CoMBoS

1st Congress of Molecular Biologists of Serbia

KNJIGA SAŽETAKA
BOOK OF ABSTRACTS

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1st Congress of Molecular Biologists of Serbia

POZDRAVNO PISMO ORGANIZATORA / *WELCOME ADDRESS*

Drage kolege,

Srpsko društvo za molekularnu biologiju (MolBioS) želi vam dobrodošlicu na Prvi kongres molekularnih biologa Srbije (CoMBoS). Program kongresa uključuje širok spektar fascinantnih tema iz molekularne biologije i srodnih oblasti i posvećen je stimulisanju radoznalosti, komunikacije i saradnje, posebno među mladim istraživačima. Nadamo se da će CoMBoS doprineti ostvarenju našeg cilja da molekularnim biolozima i naučnicima iz srodnih oblasti pružimo priliku da razmene ideje i budu inspirisani intrigantnim predavanjima renomiranih naučnika i priznatih eksperata iz 24 zemalje (Austrije, Bosne i Hercegovine, Crne Gore, Danske, Francuske, Grčke, Holandije, Hrvatske, Irske, Italije, Kanade, Makedonije, Nemačke, Poljske, Rumunije, Rusije, Sjedinjenih Američkih Država, Slovenije, Španije, Švajcarske, Švedske, Turske, Velike Britanije i Srbije).

Prof. Gordana Matić
Predsednik Srpskog društva za molekularnu biologiju
President of the Serbian Society for Molecular Biology

Prof. Goran Brajušković
Predsednik Organizacionog odbora CoMBoS 2017
Chair of the CoMBoS 2017 Organizing Committee

Dear colleagues,

The Serbian Society for Molecular Biology (MolBioS) warmly welcomes you to Belgrade, Serbia, for the First Congress of Molecular Biologists of Serbia with international participation (CoMBoS). The programme covers a wide spectrum of fascinating contemporary topics in molecular biology and related fields, and is dedicated to fostering curiosity, communication and collaboration, especially among young researchers. We hope that CoMBoS will contribute in fulfilling our aim of creating an opportunity for molecular biologists and related scientists to exchange ideas and get inspired by state-of-the-art lectures of prominent scientists and acknowledged experts from 24 countries (Austria, Bosnia and Herzegovina, Canada, Croatia, Denmark, France, Germany, Great Britain, Greece, Ireland, Italy, Macedonia, Montenegro, The Netherlands, Poland, Romania, Russia, Slovenia, Spain, Sweeden, Switzerland, Turkey, USA and Serbia).

The first CoMBoS is devoted to Academician Dušan Kanazir (1921-2010)

MolBioS is committed to preserving the memory of the great Serbian scientists who paved the way for the fruitful research and education in molecular biology in Serbia. Hence, the first CoMBoS is devoted to Academician Dušan Kanazir (1921-2010), an eminent scientist who played an essential role in the foundation of the Department of Biochemistry and Molecular Biology and the study program Molecular Biology and Physiology at the Faculty of Science, University of Belgrade, in 1972. This study quickly become attractive and acquired distinction, and has served as an incubator for many talented scientists who conduct cutting edge research in modern biology, confirming the efforts of this great visionary. In Memoriam to Academician Dušan Kanazir, warmly written by Prof. Ljubiša Topisirović and originaly published in the Archives of Biological Sciences (2010), is presented in the Special Issue of Biologia Serbica dedicated to CoMBoS.

MolBioS Award

The Serbian Society for Molecular Biology (MolBioS) has established its own award in recognition of the achievements of individuals in the field of molecular biology and their contributions to its development and promotion in Serbia. The MolBioS Award is envisioned for an active scientist and will be presented at every subsequent Congress of Molecular Biologists of Serbia, with a closing lecture given by the winner.

The Award Committee, formed by the Steering Committee of the MolBioS unanimously agreed to present the first MolBioS Award to **Dr. Gordana Matić**, the distinguished professor of Biochemistry and Molecular Biology whose research has focused on steroid biology, with a particular interest in many facets of the glucocorticoid receptor in health and disease. An inspired article about Prof. Gordana Matić, written by her close collaborators Dr. Ana Đorđević and Prof. Goran Brajušković, is presented in the Special Issue of Biologia Serbica dedicated to CoMBoS.

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Session MOLECULAR BIOLOGY OF MICROORGANISMS

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CONGRESS PLENARY LECTURES

part one

AUTOPHAGY IN MAMMALIAN SYSTEMS: FROM MECHANISMS TO APPLICATIONS

Vojo Deretić (SAD / *USA*)

Vojo Deretić, PhD
Department of Molecular Genetics and Microbiology
School of Medicine
University of New Mexico
Albuquerque, NM, USA

Research Interests:

Dr. Deretić's main contributions to science come from studies by his team on the role of autophagy in infection and immunity. Autophagy, a cytoplasmic pathway for the removal of damaged or surplus organelles, has been previously implicated in cancer, neurodegeneration, development, and aging. Dr. Deretić's group is one of those that made the discovery that autophagic degradation is a major effector and aregulator of innate and adaptive immunity mechanisms for direct elimination of intracellular microbes (such as *Mycobacterium tuberculosis* and HIV). His current work is on the role of autophagy in immunity and inflammation, interactions with lipid metabolism, and fundamental mechanisms of how selective autophagy is regulated in mammalian cells. Dr. Deretić is Chair of the Department of Molecular Genetics and Microbiology and Professor of Molecular Genetics and Microbiology, Cell Biology & Physiology, and Neurology at the University of New Mexico School of Medicine. He received his undergraduate, graduate and postdoctoral education in Belgrade, Paris, and Chicago. He was a faculty member at the University of Texas, University of Michigan, and joined the Department of Molecular Genetics and Microbiology, University of New Mexico, in 2001.

Source web site: http://mgm.unm.edu/Faculty/Deretic.html

CELLULAR PLASTICITY AND CANCER

Lynne-Marie Postovit (Kanada / *Canada*)

Lynne-Marie Postovit, PhD
Assistant Professor
Department of Oncology
Schulich Shool of Medicine and Dentistry
University of Alberta
Alberta, Canada

Research Interests:

Bidirectional communication between cells and their microenvironment is a hallmark of both cancer progression and embryological development. Indeed, in all physiological instances, cells do not survive autonomously, but rather rely on extracellular cues to direct functions as diverse as proliferation, apoptosis, invasion and differentiation. The past decade has seen an explosion of research on cells with the capacity to differentiate in response to specific microenvironmental cues. During embryogenesis, these "stem cells" are the source of all cell lineages and in adulthood they function in tissue repair and rejuvenation. Recent studies have found that cancers may similarly develop from stem cell populations, and that these rarely occuring cells care likely responsible for tumour formation, drug resistance and metastasis. The unifying goal of our research program is to determine what types of microenvironments regulate normal and cancer stem cell plasticity and function, and to elucidate the mechanisms by which such microenvironments elicit their effects. Ultimately, these studies will lead to the development of methods to maintain normal stem cell pluripotency and to inhibit cancer cell plasticity and metastasis. This research program is comprised of the following projects: Role of oxygen as a regulator of tumour cell plasticity and metastatic potential; Role of embryonic microenvironments in the regulation of stem cell life; and Microenvironmental regulation of placental development at the feto-maternal interface.

Source web site:
http://www.schulich.uwo.ca/anatomy/people/bios/faculty/postovit_lynne-marie.html

EFFICIENT TARGETED DNA METHYLATION
WITH CHIMERIC dCas9–Dnmt3a–Dnmt3L METHYLTRANSFERASE

Tomasz Jurkowski (Nemačka / *Germany*)

Tomasz Jurkowski, PhD
Junior Professor
Institut für Biochemie
Universität Stuttgart
Stuttgart, Deutschland

Research Interests:

Dr. Jurkowski is junior professor in the Institute for Biochemistry, University of Stuttgart, Germany. With his biochemical background, he significantly contributes in illuminating mechanistic basis of the DNA methylation/demethylation. DNA methylation plays an important role in epigenetic signalling, having an impact on gene regulation, chromatin structure, development and disease. Dr. Jurkowski was working on revealing structures and functions of the mammalian DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b, including their domain structures, catalytic mechanisms, localization, regulation, post-translational modifications and interaction with chromatin and other proteins, summarizing data obtained in genetic, cell biology and enzymatic studies. His research also includes the reverse direction, burning off DNA methylation, bringing new evidence for oxygen-dependent DNA demethylation. Dr. Jurkowski is interested in TET family of enzymes unveiling their role in DNA demethylation. Recently, his team disclosed an unexpected link between oxidative-stress-induced hydroxymethylation pattern changes, a set of microRNAs, and oxidative-stress-related genes. Main research focus of his team is to develop epigenome editing tools (based on CRISPR/Cas9 system) that rely on a sequence specific delivery of chromatin editors to genomic target loci, thus forcing a change of the epigenetic state in the selected region. New technological developments will likely increase the specificity of targeting devices and the efficiency of effector domains in setting the desired epigenetic marks and will supply engineered systems for spreading the modification across the whole locus, providing efficient and reliable tools for stable modification of the epigenome. Final aim is to find the most appropriate delivery of programmable epigenetic editors for epigenetic therapy.

Source web site: http://www.ibc.uni-stuttgart.de/mitarbeiter/4a44aaa7-4752-11e1-be73-000e0c3db68b/

Session
MOLECULAR BIOLOGY OF EUKARYOTES

Plenary lectures

THE ROLE OF ClpXP PROTEASE IN THE REGULATION OF MITOCHONDRIAL HOMEOSTASIS

Aleksandra Trifunović (Nemačka / *Germany*)

Aleksandra Trifunović, PhD
Professor
Institute for Genetics
University of Cologne CECAD - Cluster of Excellence
Cologne, Germany

Research Interests:

Prof. Dr. Aleksandra Trifunović and her research group are investigating mitochondrial stress responses and the cell's corresponding adaptive reactions. By deciphering the signaling cascades, they are seeking to understand the pathomechanisms of mitochondrial diseases and develop new therapeutic approaches. The group has recently started collaborating with Khondrion, one of the first European companies aiming to develop drugs to treat mitochondrial diseases. Prof. Dr. Trifunović's group will focus on evaluating the therapeutic effects in model organisms. The team led by Prof. Aleksandra Trifunović is investigating the function of mitochondria in the development of disease and during aging. When mitochondria experience stress or when dysfunction occurs, they send signals to the cell nucleus, which launches different types of adaptive cell responses. Transcription factors are activated and stimulate the expression of specific sets of genes, whose products enable the cell to adapt to the changes. The scientists aim to understand this signaling cascade in detail.

Source web site: http://cecad.uni-koeln.de/Dr-Aleksandra-Trifunovic.110.0.html

FROM NUCLEOHISTONE RECONSTRUCTION TO RETRANSPOSONS: A RETROSPECTIVE JOURNEY *(1977-2017)*

Octavian Popescu (Rumunija / *Romania*)

Academician Octavian Popescu, PhD
Professor
Babes-Bolyai University
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Research Interests:

Human erythrocyte membrane proteins. Production of heterologous proteins in E. coli. Proteoglycan mediated cell recognition and adhesion in marine sponges. Identification and characterization of glyconectins, a new class of cell adhesion molecules. Atomic force microscopy and cell adhesion. Cloning and expression of different genes from eukaryotic or prokaryotic microorganisms (enolase and glucose 6-phosphate isomerase genes from T. gondii; genes for dehydrogenases from E. coli, B. subtilis, B. stearothermophilus and Synechocystis sp.; genes for phosphoketolase from Pseudomonas aeruginosa and Synechocystis sp.). Human genotyping. Molecular taxonomy and phylogeny.

