed using multiplex PCR, genotypes were determined on agarose gel and a *β-globin* gene fragment was an indicator of a successful reaction. The sample size achieved sufficient power (99.8% for *GSTM1* and 91.3% for combined *GSTM1 null/GSTT1 null*) to detect significant differences between the cohorts, but not for *GSTT1*.

RESULTS: *GSTM1 null* carriers showed a 2.96-fold increase in ALC compared with the *non-null* genotype (95%CI, 1.85-4.74; P<0.0001). Subjects with both *GSTT1- null* and *GSTM1-null* genotypes had a significant risk of ALC (OR=10.63, 95%CI=3.16–35.76, P<0.001). The frequencies of *GSTT1 null* were similar between the groups (13.2 vs.13.0%). The median daily alcohol dose was higher in *GSTM1 non-null* carriers and carriers of the two active genes (P=0.019 and P=0.024, respectively).

CONCLUSION: Although the cohorts were small for GSTT1, we showed that *GSTM1 null* and combined *GSTM1/GSTT1 null* genotypes are independent risk factors for ALC in our study group. Further larger-scale studies including different genes involved in anti-oxidative stress are needed to understand this disease better.

Key words: Oxidative stress; *GSTM1 null*; *GSTT1 null*; alcoholic liver cirrhosis

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EFFECTS OF DIFFERENT BARIATRIC SURGERY TECHNIQUES AND TYPES OF POST-OPERATIVE DIET ON THE LEVELS OF FATTY ACID AND GLUCOSE TRANSPORTERS IN RAT LIVER

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Introduction: Obesity is commonly associated with various liver diseases. Bariatric surgery is used to treat obesity and its related health risks. Our study examined effects of different bariatric surgery techniques and post-operative diet types on the hepatic levels of fatty acid transporter CD36 and glucose transporters (GLUT1 and 2).

Methods: In both male and female rats, obesity was induced by a four-week high-fat diet (HFD). Animals were then subjected to different bariatric surgeries including sleeve gastrectomy (SG), biliopancreatic diversion (BD), or sham surgery (SHAM). Afterward, a half of each experimental group resumed consuming HFD for an additional four weeks, while the other half was fed a standard chow (SC). The hepatic levels of CD36, GLUT1, and GLUT 2 were assessed by immunoblotting.

Results: Both types of bariatric surgeries reduced body weight irrespectively of the post-operative diet type. The hepatic CD36 content was not affected by the surgery type in either males or females. However, in males, a switch to SC after surgery increased the CD36 amount. Hepatic GLUT1 content in males was reduced following the switch to SC regardless of a surgery type, while in females was reduced by both types of bariatric surgeries. GLUT2 content remained unchanged in male rats, whereas in females, it was affected by the post-operative diet type.

Conclusion: Although applied bariatric surgeries resulted in weight reduction, dietary changes following surgical procedures independently and distinctively modulated liver content of major nutrient transporters in male but not in female rats.

Key words: Obesity; Sleeve gastrectomy; Biliopancreatic diversion; GLUTs, CD36

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THE ROLE OF AUTOPHAGY IN THE EXPRESSION OF PROINFLAMMATORY CYTOKINES IN THP-1 CELLS

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Introduction: The interaction between autophagy and cytokines represents one of the mechanisms that coordinate the activity of the innate and adaptive immune systems. Understanding the balance between these two processes is necessary to realize the potential of autophagy regulation in various infectious, inflammatory, and autoimmune diseases. The aim was to investigate the role of pharmacological modulation of autophagy on the expression of mRNA for the proinflammatory cytokines TNF, IL-1, and IL-6 in the monocytic cell line THP-1.

Methods: The pharmacological modulation of autophagy by bafilomycin and trehalose was determined by measuring the autophagic flux, the conversion of LC3-II after blocking its degradation, by the immunoblot method. Using the RT-qPCR, it was determined how bafilomycin and trehalose affect the expression of the pro-inflammatory cytokines TNF, IL-1, and IL-6 by modulating autophagy, by measuring the mRNA concentrations of these cytokines. Statistical analysis was performed using the GraphPad Prism program and the t-test was used.

Results: Immunoblot analysis confirmed that bafilomycin blocks autophagic flux by increasing intracellular levels of LC3-II. Trehalose increased the level of LC3-II, both in the presence and absence of bafilomycin, inducing LC3-II conversion in THP-1 cells. RT-qPCR analysis of THP-1 cells treated with trehalose showed a significant increase in expression, and in those treated with bafilomycin, a significant decrease in the expression of mRNA for the cytokines TNF, IL-1, and IL-6.

Conclusion: Based on the results obtained in this research, it can be concluded that autophagy activates the expression of pro-inflammatory cytokines by increasing the transcription of their genes.