Source web site: http://clinicalpsychology.psiedu.ubbcluj.ro/en/staff/aa/

Session
MOLECULAR BIOLOGY OF EUKARYOTES

Invited lectures

SOX2 GENE – MASTER REGULATOR OF NUMEROUS CELLULAR PROCESSES

Milena Stevanović[1,2,3], Danijela Drakulić[1], Marija Švirtlih[1], Danijela Stanisavljević[1], Vladanka Vuković[1], Marija Mojsin[1] and Andrijana Klajn[1]

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The SOX (Sry-related HMG box) proteins comprise a group of transcription factors that act as key regulators of diverse developmental and physiological processes, ranging from blastocyst and germ layer formation to differentiation into adult tissues and organs. SOX proteins influence survival and proliferation, as well as cell fate decisions and consecutive lineage progression. Accordingly, SOX proteins are involved in multiple events, from maintenance of stem cells pluripotency, to driving their terminal differentiation into specialized cell types. The SOX2 transcription factor is pivotal for early development and the maintenance of undifferentiated embryonic stem cells (ESCs). This transcription factor plays a critical role in directing the differentiation to neural progenitors and in maintaining the properties of neural progenitor stem cells. It is a crucial transcription factor capable of reprogramming differentiated cells and reversing the epigenetic configuration of differentiated cells back to a pluripotent embryonic state. SOX2 has been found to be an immunogenic antigen in several types of cancers, and its overexpression has been reported in several types of solid tumors. Accumulating evidence suggests that SOX2 acts as an oncogene and recent evidence points toward pro-proliferative, prosurvival and/or antidifferentiation roles of the SOX2 protein. Given the crucial role of SOX2 in cell proliferation and/or antidifferentiation and its ability to endow cells with stemness potential, studying the effects of modulation of its expression has additional significance. Accordingly, we manipulated the level of *SOX2* gene expression and generated cell clones that stably overexpress *SOX2*. We have studied the effects of SOX2 overexpression and present some of our recent findings that have highlighted the important roles of SOX2 in the maintenance of pluripotency, proliferation, neural differentiation and in the regulation of the migration capacity of cells. This review also presents our findings related to the interaction and crosstalk between the *SOX2* gene and the Wnt signaling pathway.

Acknowledgements: The presented work is supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. 173051) and by the Serbian Academy of Sciences and Arts (Grant No. F 24).

THERAPEUTIC GENOME METHYLATION FOR CELL REPROGRAMMING EDITING: USE OF EPI-CRISPR-INDUCED TARGETED DNA

Melita Vidaković[1], Marija Sinadinović[1], Jelena Arambašić Jovanović[1], Anja Tolić[1], Miloš Đorđević[1], Mirjana Mihailović[1], Nevena Grdović[1], Aleksandra Uskoković[1], Jovana Rajić[1], Goran Poznanović[1], Svetlana Dinić[1], Tomasz P Jurkowski[2]

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Introduction and aim: Diabetes is the perfect candidate for cell replacement therapy since it is caused by either an absolute (type 1 diabetes) or relative (type 2 diabetes) defect of insulin-producing pancreatic beta cells (b-cells). We focused on applying a novel synthetic epigenetic tool (Epi-CRISPRs) for a straightforward, one-step transdifferentiation of mouse pancreatic alpha (a-cells) to b-cell by targeted DNA methylation and suppression of genes essential for maintaining pancreatic cell identity (homeobox Arx gene (*Arx*)).
Methods: The a-cells were transiently transfected with four different Epi-CRISPR constructs and co-transfected with a single guided RNA (gRNA) or with a mix of different gRNAs all targeting different promoter regions of *Arx*. After 5, 8 and 12 days post-transfection, DNA and RNA were isolated and the cells were immunostained. The transdifferentiated cells were analysed for key features of bona fide cells, using qPCR to assess *Arx* expression, and immunostaining of insulin/glucagon and ELISA for measuring secreted insulin.
Results: We succeeded to transiently transfect a-cells with Epi-CRISPR constructs and 275 gRNA/mix gRNA. The suppression of *Arx* in a-cells was confirmed on days 5 and 8 post-transfection. The reduction of glucagon synthesis and beginning of insulin production in transfected a-cell was confirmed and visualised by immunostaining. Whether DNA methylation-mediated suppression of *Arx* in a-cells lead to their transdifferentiation to insulin-producing cells, will be confirmed by bisulfite sequencing.
Conclusion: We are on the right course of developing a clear-cut technology capable of providing a perfect delivery system for increasing the number of insulin-producing cells *in vitro*.

Acknowledgements: This study was supported by a research grant from AstraZeneca within the European Foundation for the Study of Diabetes (EFSD): European Diabetes Research Programme in Cellular Plasticity Underlying the Pathophysiology of Type 2 Diabetes and by Grant No. 173020 from the Ministry of Education, Science and Technological Development, Republic of Serbia. The authors, MV and TPJ would like to acknowledge networking and STSM Grant support by COST Action CM1406.

THE ORIGIN AND HISTORICAL ROUTE OF MYOTONIC DYSTROPHY TYPE 2 MUTATION ACROSS EUROPE

Dušanka Savić-Pavićević[1], Jovan Pešović[1], Miloš Brkušanin[1], Stojan Perić[2], Jan Radvansky[3], Srđan Maširević[1], Vlado Kovčić[1], Zuzana Musova[4], Kristýna Stehlikova[5], Lea Leonardis[6], Kyriaki Kekou[7], Vladimir Jovanović[8], Radim Mayanec[9], Laura P Ranum[10], Goran Brajušković[1] & Vidosava Rakočević Stojanović[2]

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Introduction: Myotonic dystrophy type 2 (DM2) is predominantly a disease of the European Caucasians. Haplotype analyses have suggested a founder mutation ~4000-11000 years old. This period coincides with the Neolithic Age, when the Near Eastern farmers and later herders from the Pontic-Caspian steppe had settled in Europe, shaping the genomic architecture of the Europeans. We aimed to estimate DM2 mutation age more precisely and reconstruct its historical route.

Methods: *CL3N122*, *CL3N99*, *CL3N59*, *CL3N119*, *CL3N19* and *CL3N23* loci were genotyped in 413 individuals from Serbian, Greek, Slovenian, Slovakian and Czech DM2 families. 378 healthy and 70 DM2 haplotypes phased by family segregation analysis, and 55 DM2 published German haplotypes were used for the coalescent modeling of intra-allelic variability in DMLE+. The maximum likelihood estimation (MLE) of the mutation age was determined for the population growth rate set at 0.025-0.045, assuming DM2 mutation frequency in Finland (1/1830).

Results: The estimated DM2 mutation age is 200-280 generations (~4000–5600 years assuming 20 years/generation). It dates back in the Late Neolith and the early Bronze Age when massive migrations of Yamnaya individuals from the Pontic-Caspian steppe to Europe occurred (3500-2200 BCE), accompanied by an expansion of the Corded Ware individuals (2800-2200 BCE), who were genetically the most similar to the Yamnaya ones.

Conclusion: Presented result brings a novel insight in the origin and historical route of DM2 mutation across Europe. According to epidemiological data, the distribution of DM2 mutation seems to reflect a decreasing Yamnaya ancestry from the north to the south in the present-day Europeans.

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APOPTOSIS: *PRO ET CONTRA*; NEW VIEW ON CANCER TREATMENT

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Malignant alterations, in addition to genetic abnormalities which are connected with it, represent the ontogenetic regression since differentiated cells underwent to a process toward nondifferentiated, pluripotent phenotype. In solid tumors as well as metastatic foci, presence of poorly differentiated cells coincides with the treatment failure, classifying them as high grade tumors. Until today, the reason for their insensitivity is still searching in the apoptotic resistant phenotype, without enough respect toward multicellular cooperation in tumor tissue. However, caspase mediated cell signaling triggered by the apoptosis presents a key messenger in homeostasis maintenance. One of the leading forces in multicellularity safeguarding is a compensatory proliferation described in the earliest metazoan. This evolutionary highly conserved process is present in embryogenesis, organ and tissue regeneration but in pathological conditionssuch as cancer, is tightly connected with expansion as a response to caspase mediated cell death. The promoters of this effect are stem cells in the contact with dying neighbors. It further means that spontaneous apoptosis as well as induction of death by the conventional treatment in anaplastic tumors will force there expansion. To avoid this, induction of differentiation is proposed. There are numerous examples of *in vitro* triggered tumor cell differentiation by naturally occurring compounds, drug designed for the treatment of nonmalignant diseases, or even chemotherapeutic drugs manipulating by dosage and delivering. Once differentiated, tumor cells lose their proliferative potential and became sensitive to conventional therapy. Differentiation based therapy as alternative approach for the treatment of invasive forms of solid tumors will be elaborated.

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SYMPHONY OF MOLECULAR ADAPTATION OF TESTOSTERONE-PRODUCING LEYDIG CELLS

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Introduction: Delineating the steroid-hormones-machinery and signaling alterations that occur across lifespan could possibly help us improve the quality of life. Although testosterone-producing Leydig cells (LCs) play unequivocally critical roles in homeostasis of individual and species, their specific molecular adaptation during changes in life style have yet to be determined.
Aim: To determine molecular adaptations of testosterone-producing LCs from animals exposed to different "life-styles" comparable to humans.
Methods: Four *in vivo* experimental models (biological clock, aging, insulin/IGF1 receptors knock-out, psychophysical stress) and different *in vitro* approaches (activation/inhibition of signaling pathways). Outcomes: transcripts/proteins expression; concentration of signaling molecules in circulation and LCs; mitochondrial function/architecture/biogenesis.
Results: Leydig cells showed 24-h-rhythmic expression of clock and genes of steroidogenic regulators. Melatonin influences 24-h-rhythmicity through cAMP-signaling in LCs. Circadian rhythm of the LCs endocrine function is attenuated during aging. The nitric oxide and cGMP have opposing roles in the age-associated decline of LCs steroidogenesis, while Viagra increased testosterone production by both, adult and aged LCs. InsulinR/IGF1R knock-out caused LCs "feminization". Repeated immobilization stress disturbed mitochondrial function, steroidogenic machinery and the expression of LCs-cAMP-signaling elements and adrenergic receptors. Systemic or intratesticular *in vivo* blockade of alpha1-drenergic receptors prevented some of these effects. Stress-triggered activation of PGC1-dependent mitochondrial biogenesis does not only correlate-with, but also is an essential for LCs-testosterone-production, being both events depend on the same regulators.
Conclusion: Here we propose that all events induced by light-period-change, aging, absence of insulin/IGF1 receptors and stress provoke wide-variety of molecular-adaptive-response of testosterone-producing LCs to preserve basal steroidogenesis and homeostasis.