Key words: autophagy; proinflammatory cytokines; trehalose; bafilomycin

EFFECTS OF THE COMBINED TREATMENT WITH MOSS *HYPNUM CUPRESSIFORME* EXTRACTS AND VITAMIN B COMPLEX ON SUPPRESSING INFLAMMATORY EFFECTS OF LIPOPOLYSACCHARIDE-ACTIVATED MICROGLIA CELLS

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Introduction: Inflammatory activity of microglia cells, caused by stress, may lead to cytotoxic effects on neurons. Previous studies have shown that secondary metabolites of the moss *Hypnum cupressiforme*, as well as vitamins of the B complex, have expressed anti-inflammatory and neuroprotective effects. Therefore, our aim was to investigate the combined effect of vitamin B complex (B1, B2, B3, B5, B6, and B12) and moss extract from autumn season on suppressing inflammation caused by lipopolysaccharide (LPS)-activated microglia.

Methods: The biocompatibility of vitamin B complex and moss extracts was tested on L929 fibroblasts by MTT assay. Anti-inflammatory and neuroprotective potential of vitamin B complex and moss extract were also tested, by measuring the difference in the production of nitric oxide (NO) and reactive oxygen species (ROS) by LPS-activated BV2 microglia cells and the effect of their supernatants on the metabolic activity of SH-SY5Y neurons.

Results: The combination of vitamin B complex and moss extract from autumn season did not exhibit cytotoxic effect on fibroblasts, as shown by the MTT assay. The applied treatment decreased the production of ROS and NO by LPS-activated BV2 cells, and also led to an increase in the metabolic activity of neurons treated with BV2 supernatants.

Conclusion: The combination of vitamin B complex and moss extract from autumn season have shown anti-inflammatory effect by inhibiting the production of ROS and NO, important soluble mediators of LPS-activated BV2 cells. Also, neuroprotective effect on SH-SY5Y neurons was observed.

Key words: Microglia; Neuroinflammation; Neuroprotection; Vitamin B complex; Moss *Hypnum cupressiforme*.

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TRANSCRIPTIONAL PROFILING OF *PHF19* GENE IN COLON CANCER CELL LINES CULTIVATED IN 3D

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Introduction: A recent comprehensive pan-cancer transcriptome analysis revealed differential activity of two alternative promoters of the gene *PHF19* in malignant and non-malignant gut mucosa. Transcription from the promoter which is up-regulated in colorectal cancer results in the synthesis of transcript PHF19-207. This finding indicates that transcript PHF19-207 could potentially be used as a biomarker for this disease. Our study aimed to assess the expression profile of the *PHF19* gene in colon cancer.

Methods: Immortalized colonic epithelial cell line isolated from healthy tissue (HCEC-1CT) as well as a set of colon cancer cell lines (DLD1, SW620, HCT116) were used for transcriptional profiling of *PHF19* in cells cultivated in 3D. The transcriptional profile was obtained using RNA sequencing and the function of transcript PHF19-207 was evaluated using *in silico* tools.

Results: Our analysis confirmed the up-regulation of transcript PHF19-207 in all malignant cell cultures in comparison to the healthy cell line HCEC-1CT. The expression of transcript PHF19-207 was more notable in cell lines that originated from colon cancer in later stages. Coding Potential Calculator tool classifies this transcript as non-coding, with a probability of 0.2. Annolnc tool shows the up-regulation of this transcript in colorectal cancer cell lines and its down-regulation in healthy samples. Also, this tool predicts that transcript PHF19-207 localizes in the nucleus.

Conclusion: We conclude that transcript PHF19-207 could serve as a biomarker for colorectal cancer. Also, we hypothesize that this transcript is a lncRNA with a role in gene expression regulation and could be linked to oncogenesis.

Key words: PHF19; colorectal cancer; transcript; biomarker

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PROFILING DNA REPAIR IN THE SWITCH REGION TO PREDICT THE EFFICIENCY OF DNA REPAIR IN INDIVIDUALS

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Introduction: Upon activation, B cells can undergo class switch recombination (CSR) to change antibody class and function. CSR requires formation and repair of DNA double-strand breaks (DSBs) within B cell switch regions. These CSR prerequisites were exploited to set up a pipeline for CSR scar pattern assessment in individual DNA-repair studies. Here, we apply this approach aiming to evaluate human DNA-repair response in the absence of DNA-repair proteins and to understand B cells' ability to handle DNA-damaging irradiation.

Methods: Naïve B cells isolated from the blood of healthy human donors were subjected to siRNA-mediated gene silencing or exposed to different dosages of ionizing radiation before *in vitro* activation. DNA-repair proteins like ATM serine/threonine kinase (ATM) were targeted with siRNA delivered via electroporation-based nucleofection. The percentage of class-switched cells served as a readout for efficient DNA repair.

Results: The optimization of the nucleofection protocol for primary naïve B cells using the EH-115 program of the Amaxa4D Nucleofector and 2×10^6 cells resulted in cell viability of up to 60% post-nucleofection. siRNA-mediated *ATM* silencing in naïve B cells showed reduced levels of ATM, as detected by western blot. The switching efficacy of naïve B cells irradiated with 0.3, 1, and 3 Gray was inversely proportional to the dosage level.

Conclusion: The presented models unveil the fragility of primary B cells to genetic manipulation and underline the connection between impaired DNA repair and aberrant CSR. The present work provides a starting point to study the impact of DNA-repair malfunction and overloaded random genomic DSBs on normal DNA-repair function.

Keywords: ATM, class switch recombination, DNA repair, irradiation, siRNA

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INVOLVEMENT OF MATRIX METALLOPROTEINASES AND THEIR TISSUE IN PATHOGNESIS OF APICAL PERIODONTITIS

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Introduction: Chronic apical periodontitis (AP) is an inflammatory process that affects the periapical region of tooth with necrotic pulp. It is a consequence of the propagation of the inflammatory process from the infected canals of the teeth to the periapical tissue. As a result, the alveolar bone resorption occurs, which is caused by numerous inflammatory mediators. Disorder in balance between osteoclasts and osteoblasts have an immunopathologic implication connected with reduction or increase of bone mineral matrix density. This process is based on controlling factors osteoprogeterin (*OPG*) and Receptor activator of nuclear factor kappa-beta ligand (*RANKL*), known as osteoprogeterin ligand.

Methods: The study group consisted of 60 AP lesions harvested from 60 adult voluntary participants (33 females, 27 males, with an average age of 56.1 years) in conjunction with apicoectomy. Twenty pulp tissues of intact teeth extracted due to orthodontic reasons, collected from 30 voluntary individuals (17 females, 13 males, with an average age of 24.3 years), served as healthy control (HC). The relative expression level of the investigated transcripts (*RANKL* and *OPG*) in all tissue samples was analyzed using RT-qPCR. Hi-square and Mann-Whitney U test were used for statistical analysis.

Results: There was no significant difference in gene expression between gender groups. Patients of experimental group were significantly older compared to control group (P=0.001). Level of relative gene expression of *RANKL* and *OPG* was significantly higher in AP compared to HC (P=0.001, P=0.001).

Conclusion: These findings suggest that RANKL and OPG are involved in pathogenesis of AP.

Key words: apical periodontitis; pulp; RANKL; OPG; gene expression

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LIVER REGENERATION AND THE USE OF ORGANOIDS IN REGENERATIVE BIOLOGY

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Liver disease is a leading cause of death worldwide with increasing morbidity and mortality and limited therapeutic options. The only curative treatment for end-stage liver disease is liver transplantation, restricted by shortage of available organs, graft rejection and failure. One of the most common causes for graft loss after liver transplantation is biliary damage. There is an urgent clinical need for treatments which could prevent or repair bile ducts, increasing the availability of organs for transplantation and reducing the ever-increasing demand for organ transplantation.

Regenerative biology could address this pressing need by using cells or artificial tissue grown in the lab to regenerate or replace damaged ducts, and the Sampaziotis lab has shown proof-of-principle for the feasibility of this approach. The lab developed a method to grow primary biliary epithelial cells (cholangiocytes) as organoids, which resemble their native counterparts and maintain genetic stability in culture. These characteristics make cholangiocyte organoids a valuable tool for investigating the molecular and cellular events involved in liver regeneration, as well as for cell-based therapy and tissue engineering. The group transplanted cholangiocyte organoids in human livers perfused ex-situ and repaired damaged bile ducts. These results show great promise for the application of regenerative biology in hepatobiliary disorders; however, some challenges remain prior to tangible clinical translation of this technology.

Key words: liver regeneration, cholangiocytes, organoids

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PROJECT CORNER

Strengthening regional stem cells based research for advancement of multi modal innovative strategy for modelling neurodevelopmental disorders – STREAMLINE

Neurodevelopmental disorders (NDDs) are a group of complex and heterogeneous disorders that give rise to the psychiatric conditions such as autism spectrum disorders, intellectual disability, schizophrenia and bipolar disorder. These disorders are caused by alterations in early brain development affecting cognitive, social and motor abilities of patients. STREAMLINE aims to develop Institute of Molecular Genetics and Genetic Engineering (IMGGE) as a high capacity hub for research of NDDs in the Western Balkans by twinning IMGGE with three top-class research institutions in Europe: Cardiff University, University of Maastricht and Centre for Research and Technology.
The project objectives are: a) to enhance the current and scale up the overall strategic networking activities between the IMGGE and internationally-leading European partners and regional partners; b) to raise the research profile of research stuff and scientific attractiveness of IMGGE in the field of NDDs; c)to strengthen research management capacities and administrative skills of the IMGGE staff and d) to become a regional leader in innovations tackling NDDs.
Funded by European Union, under the Horizon Europe programme Widening Participation and Spreading Excellence, Grant Agreement number: 101060201