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THE ROLE OF GLUCOCORTICOID HORMONES IN DIET-INDUCED METABOLIC DISEASES

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Excessive fructose intake promotes the development of metabolic syndrome through the deregulation of metabolic pathways in the hypothalamus, liver and adipose tissue, which play crucial roles in metabolic homeostasis by responding to the body's nutritional and energy requirements. Variable amounts and modes of fructose intake have been shown to result in different patterns of expression of metabolic disturbances, which generally include adiposity, insulin and leptin resistance, dyslipidemia and hypertension. We explored the possible mediatory role of glucocorticoid signaling on the effects of two different dietary fructose loads on hypothalamic leptin sensitivity and hepatic and adipose tissue lipid metabolism, which are responsible for the development of signs of metabolic syndrome. Experimental rats were provided with 10% and 60% fructose solutions *ad libitum* over a period of nine weeks. Our results revealed that the applied fructose had different impacts on leptin and glucocorticoid signaling and different consequences on visceral adiposity and hepatic lipid metabolism. Only rats maintained on the high-burden 60% fructose diet accumulated visceral fat through the activation of adipogenic transcription factors and adipogenesis. This was paralleled by diminished glucocorticoid signaling in the adipose tissue and the establishment of the state of hypothalamic leptin resistance. The high-burden dietary fructose triggered hepatic *de novo* lipogenesis and a concomitant inhibition of β oxidation. Consumption of 10% fructose enhanced glucocorticoid signaling and lipolysis in the adipose tissue, creating a circulatory influx of free fatty acids and providing substrates for enhanced β oxidation and triglyceride synthesis in the liver. In summary, our results show that a long-term high dietary fructose load leads to hypothalamic leptin resistance, the development of visceral adiposity and increased hepatic *de novo* lipogenesis. Glucocorticoids regulate adipocyte storage functionality and thus may indirectly contribute to the observed changes in hepatic lipid metabolism, aggravating the metabolic disturbance.

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THE AGING BRAIN – MOLECULAR AND METABOLIC CHANGES

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Aging is a complex set of events that involves the whole body. However, disruption of the central nervous system (CNS) function is the aspect of aging that elderly people worry about most. Aging has different effects on different aspects of neurological function. Our knowledge of the basic molecular mechanism of brain aging has significantly improved over the past few decades. The rate of aging is not fixed, but is plastic and subject to modifications. The environmental factor proven to be very potent in modulating aging is reduced dietary intake. Dietary restriction (DR) is a vigorous nongenetic and nonpharmacological intervention that is known to delay ageing and increase an active and healthy lifespan in diverse species, from yeast to mammals. Additionally, DR can improve various brain functions, including learning and memory, synaptic plasticity and neurogenesis.

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INTERACTION BETWEEN MITOCHONDRIAL AND NUCLEAR GENOMES: THE ROLE IN LIFE-HISTORY EVOLUTION

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The evolution of eukaryotes is based on dynamic coevolutionary interactions between the two genomes, nuclear (nDNA) and mitochondrial (mtDNA). Current evidence suggests that the origin of eukaryotes corresponds to the origin of mitochondria. The primary center of adenosine triphosphate (ATP) production through the process of oxidative phosphorylation (OXPHOS), which is based on the functioning of four large protein complexes that are responsible for the proton gradient across the inner mitochondrial membrane. These complexes in the electron transport chain (ETC) are composed of polypeptides encoded by both mitochondrial and nuclear genes. In order to preserve the uncompromised functionality of mitochondria, i.e. the adequate coupling of all interacting subunits in OXPHOS, the two genomes had to coevolve. In other words, mitonuclear compatibilities are required for optimal life-history of an organism because even minor biochemical inefficiency can have major fitness consequences by modulating energetic efficiency and oxidative stress levels. The link between life-history evolution and mitonuclear interactions is deeply rooted within the mechanisms of energy metabolism. The coevolved epistatic interactions between mitochondria and nucleus determine the amount of energy available for all biological functions. Selective optimization of one life-history function (e.g. reproduction) may come at the cost of reduced competence for somatic maintenance, viability and survival due to mutually exclusive energy allocation to distinct functions. Different approaches in investigating the central roles of mitochondrial metabolic processes and mitonuclear epistasis in life-history evolution are discussed in this paper.

Session
MOLECULAR BIOLOGY OF EUKARYOTES

Flash presentations

MITOCHONDRIA PROTEIN CARBONYLATION IN THE SHORT- AND LONG-LIVED LINES OF THE SEED BEETLE (*Acanthoscelides obtectus* Say)

Mirko Đorđević[1], Uroš Savković[1], Jelica Lazarević[1], Biljana Stojković[2],Darka Šešlija Jovanović[1], Aleksandra Trifunović[3]

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Introduction: As the main site of both energy and oxygen radical production, mitochondria plays important role in ageing. Since accumulation of carbonyl groups on protein side chains occurs as a result of oxidation, protein carbonylation is considered as a robust biochemical marker of oxidative stress. To test whether long life is related to lower level of oxidative stress we compared mitochondrial protein carbonyl content between different longevity lines of the seed beetle.
Material and Methods: We analyzed longevity and ageing dynamics of long-lived late reproduction (L) and short-lived early reproduction (E) lines. Mitochondrial carbonylated proteins from 1 day old L and E beetles were identified by derivatization of the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH), which leads to the formation of a stable 2,4-dinitrophenyl (DNP) hydrazone. The DNP adducts were then assessed by two-dimensional gel electrophoresis followed by Western blot immunoassay (Oxyblot).
Results: The level of mitochondrial carbonyl proteins was significantly higher in E than L females, which was accompanied with significantly higher baseline mortality and accelerated ageing. In contrast, E and L males revealed no differences in protein carbonylation and baseline mortality. Sexual dimorphism in the level of oxidative stress was detected only in E line where females were shown to be more sensitive than males.
Conclusion: Our results show that, selection for delayed reproduction in long-lived females is correlated with lower oxidative stress level and concomitant decrease in baseline mortality.

Acknowledgement: This study was supported by the grant no. 173007 from the Ministry of Education, Science and Technological Development, Republic of Serbia

FIFTY'S NOT TOO THRIFTY! 5' END OF MITOCHONDRIAL CYTOCHROME C SUBUNIT I YIELDS FIFTY HAPLOTYPES AND FAILS AS A BARCODE FRAGMENT FOR *Culex pipiens* MOSQUITO IDENTIFICATION (DIPTERA: CULICIDAE)

Nemanja Gojković[1], Ljubinka Francuski Marčetić[1], Bosiljka Krtinić[2], Vesna Milankov[1]

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Aims: We wanted to test the validity of the 5' end of mitochondrial cytochrome c subunit I (*MT-CO1*) as a DNA barcode fragment for *Culex pipiens pipiens* molecular identification by comparing its intraspecific variability with the interspecific differences of closely related taxa: subspecies *Cx. p. pallens* and species *Cx. quinquefasciatus*, *Cx. torrentium*, *Cx. vagans*, *Cx. australicus* and *Cx. globocoxitus*.
Methods: Ten female *Cx. p. pipiens* adults from Novi Sad (Serbia) were genotyped at the barcode fragment and along with 664 sequences from the GenBank used to define the global intraspecific variability of the species. Additionally, one sequence was retrieved from the GenBank for each of the related taxa, while the degree of genetic divergence between the haplotypes was quantified using *p* distance.
Results: In total, 50 *Cx. p. pipiens* haplotypes and five additional haplotypes belonging to the related taxa were recovered. Although the average *p* distance among the *Cx. p. pipiens* haplotypes was lower than the average *p* distance between all the taxa (0.78% and 2.64%, respectively), there were several instances where the interspecies and intraspecies variability overlapped, exhibiting the lack of a barcode gap and thus violating the criteria for successful molecular identification.
Conclusion: Despite its widespread use, 5' end of *MT-CO1* is not an adequate candidate for a barcode fragment for *Cx. p. pipiens* identification. Since the analysed mosquito species are highly important from the epidemiological standpoint, there is an increasing pressure to identify suitable genome regions for their unambiguous molecular identification which would accompany traditional approaches.

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BENCHMARKING AND OPTIMIZATION OF TOOLS FOR RNA-SEQ ANALYSIS OF LOW COVERAGE MAIZE TRANSCRIPTOME DATA

Dragana Dudić[1], Vesna Pajić[1], Ana Nikolić[2], Dragana Ignjatović-Micić[2], Nenad Delić[2], Bojana Banović[3]

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Introduction: RNA-seq data are mapped to reference genomes requiring complex computing methods that address the presence of introns, splice junctions and differential gene expression in eukaryotes. With repetitive nature of some genomes like maize's, a high number of multi-mapped reads and high duplication rate also need to be addressed. We tested different bioinformatics tools on low-coverage maize total transcriptome data, setting an optimal integrative approach.
Methods: 151bp pair-end RNA-seq low-coverage data for 6 maize inbred lines; Three splice aware mapping tools: STAR, Subread, Tophat (insert size 130, standard deviation 50, and maximum intron size 100.000) and three assembly tools: Oases, TransAbySS, Trinity (kmer sizes 15-31, minimum contig length 30) were tested. Data processing was conducted in 3 ways: mapping (M), assembling-followed-by-mapping (AM), mapping-followed-by-assembling-followed-by-mapping (MAM).
Results: Mapping tools results varied: for mapping 93.7-97.5%, for duplicate rates 48.66-55.01%, for unique reads 16.4-48.4%, for reads assigned to exons 6.57-8.42%/introns 13.18-23.28%, with STAR giving the best results. Assembly tools revealed total lengths from 3166928 to 9784426bp, total number of sequences from 8413 to 57524, with Trinity giving the best results. Integrative methods processing outputs: for mapping 99.78-99.99%, for duplicate rates 0.16-9.56%, for unique reads 86.59-93.21%, for reads assigned to exons 43.82-53.12%/introns 23.28-53.12%, with AM giving better results. Presented results are given for one inbred line and detailed results for 6 inbred lines will be discussed during the conference.
Conclusions: For low-coverage RNA-seq data derived from highly repetitive genomes such as maize's/most-of-plant genomes, we advise using integrative methods like AM and MAM.

Acknowledgements: Numerical simulations were run on the PARADOX supercomputing facility at the Scientific Computing Laboratory, National Center of Excellence for the Study of Complex Systems, Institute of Physics Belgrade, supported in part by the Ministry of Education, Science, and Technological Development of the Republic of Serbia under project No. ON171017.

JNK AND ERK DEPENDENT AUTOPHAGY INDUCTION DURING PHORBOL MYRISTATE ACETATE DIFFERENTIATION OF HL-60 LEUKEMIA CELLS

Miloš Mandić[1], Ljubica Vučićević[2], Maja Misirkić-Marjanović[2], Maja Jovanović[1], Mihajlo Bošnjak[3], Vladimir Perović[1], Nataša Latinović[1], Ljubica Harhaji-Trajković[2], Vladimir Trajković[1]

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Introduction: We investigated the mechanism and the role of autophagy in phorbol myristate acetate (PMA)-induced myeloid differentiation of HL60 human leukemia cells. The mitogen-activated protein kinases (MAPKs) regulate myeloid differentiation, therefore our aim was to investigate whether autophagy induction during differentiation is dependent of MAP kinases.
Methods: Colony stimulating factor 1 receptor (CSF1R), early growth response protein 1 (EGR1) and interleukin 8 (IL-8), all markers of differentiation, were assessed by real-time RT-PCR quantification. Cell cycle arrest and expression of surface marker CD11b were analyzed by flow cytometry. Autophagy was monitored by acridin orange staining, RT-PCR analysis of autophagy-related (ATG) genes expression and immunoblotting. The role of autophagy was analyzed using RNA interference-mediated knockdown of ATG5 and p62. Pharmacological inhibition and siRNA were used to determine the role of MAPKs in autophagy induction.
Results: PMA differentiated HL-60 cells into macrophage-like cells by inducing cell-cycle arrest with high p21 gene expression and elevated expression of CD11b, CSF1R, EGR1 and IL-8. The induction of autophagy was demonstrated by accumulation of LC3-II, the increase in autophagic flux and protein expression of beclin-1, ATG5, p62 and mRNA encoding ATG genes. The suppression of autophagy by siRNA–mediated knockdown of ATG5 and p62 counteracted differentiation, while inhibition of MAPKs decreased expression of LC3-I, beclin 1 and p62 and reduced mRNA level of EGR1, CSF1R and IL-8.
Conclusion: Our study revealed the involvement of MAP kinases in induction of autophagy during PMA differentiation and indicated autophagy modulation as a possible target for treatment of acute myeloid leukemia.

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Poster Session
MOLECULAR BIOLOGY OF EUKARYOTES

FRESHWATER SPONGES IN DANUBE AND SAVA RIVERS – MOLECULAR AND MORPHOLOGICAL IDENTIFICATION

Stefan Andjus[1], Nađa Nikolić[2], Valerija Dobričić[3], Ana Marjanović[3], Maja Raković[1], Momir Paunović[1]

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Introduction: Sponges in the large rivers within the Danube River Basin have not been extensively studied. Hence, the aim of this work was to undertake a pilot investigation of the distribution of sponge species in the Danube and Sava Rivers.
Methods: 88 localities were covered by the study and sponges were collected at 24 sites only (46 samples in total). Morphological and genetic (28S rDNA sequencing) analysis were used for species determination.
Results: Three species were found only. In the Danube, the predominant species was *Ephydatia fluviatilis,* making approximately 80% of collected samples, whilst in the Sava River it was *Spongilla lacustris* representing 46% of Sava River sponges. *Eunapius fragilis* was the rarest species detected in both rivers.
Conclusion: the Danube and Sava Rivers are not characterized by high abundance and diversity of Porifera.

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INVOLVEMENT OF LUTEINIZING HORMONE IN RESETTING OF PERIPHERAL CLOCK IN RAT LEYDIG CELLS

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Introduction: Circadian rhythm in testosterone concentration is a long-established phenomenon; however, the data concerning the presence, role and resetting mechanism of clock elements in testis are poorly understood. Previously, we characterized pattern of circadian transcription of core clock genes in testosterone-producing Leydig cells (LCs).
Aim: Here we wanted to examine potential role of LH-cAMP signaling pathway in resetting of LC's clock.
Methods: RTq-PCR (gene expression analysis), RIA (androgen level), ELISA (LH and cAMP levels).
Results: *In vitro* activation of LH receptor in primary culture of LCs by hCG (LH analog) led to the changes in transcription of many clock genes (upregulation of *Per1, Dec1/2, Rorb, E4bp4* and downregulation of *Bmal1, Npas2, Rev-erba/b*). Elevation in transcription of *Per1* and *Dec1/2* genes happened within the first 30 min to 1 h after adding hCG. Further, stimulation of LC's culture with 8br-cAMP showed compatible pattern of transcriptional changes as previous, indicating the role of cAMP in mediating the response of clock genes to LHR activation. To see if *in vivo* disruption of LH-cAMP signaling can affect expression of clock genes in LC, we used hypogonadotropic hipogonadal rats. Disruption of LH-cAMP signaling in this model led to increase in transcription of many clock genes (*Per2, Dec1, Rorb, Rev-erbb, E4bp4, Hlf, Tef*) and decrease in transcription of *Npas2*.
Conclusion: Altogether, LH-cAMP signaling pathway may affect expression of clock genes in LC both *in vitro* and *in vivo*. The results also suggest potential role of LH in resetting LC's clock via cAMP and activation of *Per1* transcription.

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TIME-DEPENDENT CHANGES IN THE RAT LEYDIG CELLS PRIMARY CULTURE

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Introduction: Isolation and purification of Leydig cells from testis allows the study of the cell responses that are independent of systemic and paracrine regulation. However, this type of studies is time limited because over the time Leydig cell primary culture decreases abilities to produce testosterone.
Aim: To describe changes in steroidogenic capacity of Leydig cells primary culture over 24h after isolation.
Methods: Leydig cells from adult rat purified on Percoll gradient; after initial attachment/recovering in the presence of 10% FCS for 4 h, cells were cultured in DMEM/F12 for 6, 18 and 24 h; Leydig cells were determined using NBT staining; testosterone level measured by RIA; relative gene expression quantified with qRT-PCR and mitochondrial membrane potential ($\Delta\psi$i) analyzed using TMRE fluorescence.
Results: Basal testosterone production significantly decreased over 24 h of Leydig cell culturing. This is followed by reduced transcription of main steroidogenic elements (*Star*, *Cyp11a1*, *Hsd3b*, *Cyp17a1*). In 24 h old culture transcription of *Cyp11a1*, *Hsd3b1/2*, *Cyp17a1* was only 5-10% of that registered in 6 h old culture. The reduced gonadotropin sensitivity observed in 18 h old culture was associated with reduced mRNA expression of gene encodes luteinizing hormone receptor (*Lhcgr*). hCG-treatment of 6 and 18 h old cultures stimulated *Star*, *Cyp11a1*, *Hsd3b1/2* and *Cyp17a1* transcription which is not registered in the 24 h old culture. Culturing of Leydig cells increased $\Delta\psi$i. Changes in morphology of Leydig cells were apparent in some cells at 18 h and were more pronounced at 24 h of culturing.
Conclusion: Leydig cells primary culture in short-term cultivation, provide a valuable tool for studying Leydig cell function.

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DIFFERENTIAL EXPRESSION OF GALECTIN-1 IN NORMAL AND TRANSFORMED TROPHOBLAST

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Introduction: Galectin-1 (gal-1) is shown to mediate trophoblast cell migration and invasion. We investigated the expression pattern of this protein in three different trophoblast cell lines- normal first trimester of pregnancy extravillous trophoblast cell line - HTR-8/SVneo and two transformed choriocarcinomic cell lines - JAr and Jeg-3.
Methods: Expression of gal-1 was investigated at mRNA level using quantitative real-time PCR. Cellular distribution was studied using immunocytochemistry. The level of protein was determined by densitometry of immunofluorescently stained cells, as well as by Western blot.
Results: Significant difference in the levels of both gal-1 mRNA and protein was shown. qPCR analysis revealed 5-fold and 25-fold greater expression of gal-1 mRNA in HTR-8/SVneo cells compared to JAr and Jeg-3, respectively. This was in accordance with protein levels. Immunocytochemistry followed by densitometry demonstrated significantly lower levels of gal-1, which were 56% of that in HTR-8/SVneo cells for JAr and 25% for Jeg-3. Similar results were obtained by Western blot.
Conclusion: Difference in cell type specific distribution and the significantly lower level of expression of gal-1 in transformed compared to normal trophoblast could mean that gal-1 acts, not only as stimulator but also possibly as cotrolling factor in trophoblast invasion process and cell cycle regulator.

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EFFECT OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON THE CELL-CELL COMMUNICATION *VIA* EXTRACELLULAR VESICLES

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Introduction: Extracellular vesicles (EVs) comprise a heterogeneous group of vesicles of different sizes that are able to transfer signaling molecules between cells. EVs are of pathophysiological relevance for inflammatory, infectious, cardiovascular, hematological, and other diseases. In order to successfully deliver their cargo, EVs need to fuse with or to be taken by the target cells. Several studies suggest that successful cellular uptake of nanoparticles is affected by their mechanical properties, in addition to chemical make-up. However, the possible importance of nonsteroidal anti-inflammatory drugs (NSAIDs) on mechanical properties of EVs in blood has not been addressed up to date.
Aim: We propose that mechanical properties of EVs can be influenced by the two most widely used NSAIDs-ibuprofen and aspirin. This can lead to unintended consequences due to influence on the cell-cell communication via extracellular vesicles.
Methods: To test our hypothesis, we examine vesicles from red blood cells (RBC). Treatment in duration of 12 h was maintained with ibuprofen and aspirin. Moreover, we examine the effect of cell temperature treatment on the mechanical properties of the secreted vesicles. To do so we perform a detailed AFM force spectroscopy study and analyze our results using a Helfrich-model based theoretical framework to estimate the bending modulus of different vesicle populations.
Results: We find surprising variations in bending modulus values of different vesicle populations. Treated EVs possess lower bending modular i.e. they are softer.
Conclusion: Our results can provide better understanding of drug effect on EVs-mediated functions in blood and provide new insights into the vesiculation process in health and disease.

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NEURONAL DIFFERENTIATION TRIGGERED BY RETIONOIC ACID INDUCES *SNORD115* EXPRESSION AND IS ACCOMPANIED BY POST-TRANSCRIPTIONAL CHANGES OF SEROTONIN RECEPTOR 2c mRNA

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Introduction: The serotonin (5-hydroxytryptamine) system is the most widespread neurotransmitter system in the brain, implicated in modulation of neuronal responses to other neurotransmitters. Among 14 serotonin receptor subtypes, Htr2c plays a pivotal role in controlling neuronal network excitability. Serotonergic activity conveyed through receptor Htr2c is regulated post-transcriptionally via two mechanisms, alternative splicing and A-to-I RNA editing, resulting in modification of receptor's genetically encoded primary structure. Brain-specific small nucleolar RNA SNORD115 harbours a phylogenetically conserved 18-nucleotide antisense element with perfect complementarity to the region of *Htr2c* primary transcript that undergoes post-transcriptional changes. Previous *Htr2c* minigene studies have implicated SNORD115 in fine-tuning of both post-transcriptional events.
Methods: We used an established neuronal differentiation protocol and monitored post-transcriptional changes of endogenous *Htr2c* transcripts throughout neuronal differentiation by qPCR, RT-PCR, and amplicon sequencing.
Results: Both *SNORD115* and *Htr2c* were upregulated upon neuronal commitment. In conjunction, we detected increased exon inclusion and inhibition of A-to-I editing of key adenosines at sites E, C and D while adjacent, non-targeted, sites A and B show differentiation dependent increase in editing.
Conclusion: Our data provide *in vivo* support for the model where SNORD115 blocks conversion of key adenosines to inosines and promotes exon inclusion to favour production of full-length receptor isoforms with higher potency.

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HYPOTHALAMIC INSULIN EXPRESSION INCREASES AFTER THE SHORT-TERM FASTING

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Introduction: Among other processes, insulin in the brain regulates glucose metabolism, appetite and neuronal growth. Here we examined effect of six-hour fasting on insulin expression in rat hypothalamus and its possible involvement in the hypothalamic glucose transport.
Methods: Insulin mRNA expression was assessed by PCR. Content of insulin, phosphorylated insulin receptor (pIR) and glucose transporters (GLUT1, 2, 3) was detected by immunoblotting. Anatomical and cellular localization of insulin immunopositivity was analyzed by immunofluorescence. Serum and cerebrospinal fluid glucose levels were measured with an Exac-tech glucose analyzer.
Results: Short-term fasting increased both insulin II mRNA expression and insulin content in the hypothalamus. Increased insulin immunopositivity was observed in the periventricular NeuN-positive cells and in the ependymal cells surrounding the third ventricle. The amounts of pIR, GLUT1 and GLUT3 were increased, whereas GLUT2 remained unchanged. Unlike blood glucose which was lowered, cerebrospinal fluid glucose was not affected by six-hour fasting.
Conclusion: Our results showed that *de novo* insulin production occurred in the hypothalamus during the short-term fasting. The fact that hypothalamic GLUT expression was either increased or unchanged suggests that centrally produced insulin may be involved in the regulation of glucose transport in the state of acutely disturbed energy homeostasis.