Pharmacogenomics Hub in a strengthened IMGGE – PharmGenHUB

Pharmacogenomics (PGX) aims to individualize therapy upon patients' unique DNA profiles. IMGGE (Institute of Molecular Genetics and Genetic Engineering, University of Belgrade) is a pioneer in the PGX in the Western Balkans (WB). Through PharmGenHUB project IMGGE will become WB central place for PGX diagnostics and R&I, education and translation of PGX knowledge into clinically applicable digital solutions.
PharmGenHUB scientific goals are:
1. To design PGX-WB panel containing specific PGX markers relevant for WB region
2. To develop ePGA-WB (electronic pharmacogenomics assistant for WB) as a tool for personalized treatment recommendations
3. To discovery novel PGX markers relevant for WB

Funded by European Union, under the Horizon Europe programme Widening Participation and Spreading Excellence, Grant Agreement number: 101059870

Zebrafish ankrd1a as a common player in heart regeneration and skeletal muscle repair - a new prospect for unlocking regenerative potential of human heart – ZEBARR

Heart diseases are the leading cause of death worldwide due to incapability of the injured human heart to regenerate. In contrast to humans, zebrafish has remarkable ability to regenerate injured heart, while both humans and zebrafish efficiently repair the wounded skeletal muscle. This implies that the key for unlocking endogenous regenerative potential of human heart may be hidden in regeneration-competent skeletal muscle. We use this novel approach to identify targets that might contribute to restoration of impaired cardiac function by promotion of myocardial regeneration. ZEBARR will expand the understanding of cellular and molecular mechanisms involved in heart regeneration and skeletal muscle repair.

Funded by the Science Fund of the Republic of Serbia, program IDEAS, Grant No. 7739807

Modulation of gut ILC3 by a FFAR2 agonist for the treatment of autoimmune diseases – GUTtoAID

The steadily increasing number of individuals affected by autoimmune diseases in modern societies mandates the study of the etiopathology and therapy of such diseases. Two organ-specific autoimmune diseases are investigated in this project: type 1 diabetes (T1D) and multiple sclerosis (MS), in which the pancreas and the central nervous system (CNS), respectively, are targeted by autoimmunity. The aim of this project is to explore a possibility to potentiate immunoregulatory properties of gut type 3 innate lymphoid cells (ILC3) through the engagement of free fatty acid receptor 2 (FFAR2) towards prevention and/or therapy of T1D and MS.

Funded by the Science Fund of the Republic of Serbia, Program IDEAS, Grant No. 7742898

Understanding repeat expansion dynamics and phenotype variability in myotonic dystrophy type 1 through human studies, nanopore sequencing and cell models – READ-DM1

Myotonic dystrophy type 1 (DM1) is an incurable disease with the most variable clinical presentation among monogenic conditions. It is caused by a repeat expansion in the DMPK gene that is inherently unstable throughout the patient's life leading to disease progression. One of the main barriers to understanding individual patient variability, predicting disease course and developing new therapies is limited knowledge about DM1 expansion instability. READ-DM1 studies genetic and epigenetic factors influencing DM1 mutation instability. We use nanopore sequencing to explore DM1 mutation in patients and genome engineering to design cell models for studying DM1 mutation instability. READ-DM1 will pave the way toward a personalized approach and standardized genetic testing for DM1.

Funded by the Science Fund of the Republic of Serbia, program IDEAS, Grant No. 7754217

Identification and functional characterization of extracellular and intracellular genetic regulators of ferroptosis related processes in multiple sclerosis - FerroReg

FerroReg will provide new knowledge on intracellular (mRNA, miRNome/miRNA, SNPs) and extracellular (exosomes) transcriptional and post-transcriptional regulation with functional confirmation and association with metabolic indicators of iron dependent cell death-ferroptosis. Multiple sclerosis, a chronic inflammatory and neurodegenerative disease with no current cure, in its etiology comprehend: increased susceptibility of CNS to oxidative damage, and mitochondrial dysfunction, impaired iron metabolism, which all lead to accumulation of lipid peroxidation products, a main driving force for ferroptosis. Project integrates genomics, bioinformatics, biochemistry humans research, using next-generation sequencing, gas chromatography, qPCR, cell culture to provide most accurate multilevel molecular data (proteins, PUFAs, metabolic products).

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