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THE EFFECT OF BORIC ACID AND BORON DERIVATIVES IN GLIOBLASTOMA

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Aim: The aim of this study was to investigate the effect of boric acid (BA) and two boron derivatives, borax pentahydrate (BPH) and disodium octaborate tetrahydrate (Etidot-67, EDT-67) on glioblastoma cell line in terms of inflammation.
Methods: Cytotoxic effect of BA, BPH and EDT-67 in U87MG cell line was evaluated by using the MTT method. Besides, effect of BA, BPH and EDT-67 treatment on mRNA expressions of genes related to inflammation were determined by quantitative RT-PCR (Q-PCR).
Results: At 72 h, IC_{50} value was calculated as 4.8 mM, 2.4 mM and 4.9 mM for BA, BPH and EDT-67, respectively. According to the Q-PCR analysis, in BA treated cells, while IL-1a and IL-8 levels decreased in average by 2 fold, IL-8R1 and RelA levels increased in average by 1.5 fold. Besides, in BPH treated cells, while IL-1a, IL-8, p50 and RelA levels decreased in average by 2 fold, IL-8R1 level increased by 1.5 fold. Finally, in EDT-67 treated cells, while IL-1a and IL-8 levels decreased by 1.5 and 252 fold, respectively, IL-8R1, p50 and RelA levels increased by 4.2, 1.3 and 1.2 fold respectively. Interestingly, IκBa levels increased in BA, BPH and EDT-67 treated cells by 98612, 63872 and 73468 fold, respectively.
Conclusion: Our preliminary results showed that boric acid and boron derivatives might play role in decreasing inflammation in glioblastoma and NF-κB signaling pathway can be effective in this process.

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DYNAMIC RNA-PROTEIN INTERACTIONS UNDERLIE THE ZEBRAFISH MATERNAL-TO-ZYGOTIC TRANSITION

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Introduction: During the maternal-to-zygotic transition (MZT), transcriptionally silent embryos rely on post-transcriptional regulation of maternal mRNAs until zygotic genome activation (ZGA). RNA-binding proteins (RBPs) are important regulators of post-transcriptional RNA processing events, yet their identities and functions during developmental transitions in vertebrates remain largely unexplored.
Results: Using mRNA interactome capture, we identified 227 RBPs in zebrafish embryos before and during ZGA, hereby named the zebrafish MZT mRNA-bound proteome. This protein constellation consists of many conserved RBPs, some of which are stage-specific mRNA interactors that likely reflect the dynamics of RNA–protein interactions during MZT. The enrichment of numerous splicing factors like hnRNP proteins before ZGA was surprising, because maternal mRNAs were found to be fully spliced. To address potentially unique roles of these RBPs in embryogenesis, we focused on Hnrnpa1. iCLIP and subsequent mRNA reporter assays revealed a function for Hnrnpa1 in the regulation of poly(A) tail length and translation of maternal mRNAs through sequence-specific association with 3′ UTRs before ZGA. Comparison of iCLIP data from two developmental stages revealed that Hnrnpa1 dissociates from maternal mRNAs at ZGA and instead regulates the nuclear processing of zygotic *pri-mir-430* transcripts. Moreover, the global shift from cytoplasmic to nuclear RNA targets was accompanied by a dramatic translocation of Hnrnpa1 and other pre-mRNA splicing factors to the nucleus in a transcription-dependent manner.
Conclusion: Thus, our study identifies global changes in RNA–protein interactions during vertebrate MZT and shows that Hnrnpa1 RNA-binding activities are spatially and temporally coordinated to regulate RNA metabolism during early development.

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EFFECTS OF BMP RECEPTOR INHIBITORS ON EARLY DEVELOPMENT OF ZEBRAFISH (*Danio rerio*) EMBRYOS

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Introduction: Bone morphogenic protein (BMP) pathway has been proven to regulate a wide variety of biological processes during development and adulthood. K02288 and LDN-214117 are potent and selective type I BMP receptor inhibitors. Acute toxicity of these compounds has not yet been evaluated on zebrafish embryos.
The aim: The aim of this study was to analyze the effects of BMP receptor inhibitors on early development of zebrafish embryos.
Methods: Embryos were treated with 1, 10, 20 and 50 μM K02288 or LDN-214117. The treatments started at 6 hours post fertilization (hpf) or at 24 and 48 hpf for dechorionated embryos. They were analyzed using light microscopy and the end-points (mortality, hatching rate and malformations) were recorded at 24, 48 and 72 hpf.
Results: The lowest tested concentration of K02288 (1 μM) did not show toxic effects on early zebrafish development. Exposure to 10 μM K02288 resulted in yolk edema and hemagglutination, whereas 20 μM K02288 had significant effect on mortality and induced yolk and pericardial edema, widening of yolk extension, hemagglutination, abnormal eye pigmentation and deformed tail. None of the embryos survived treatment with 50 μM K02288. Exposure of embryos at 6 hpf, or dechorionated embryos at 24 and 48 hpf to 1, 10, 20 or 50 μM LDN-214117 did not cause developmental malformations in comparison to untreated embryos.
Conclusion: Our results show that although K02288 and LDN-214117 are both BMP receptor inhibitors, only K02288 has toxic effects on early zebrafish development. Further analyses are needed to explain observed differences.

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IN VIVO ANTIOXIDANT CAPACITY OF THREE MERLOT WINES – RELATION TO RESVERATROL AND ANTIOXIDANT ENZYMES

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Introduction: Protective effects of moderate wine consuption are, at least partly, mediated by polyphenolic antioxidants, such as resveratrol. They have large array of biological actions including antioxidant enzyme modulation. Here we examined *in vivo* protective capacity of three selected Merlot wines in H_2O_2 – stressed *S. cerevisiae* cells by monitoring survivor and activities of antioxidant enzymes catalase (Cat) and glutathione peroxidase (GPx).
Methods: Merlot wines [commercial wine and two clones (C I and C II)], vintage 2011 were obtained from "Plantaže 13. juli" A.D. winery (Podgorica, Montenegro). Content of resveratrol and its glucosides was determined by liquid chromatography – tandem mass spectrometry (LC-MS/MS). The enzymes activities in control groups (13% ethanol/wines/H_2O_2) and H_2O_2 – stressed cells pretreated with selected wines were measured spectrophotometrically in cell-free extracts.
Results: Yeast exposure to H_2O_2 decreased survival rate to 33% compared to 13% ethanol-treated control yeast cells (100%). Pretreatment with all tested wines increased survival in H_2O_2-stressed cells, whereby the effect of CI sample was the most prominent. This sample also had the highest resveratrol content. With regard to antioxidant enzymes, significant increase in Cat and decrease in GPx activity was found in H_2O_2 – stressed cells. Pretreatment with all selected wines increased GPx activity in these cells.
Conclusion: This study revealed *in vivo* protective effects of all tested wines, whereby the highest resveratrol content wine was the most effective. Besides, these results indicate that intensified GPx-mediated defense may be one of the biological mechanisms underlying wine-induced increase in survival rate in yeast cells under oxidative insult.

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ESTABLISHMENT AND INITIAL CHARACTERIZATION OF NT2/D1 CELL CLONES WITH INDUCIBLE *SOX2* OVEREXPRESSION

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Introduction: *SOX2*, a universal marker of pluripotent stem cells, plays key roles in regulation of various developmental and physiological processes, such as maintenance of pluripotency and self-renewal of stem cells, determination of stem cell fate and differentiation, proliferation, lineage specification, morphogenesis and neural differentiation. Mutations and aberrant expression of this gene have been revealed in a numerous developmental diseases and cancer. The aim of this study was to modulate *SOX2* expression in order to elucidate the role of this transcription factor in NT2/D1 cell pluripotency, viability and proliferation.
Methods: To generate NT2/D1 cell clones with inducible *SOX2* overexpression we used Tet-on system which contained the regulator plasmid pCAG-rtTAon-Hyg and response plasmid pTRE-SOX2-neo. Integration of regulatory plasmid into the genome of NT2/D1 cell clones was analyzed by using oligonucleotides specific exclusively for hygromycin resistance gene cassette of regulatory plasmid. Doxycycline inducibility was tested by transient transfection of cell clones with a luciferase expression plasmid pTRE-Luc. Cell clones with integrated regulator plasmid were transfected with pTRE-SOX2-neo expression vector. *SOX2* overexpression after transfection was tested by reverse transcriptase-PCR and Western blot analysis. Viability, proliferation and pluripotency of cell clones with *SOX2* overexpression were tested by MTT test, Ki67 immunostaining and analyzing the expression of pluripotency markers, respectively.
Results: *SOX2* overexpression modulated expression of pluripotency-related genes and altered viability, but did not change proliferation of NT2/D1 cell clones with inducible *SOX2* overexpression.
Conclusion: These results additionally contributed to understanding the effects of altered *SOX2* expression on pluripotency, proliferation and viability of cells.

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AN IMPROVED METHOD FOR DNA EXTRACTION FROM CHEESE

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Introduction: For the purpose of monitoring cheese quality and authenticity of the information provided to the consumers a multiplex PCR method is developed. The method specifically identifies bovine, caprine and ovine DNA and performs well with DNA of sufficient quality, purity and concentration. The aim of the study was to improve the existing, generic, DNA extraction protocol from cheese that would yield sufficient quantity of PCR grade DNA for multiplex PCR analysis.
Methods: Three previously described generic DNA extraction methods were evaluated for yield and quality of procured DNA. Those parameters were tested in multiplex PCR previously validated for DNA targets isolated from blood. Finally, a variation to the one of the tested generic protocols was introduced, which provided DNA of satisfactory properties.
Results: Two of the tested extraction methods failed to produce PCR grade DNA from cheese. One of the methods yielded PCR grade DNA as confirmed by amplification cytB fragment specific for Bos/Ovis/Capra, however, multiplex PCR reaction failed. Following the introduced variation to the one of the tested methods, we obtained DNA of sufficient quantity and quality for multiplex PCR analysis. The improved method was tested on a series of cheese samples of various consistency and maturity. Specific PCR targets were obtained in all cases and two irregularly labeled cheese products were detected.
Conclusion: Due to the complexity of cheese as a matrix, the analysis of therein contained eukaryotic DNA is challenging. The improved extraction method provides sufficient quantity of PCR grade DNA for multiplex PCR analysis.

BORIC ACID REDUCES THE FORMATION OF DNA DOUBLE STRAND BREAKS

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Introduction: Boron is absorbed by digestive and respiratory system and it was considered that it is converted to boric acid (BA), which was distributed to all tissues above 90%. The biochemical essentiality of boron element is caused by boric acid because it affects the activity of several enzymes involved in the metabolism. DNA damage repair mechanisms and oxidative stress regulation is quite important in the transition stage from normal to cancerous cells, thus this study was conducted to investigate the protective effect of boric acid on DNA damage and wound healing in human epithelial cell line.
Methods: The amount of DNA damage occurred with irinotecan (CPT-11), etoposide (ETP), doxorubicin (Doxo) and H_2O_2 were determined by immunofluorescence through phosphorylation of H2AX$^{(Ser139)}$ and pATM$^{(Ser1981)}$ in the absence and presence of BA. Moreover the effect of BA on wound healing has been investigated in epithelial cells treated with these agents.
Results: Our results demonstrated that H2AX$^{(Ser139)}$ foci numbers were significantly decreased in the presence of BA while wound healing was accelerated by BA compared to control and only drug-treated cells. Eventually, the results indicate that BA reduced the formation of DNA double strand breaks caused by agents as well as improving the wound healing process.
Conclusion: We suggest that boric acid has important therapeutical effectiveness and may be used in the treatment of inflammatory diseases where oxidative stress and wound healing process plays an important role.

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ASSESSMENT OF GLOBAL AND GENE-SPECIFIC DNA METHYLATION IN THE FIRST TRIMESTER PLACENTA AND FETAL LIVER EXPOSED TO MATERNAL CIGARETTE SMOKING

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Introduction: Maternal cigarette smoking is associated with low birth weight in newborns and various pathologies later in life. Maternal smoking was also shown to induce certain epigenetic changes in newborn, some of which are maintained until adulthood.
Aim: Assessment of the impact of exposure to maternal smoking during first trimester (6 to 12 weeks), in human placenta and fetal liver by determining level of DNA metylation.
Methods: Global DNA methylation levels were quantified with ELISA using a methylcytosine antibody as well as with the bisulfite pyrosequencing of surrogate markers for global methylation status, *LINE-1*, and *AluYb8*. Gene-specific DNA methylation was determined for two genes involved in detoxification, *CYP1A1* and *AHRR,* by bisulfite pyrosequencing. In addition, *CYP1A1* and *AHRR* mRNA expression was examined by RT-qPCR.
Results: We observed no significant global methylation changes associated with maternal smoking. Similarly, no exposure-associated *CYP1A1* DNA methylation changes were detected, but exposure associated increases in *CYP1A1* mRNA expression was detected in placenta and male fetal liver. Exposure was associated with *AHRR* DNA hypermethylation in placenta of female fetuses, whereas placenta *AHRR* mRNA expression was not affected. Exposure was not associated with *AHRR* DNA methylation changes in fetal liver, whereas herein an exposure associated increase in *AHRR* mRNA expression was detected.
Conclusion: Our results indicate that exposure to maternal smoking in first trimester relates to changes in *CYP1A1* mRNA expression and *AHRR* mRNA expression and DNA methylation. However, results also indicate that exposure in first trimester has not mediated DNA methylation changes observed at birth.

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GLUCOCORTICOID-MEDIATED EFFECTS OF MIF DEFICIENCY AND FRUCTOSE-ENRICHED DIET ON LIPID METABOLISM IN THE MOUSE INTRA-ABDOMINAL ADIPOSE TISSUE

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Introduction: The macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine involved in metabolic inflammation and regulation of glucocorticoid action. Low-grade inflammation in adipose tissue is associated with obesity and dyslipidemia and may be caused by fructose-enriched diet. We hypothesized that the effects of MIF deficiency on lipid metabolism in adipose tissue, after high fructose consumption, could be mediated by glucocorticoids (GCs) as potent regulators of energy metabolism.
Methods: We analyzed the effects of 9-week 20% fructose-enriched diet on energy intake, body mass, intra-abdominal adipose tissue mass and histology in MIF wild type (WT) and MIF deficient ($MIF^{-/-}$) C57Bl/6J mice. Expression of key transcriptional regulators involved in adipogenesis and lipogenesis, peroxisome-proliferator-activated receptor γ (PPARγ) and sterol regulatory element-binding protein-1 (SREBP-1), was also assessed. Glucocorticoid signaling was characterized by prereceptor metabolism, glucocorticoid receptor (GR) protein level and phosphorylation, and expression of GC-target genes involved in lipogenesis.
Results: Both WT and $MIF^{-/-}$ mice on fructose diet had increased energy intake, but the elevation of adipose tissue mass and enlargement of adipocytes were observed only in fructose-fed $MIF^{-/-}$ mice. Elevated GR protein level and its activating Ser^{220} phosphorylation, enhanced glucocorticoid prereceptor metabolism, an increase in PPARγ and SREBP-1 levels and induced expression of all examined lipogenic genes were also observed in $MIF^{-/-}$ mice on fructose diet.
Conclusion: The results show that only under fructose caloric overload MIF deficiency results in lipogenesis and adipocyte hyperthrophy and that this effect might be mediated by enhanced glucocorticoid signalling in intra-abdominal adipose tissue.

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FLOWER COLOR MORPHS OF *Iris pumila* DIFFER IN THE AMOUNT OF HEAT SHOCK PROTEIN 70 AND PIGMENTS WITH ANTIOXIDANT PROPERTIES

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Introduction: Natural populations of the dwarf bearded iris, *Iris pumila*, are markedly polymorphic for flower color. Selection pressures exerted by a range of abiotic and biotic factors could be involved in maintaining the polymorphism. Here we quantified the amount of the Hsp70 and the two groups of pigments with antioxidative properties: anthocyanins and carotenoids.These molecules impact abiotic stress tolerance, ultimately influencing the fitness of individual plants.
Methods: A total of fifty genotypes raised in a common-garden and belonging to a corresponding color class (yellow, blue, violet, dark blue and dark violet) were surveyed. One fully developed leaf and a flower from each genotype were analyzed for the Hsp70 amount and pigments concentration.
Results: The Western blot analysis revealed the presence of one isoform for the Hsp70 in leaf and two isoforms in flower organs. In both vegetative and reproductive tissues the amount of Hsp70 was found to be the lowest in yellow colored genotypes compared to other color classes. In violet and blue flowers, the concentration of Hsp70 decreased gradually from light to dark colored variants. Conversely, the concentration of anthocyanins was found to be higher in darker than in the lighter color morphs. An inverse trend was observed for the total carotenoids concentration.
Conclusion: This study revealed that each *I. pumila* color genotype produces a unique amount of Hsp70 and antioxidative pigments in both the vegetative and reproductive plant parts, in order to protect cellular homeostasis under fluctuating temperature conditions prevailing in its population.

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THE ROLE OF AMPK IN DIETARY FRUCTOSE- AND STRESS-INDUCED METABOLIC INFLAMMATION

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Introduction: Both chronic stress and fructose overconsumption trigger metabolic disturbances, including obesity, insulin resistance and hepatic steatosis, by modulating metabolic and energy sensor pathways in the liver and other tissues. Two energy sensors that may mediate these processes are AMP-activated protein kinase (AMPK) and Sirtuin1 (SIRT1), but their role in the regulation of metabolic inflammation is not clarified. The aim of this work was to examine possible role of hepatic AMPK and SIRT1 in the development of metabolic inflammation induced by fructose overload and/or chronic stress.
Methods: We analyzed the effects of 4-week chronic unpredictable stress and 9-week 20% fructose-enriched diet, separately and in combination, on energy intake, body and liver mass, blood biochemistry, liver corticosterone and glucose tolerance. The expression of hepatic proinflammatory cytokines, NFκB, ERK, JNK and AMPK kinases, SIRT1 and peroxisome proliferator-activated receptor gamma coactivator (PGC-1a) were analyzed by enzyme immunoassays and Western blot.
Results: Both unstressed and stressed groups on fructose diet had an increased energy intake, hypoglycemia and hyperinsulinemia, while insulin sensitivity was impaired only in the unstressed fructose-fed group. The same group displayed hepatic metabolic inflammation, characterized by increased proinflammatory cytokines and activated NFκB and ERK, as well as decreased level of activated AMPK. Neither SIRT1 nor PGC-1a were changed after fructose and/or stress treatment.
Conclusion: These results show that fructose-enriched diet leads to development of metabolic inflammation in the liver, which is paralleled with impaired glucose tolerance. Fructose-mediated deregulation of AMPK activation might be considered as a link between these metabolic disturbances.

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THE ROLE OF MATRIX METALLOPROTEINASE-9 IN STRUCTURAL PLASTICITY OF MURINE HIPPOCAMPUS AND RETROSPLENIAL CORTEX IN CONDITIONS OF ENRICHED ENVIRONMENT

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Introduction: Matrix metaloproteinase-9 (MMP-9) is a component of the extracellular matrix that is functionally involved in the regulation of brain plasticity. Previous studies have shown that enriched environment can influence brain structural plasticity by changing the activity of MMP-9 (Stamenković et al, 2016).
Methods: MMP-9 $^{-/-}$ deficient and wild type mice were reared under standard conditions and enriched environment for 8 weeks starting from postnatal day 21. Immunohistochemistry was employed to fluorescently stain brain sections and images were acquired and analysed on a confocal laser scanning microscope (Zeiss LSM 510).
Results: Preliminary results show that the MMP-9 deficiency decreases the intensity of the signal for GABAergic terminals (VGAT) in the hippocampus regardless of rearing conditions. In addition, the GABAergic signal seems to be increased in the hippocampus and retrosplenial cortex in wild type mice reared in enriched environment. The intensity of the signal for glutamate terminals (VGLUT1) was decreased in MMP-9 $^{-/-}$ mice exposed to enriched environment as compared to standard conditions, and increased in wild type mice regardless of rearing conditions.
Conclusion: The results indicate that MMP-9 can be a modulator of the balance between excitation and inhibition in the hippocampus and retrosplenial cortex under the conditions of enriched environment.

GLUCOCORTICOID PRERECEPTOR METABOLISM IN THE LIVER OF 5a-DIHYDROTESTOSTERONE-TREATED RATS AS ANIMAL MODEL OF POLYCYSTIC OVARY SYNDROME

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Introduction: Polycystic ovary syndrome (PCOS) is a reproductive and metabolic disorder characterized by hyperandrogenism, ovulatory dysfunction, visceral obesity and insulin resistance. PCOS is also associated with enhanced cortisol metabolite excretion, as well as with altered peripheral glucocorticoid metabolism, which is inevitably linked to insulin resistance characteristic for women with PCOS. The main enzymes involved in glucocorticoid prereceptor metabolism are 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) that regenerates corticosterone from its inactive precursor, and 5a and 5β reductases (5aR and 5βR) that inactivate corticosterone. In this study, female rats treated with nonaromatizable 5a-dihydrotestosterone (DHT) were used as an animal model of PCOS and the aim was to examine whether this treatment affects hepatic glucocorticoid prereceptor metabolism.
Methods: We analyzed the effects of prolonged treatment of prepubertal rats with DHT on body and liver masses, and plasma and liver corticosterone levels. The expression of hepatic 11βHSD1, hexose-6-phosphate dehydrogenase (H6PDH), 5aR, 5βR and glucocorticoid receptor (GR) were analyzed by real-time PCR and Western blot methods.
Results: DHT treatment induced an increase in body and liver masses, an elevation of hepatic 11βHSD1 expression and a reduction of 5aR mRNA level, leading to tissue corticosterone rise and GR nuclear accumulation. In addition, H6PDH and 5βR mRNA levels remained unchanged.
Conclusion: DHT treatment affected hepatic glucocorticoid prereceptor metabolism through enhanced corticosterone availability and its decreased inactivation, which led to enhanced GR activation. Further studies should reveal possible link between enhanced hepatic glucocorticoid signaling and metabolic disturbances observed in PCOS.

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THE EFFECT OF PRENATAL MILD UNPREDICTABLE STRESS ON THE EXPRESSION OF MONOAMINOXIDASE A, BETA 1 AND 2 ADRENERGIC RECEPTORS IN THE HEART OF ADULT FEMALE RAT

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Introduction: Prenatal stress exposure may increase the risk of developing cardiovascular disorders later in life. Cardiotoxic effects of catecholamines are mediated via prolonged adrenergic receptors stimulation and increased oxidative stress levels upon catecholamine degradation by monoamine oxidase A (MAO-A). The aim of this study was to examine long-term effects of prenatal stress on adrenergic receptors β1 (β1AR), β2 (β2AR) and MAO-A gene expression in the heart of adult female rats.
Methods: Pregnant Wistar rats were exposed to chronic unpredictable mild stress during the third week of gestation. Adult female offspring were sacrificed by decapitation and RNA was isolated from left ventricular apex and base. Real-time PCR was used to measure β1AR, β2AR and MAO-A gene expression in collected ventricular tissue samples.
Results: Our results show that β1AR gene expression was higher than β2AR in both left ventricular regions in female offspring of unstressed mothers. In prenatally stressed females, regional difference in beta adrenergic receptors gene expression was lost in the left ventricular apex but not in the base. We showed that in control female offspring, MAO-A gene expression was higher in the left ventricular base compared to the apex. However, in prenatally stressed females, MAO-A gene expression was not significantly different in the examined regions.
Conclusion: Our results suggest that prenatal stress modulates beta adrenergic receptors and MAO-A gene expression in the left ventricle in female offspring. Whether these changes are gender specific and whether they predispose to development of cardiovascular diseases during a lifespan, will be a subject of future experiments.

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PRO-INFLAMMATORY AND ANTI-INFLAMMMATORY ROLE OF HMGB1 IN THE LIVER OF DIABETIC RATS

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Introduction: Oxidative stress and chronic low-grade inflammation in diabetes leads to liver injury. During diabetes, extracellular level of high-mobility group box-1 (HMGB1) protein increases. *Considering that* extracellular HMGB1 (eHMGB1) protein functions as an pro-inflammatory mediator, triggering inflammatory responses by promoting the expression of inflammatory cytokines, *the aim of this study was to investigate its contribution to the maintenance of inflammatory condition in the liver of diabetic rats.* This may help to better understand diabetes-induced liver pathologies and potentially provide target to develop efficient therapies.
Methods: Diabetes was induced by a single intraperitoneal (ip) injection of STZ (65 mg/kg). Inflammatory status in the rat liver was determined in the fourth week after diabetes induction by measuring expression of pro-inflammatory cytokines (TNFa, IL-6) and related production of anti-inflammatory protein haptoglobin (Hp). We also studied the effects of HMGB1 on inflammation through its interaction with TLR4 and related downstream signaling pathways in terms of inhibited HMGB1 secretion in diabetic rats by ethyl pyruvate (EP) treatment (80 mg/kg/ip/daily).
Results: The results show that decrease in eHMGB1 expression caused by EP treatment, correlates with reduced level of TNFa, IL-6 and Hp in the serum and liver of diabetic rats. These changes are in accordance with significant decrease in HMGB1/TLR4 interaction and decreased activation of MAPK (p38, ERK, JNK), NF-κB p65 and JAK1/STAT3 signaling pathways in diabetic liver.
Conclusion: In diabetic liver eHMGB1 is involved in the inflammatory response dually. It acts pro-inflammatory by enhancing production of inflammatory mediators and anti-inflammatory by increasing Hp expression.

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FISH OIL SUPPLEMENTATION SUPRESES NEUTRIC DYSTROPHY AND Aβ PATOLOGY IN PARIETAL CORTEX OF 5xFAD MICE

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Introduction: Alzheimer's disease (AD) is the most prevalent neurodegenerative disease in elderly. Defining feature of AD pathology is the formation of amyloid plaques, structures that are composed of fibrillar Amyloid-β organized in a β-sheet conformation. In AD pathology the clinical symptoms mirror the pathological changes in the brain, where the neuronal loss and plaque pathology occur in the memory related areas. The onset of neuritic dystrophy represents the initial phase of neurodegeneration. It occurs in an early phase of pathology called latent phase, which leaves time frame for potential treatments. So far, omega 3 fatty acids (omega-3 FA), one of the main compounds of fish oil (FO), represent one of the most promising treatment. Here we investigated influence of omega-3 FA supplementation on number of plaques, Aβ load and neuritic dystrophy in parietal cortex in 5XFAD mice.

Methods: Three-month old female 5xFAD mice received FO (100 µl/animal/day) via oral gavage during a 3-week period. Number of plaques, total Aβ levels and neuritic dystrophy were visualized by immunohistochemistry and quantification was done by Image J software.

Results: Our results showed that 3 weeks of FO supplementation significantly decreased number of plaques, total Aβ levels and neuritic dystrophy in the parietal cortex of FO-supplemented 5xFAD animals as compared to non-supplemented 5xFAD animals.

Conclusion: Since fish oil supplementation proved to be able to stop neuritic dystrophy in the parietal cortex of 5xFAD mice it may represent good approach for long term treatment in AD prevention.

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THE MOST RECENT FINDING OF ATLANTIC TROUT INTROGRESSION IN NATIVE BROWN TROUT *Salmo trutta* L. STOCKS OF SERBIA

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Introduction: Phenetic variations of brown trout, *Salmo trutta* together with different behavioural life histories throughout its natural range created confusion among taxonomists making evolutionary history of this species difficult to unravel. The control region of mitochondrial DNA (CR mtDNA) and nuclear lactate dehydrogenase *(LDH)* gene have proved to be very good and widely applied molecular markers in resolving genetic and phylogenetic problems of this species.
Methods: To extend present knowledge of brown trout phylogeography in Serbia and introduction patterns, 33 samples from five streams: Srndaljska, Dojkinačka, Rasina, Mlava and Visočica were analysed for CR mtDNA. Since mtDNK expresses only maternal inheritance, in Rivers with both haplogroups detected, a RFLP of nuclear LDH gene was used, in order to reveal completely interbreeding between both parents from Da and At haplogroups.
Results: The results of this study indicated the presence of two main haplogroups of brown trout, namely Da and At. Of the 13 specimens that were included in RFLP analysis six were characterized as At1 haplotype, one was heterozygous for the LDH marker (LDH-C*90/LDH-C*100), two were homozygous for LDH-C*90 allele and remaining 10 were homozygous for LDH-C*100 allele.
Conclusion: According to the presented results, brown trout of the Da lineage are remarkably dominant in the Balkans and considered indigenous, whereas an occurrence of At lineage is thought to be a consequence of introductions. These results also pointed to the potential interbreeding between individuals of the two haplogroups, although it is uncertain whether the hybridization occurred in the hatchery or in nature.

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CHARACTERIZATION OF MUSCLE REGULATOR ANKRD1 IN GLIOBLASTOMA CELL LINES U87-MG AND LN229

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Introduction: ANKRD1, predominantly expressed in cardiac muscle, is a member of the striated muscle specific MARP (muscle ankyrin repeat proteins) family. Its expression is induced in skeletal muscle upon mechanical stress and in pathological conditions (spinal muscular atrophy, Duchene muscular dystrophy, amyotrophic lateral sclerosis), while mutations in *ANKRD1* gene have been detected in patients with hypertrophic and dilated cardiomyopathies. Regarding tumors, it has been studied in ovarian tumor and rhabdomyosarcoma, but its role in molecular mechanisms of carcinogenesis is not well understood.
Methods: To study ANKRD1 in tumors we choose glioblastoma cell lines U87-MG and LN229 which differ in p53 status. Expression levels of ANKRD1 were determined by qRT-PCR and western blot analysis. Intracellular localization was assayed by immunocytochemistry.
Results: U87-MG and LN229 express very low levels of ANKRD1 which is mainly localized in the nucleus. Both diffuse and spotted patterns were observed. Sequence analysis of ANKRD1 cDNA originating from U87-MG and LN229 cells revealed several cell-specific single point mutations; three of them lead to amino acid substitutions. We also investigated how doxorubicin, anti-tumor drug with limited application in glioblastoma treatment due to difficult delivery, affects ANKRD1 expression in U87-MG and LN229 cells. In contrast to inhibition of ANKRD1 in cardiomyocytes, we detected mild increase in expression of ANKRD1 in glioblastoma cells treated with doxorubicin, both on mRNA and protein levels.
Conclusion: We preliminary characterized endogenous ANKRD1 in two glioblastoma cell lines and set the stage for future studies of ANKRD1 in tumor development and progression.

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IMMUNE RESPONSE WITHIN GUT-ASSOCIATED LYMPHOID TISSUE IN 5xFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease (AD) is characterized by accumulation of amyloid-β plaques that further promotes microglia-mediated neuroinflammatory responses and inflammation in the brain. Emerging data are revealing the relation between gut-associated lymphoid tissue (GALT) cells and CNS, as effector cells primed in the gut might home to the brain. This study aimed to determine cell composition of GALT in 5xFAD mice, an established model for AD.
Methods: Immune cells isolated from Payer's patches (PP) and mesenteric lymph nodes (MLN) were stained with surface and intracellular markers for T helper (Th) subpopulations, B lymphocytes and macrophages and analyzed cytofluorimetrically, while cytokine expression and production were determined by qPCR and ELISA, respectively.
Results: Inflammation was detected in GALT of 5xFAD mice with established AD-pathology. Interestingly, lower IL-17 production could be observed in PP and MLN cells. However, this phenomenon could not be attributed to a lower abundance of Th17 cells, or cytokines that initiate their formation (TGF-β and IL-6). Also, the observed production of IL-17 protein was not a consequence of irregular *Il-17* mRNA transcription or deficiency of Rorγt, a key transcription factor for IL-17. However, miR-155, a non-coding micro RNA that promotes the development of Th17 cells, had significantly lower expression in MLN cells of 5xFAD mice. In contrast, young mice without AD neuropathology did not have inflammation in GALT or altered number of Th17 cells, nor decreased IL-17 production.
Conclusion: The observed changes in GALT of 5xFAD mice are accumulating with the disease progression.

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PHYLOGENY OF MITOCHONDRIAL DNA SUPER-HAPLOGROUP U LINEAGES DETECTED IN SERBIANS

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Introduction: Available mitochondrial DNA (mtDNA) data show genetic differentiation among South-Slavic populations inhabiting the Balkan Peninsula. However, their resolution is insufficient to elucidate the female-specific aspects of the genetic history of South Slavs, including the genetic impact of various migrations which were rather common within the Balkans, a region having a turbulent demographic history. The aim of this study was to thoroughly analyze complete mitogenomes of Serbians, a population linking westward and eastward South Slavs.
Methods: Serbian mitogenomes belonging to super-haplogroup U were completely sequenced and analyzed phylogenetically against ~4000 available complete mtDNAs of modern and ancient Western Eurasians. The most-parsimonious trees of the complete mtDNA sequences were reconstructed using mtPhyl software. Values of mutation rates based on complete mitogenome variability data, synonymous substitutions and coding region substitutions were employed.
Results: Serbians share a significant number of U mtDNA lineages with Southern, Eastern-Central and North-Western Europeans. Putative Balkan-specific lineages (e.g. U4c1b1, U5b3j and K1a13a1) and lineages shared among Serbians (South Slavs) and West and East Slavs were detected (e.g. U2e1b1, U4a2c, U4d2b and U5b1a1). U lineages that are shared between Serbians and populations from the Middle East and/or the Caucasus were also detected (e.g. U3a3, U5a1a2a, K1a2 and K1b2a2).
Conclusion: The exceptional diversity of maternal lineages found in Serbians may be associated with the genetic impact of both autochthonous pre-Slavic Balkan populations whose mtDNA gene pool was affected by migrations of various populations over time (e.g. Bronze Age pastoralists) and Slavic and Germanic newcomers in the early Middle Ages.

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MIXTURES OF ANTHOCYANINS AND THEIR GUT METABOLITES ATTENUATE MONOCYTE ADHESION AND TRANSENDOTHELIAL MIGRATION THROUGH NUTRIGENOMIC MECHANISMS REGULATING ENDOTHELIAL CELL PERMEABILITY

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Introduction: Cardioprotective effects of dietary anthocyanins are partly attributed to their ability to improve endothelial function. However, the underlying mechanisms of action are not fully understood. The aim of this study was to investigate the effect of mixtures of anthocyanins and their gut metabolites on endothelial cell function and decipher underlying molecular mechanisms.
Methods: Primary endothelial cells were treated with a mixture of cyanidin-3-arabinoside, cyanidin-3-galactoside, cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside and degradation product 4-hydroxybenzaldehyde or a mixture of protocatechuic, vanillic, ferulic and hippuric acid, at physiologically-relevant conditions. Successive treatment with both mixtures mimicked profiles of circulating anthocyanin forms following their dietary intake. Inflammation was induced and monocytes added to investigate adhesion and transmigration. Gene and miRNA expression, cell-signaling and in-silico docking were analyzed.
Results: All mixtures significantly decreased monocyte adhesion and transendothelial migration by 24.5% and 46.2% in average. Transcriptomic analysis showed that mixtures modulated expression of genes involved in cell-cell adhesion, leukocyte transendothelial migration, cytoskeleton organisation or focal adhesion. This nutrigenomic effect was associated with the observed modulations of phosphorylation of p65 or p38 signaling proteins. Using docking analyses, we identified 25, among 63 tested, signaling proteins to which these molecules can bind. The mixtures also affected miRNA expression and bioinformatic analysis suggested that target genes of modulated miRNAs are also involved in regulation of endothelial cell permeability and function, contributing to the observed nutrigenomic effect.
Conclusion: These results revealed potential mechanisms by which anthocyanins and their metabolites modulate monocyte adhesion and transmigration and therefore affect endothelial cell permeability and function.

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EVALUATION OF ANTIOXIDATIVE EFFECT OF L-CARNITINE WITH VITAMIN B6 FOOD SUPPLEMENT IN CULTURED LYMPHOCYTES

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Introduction: Oxidative stress is an imbalance in cellular oxidation - reduction reactions with important role in pathogenesis of cardiovascular and infectious diseases, cancer, fibrosis, hemolysis and the aging process. It is known that many nutritional supplements, because of its antioxidant and anti-inflammatory effects have overall positive effects on the health of individuals. L-carnitine, which is commercially available in various forms, contains levoenantiomere and carnitine tartrate with a chemoprotective and antioxidant potential. The aim was to determine the effects of combined L-carnitine and vitamin B_6 in oxidative stress *in vitro* by monitoring the activity of genes involved in these processes.

Material and methods: In this study we tested the effect of liquid carnitine, supplemented with vitamin B_6, on gene expression in cultured lymphocytes. Total mRNA was isolated from treated and non-treated cells. Real-Time PCR was used to assess relative gene expression of HIF1A, CAT, SOD1, GPX1, IL1B, IL2, IL6 genes in cells before and after treatment with active substance in concentration of 50mg/ml and *GAPDH* as housekeeping gene for normalization. REST software was used to calculate difference and statistical significance in expression of treated and non-treated lymphocytes.

Results: L-carnitine supplemented with vitamine B_6 in concentration of 50mg/ml significantly downregulates gene transcription of both HIF1A (P=0,007), CAT (P=0,018) genes and upregulates SOD1 gene (P=0,042) in cultured lymphocytes.

Conclusion: Experimental treatment significantly modulates activity of antioxidative enzymes gene expression level and HIF1A gene regulation suggesting inhibition of proinflamatory processes.

MECHANISMS OF INTESTINAL NON-HAEM IRON ABSORPTION BY QUERCETIN

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Introduction: Inhibitors of iron absorption are great threat to human health as the rate of non-haem iron absorption is generally low. Dietary polyphenols block iron uptake, but mechanism by which they exert this action is not fully understood. Since the polyphenol quercetin is ingested daily in significant amounts it was considered worthwhile to investigate chelation effect of quercetin on duodenal non-haem iron absorption *in vivo*, as well as its effect on systemic iron metabolism.
Methods: Rats were subject to gavage and systemic quercetin treatments. Treatments were followed with uptake studies using radiolabeled iron, serum iron and transferrin saturation measurements, LC–MS/MS analysis of quercetin metabolites in serum, determination of tissue non-haem iron content and analysis gene expression of iron-related proteins.
Results: Both oral and intraperitoneal (IP) quercetin caused iron deficiency *in vivo* by two mechanisms, firstly by increasing mucosal iron uptake and inhibiting iron efflux from duodenal mucosa and secondly by decreasing levels of duodenal DMT1, Dcytb and FPN. Additionally, IP quercetin induced highly significant increased expression of hepcidin. Only after IP quercetin administration, some quercetin metabolites were detected with LC-MS/MS in serum. Thus, possibility that quercetin metabolites could also be involved in the observed effects on iron uptake and metabolism should not be overlooked.
Conclusion: Oral quercetin had a great effect on iron absorption, while IP quercetin mainly affected iron-related genes. These results could lead to development of new effective ways of preventing and treating iron deficiency anaemia, the most frequent and widespread nutritional disorder in the world.

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IN VITRO CULTIVATION OF HONEY BEE MIDGUT AND THE EFFECT OF ACUTE PARAQUAT EXPOSURE

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Introduction: Honey bee (*Apis mellifera*, L.) is the most important managed pollinator species in the world. During recent decades, a decrease in honey bee colony numbers has been observed. Multiple factors contribute to this, including pathogens and exposure to xenobiotics, especially pesticides. Exposure to pesticides may be direct or indirect, and intoxication can be oral, contact or inhalation.
Gastrointestinal tract of honey bee play a significant role in defense against pathogens; intestinal epithelium is the first line of defense against many bacteria, viruses and fungi. On the other side, the absorption of xenobiotics largely takes place in midgut, and thus begins their biotransformation.
Methods: This paper describes a method for *in vitro* cultivation of honey bee midgut in DMEM medium. MTT test was used to access the cell viability. Thereafter, the explant was tested by looking the effect of acute paraquat exposure, which is known to induce oxidative stress, on expression of selected genes involved in antioxidative defense.
Results: The results showed that 2 hours exposure led to upregulation of *Sod1* and *Cat* genes, whose products are directly involved in antioxidant defense. On the other hand, paraquat did not affect the transcription of *Nrf* transcription factor. The reason might be short exposure period, after which Nrf was activated only at the posttranslational level.
Conclusion: Our results confirmed the response of the explant to the oxidative stress induced by paraquat which makes this model system suitable for testing the effects of various biological and chemical agents on the bees.

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MOLECULAR DOCKING AND FLUORESCENT YEAST ASSAY IN TARGETING POTENTIAL STEROID RECEPTOR LIGANDS

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Introduction: Hormone-dependent breast and prostate cancers are leading causes of death worldwide, and targeting steroid receptor signaling is one of the effective treatment strategies in fighting these diseases.
Aim: The aim of this study was to identify potential ligands for androgen receptor and estrogen receptor α and β isoforms.
Methods: To identify potential ligands for selected steroid receptors, we have performed *in silico* screening. For further estimation of their binding affinities, we have developed fluorescent assay in yeast. Ligand-binding domains of steroid receptors were expressed in-frame with yellow fluorescent protein in *Saccharomyces cerevisiae*. Binding of a ligand to a steroid receptor resulted in receptor dimerization and fluorescence resonance energy transfer between two molecules of yellow fluorescent protein. Fluorescence intensity was measured by fluorescence microscopy and fluorimetry.
Results: We have optimized molecular docking simulation and yeast fluorescent assay for testing steroid compounds. In both methods used in this study we have achieved selectivity of ligands towards steroid receptors they naturally bind to.
Conclusion: This economical, effective and safe assay in yeast could be applied in screening of potential therapeutics for treatment of breast and prostate cancer.

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EFFECT OF MELATONIN ON OXIDATIVE AND INFLAMMATORY STRESS IN SPLEEN AND LIVER OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Introduction: Oxidative stress and inflammation are involved in the pathogenesis of diabetes. Previously, we showed that melatonin exerts potent anti-oxidative and anti-inflammatory actions in the liver of streptozotocin (STZ)-induced diabetic rats, thus correcting diabetes-associated abnormalities. The concept of a liver-spleen axis has been proposed as an intersection linking immunity and metabolism in various conditions, including chronic liver diseases. We therefore compared the effect of melatonin on oxidative stress and the inflammatory response in the liver and spleen of STZ-induced diabetic rats.
Methods: Male Wistar rats were injected with 65 mg/kg STZ to induce diabetes. Melatonin was administrated daily (0.2 mg/kg/i.p) until the end of the study at 4 weeks after diabetes induction. Oxidative stress was assessed by measuring the level of lipid peroxidation and the changes in antioxidative enzyme activities. Inflammation was evaluated by examining the levels of proinflammatory cytokines, inflammatory mediators and the acute-phase protein haptoglobin (Hp).
Results: In both tissues, melatonin lowered oxidative stress, which was observed as a decrease in lipid peroxidation and increased expression and activity of CAT, MnSOD and CuZnSOD. By suppressing the activation of NF-κB p65 and MAPK (p38, JNK, ERK) signaling cascades and by decreasing the production of TNF-a, IL-6, HMGB1 and Hp, melatonin also reduced inflammation.
Conclusion: Melatonin stimulated the antioxidative defense in both, the spleen and liver of diabetic rats and attenuated inflammation via the same molecular mechanisms.

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IMMOBILIZATION STRESS RESETS THE CIRCADIAN RHYTHM OF THE ENDOCRINE FUNCTION OF RAT LEYDIG CELLS

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Introduction: It is well known that immobilization stress (IMO) decreases serum testosterone level and expression of Leydig cells steroidogenic genes, but the IMO effects on circadian rhythm of Leydig cells endocrine function are poorly understood.
Aim: This study was designed to evaluate the effect of acute (3 h) and repeated (3 h daily for 10 consecutive days) IMO, applied at different times during the 24 h on circadian rhythm of testosterone secretion, expression of clock and steroidogenic genes in Leydig cells.
Methods: Hormones level was measured by radioimmuno assay and gene expression was followed by using qPCR.
Results: The result showed that acute IMO decreased level and canceled circadian pattern of testosterone secretion, which is associated with changed transcription of some genes involved in steroidogenesis (decreased level and canceled rhythm of *Cyp11a1* and *Cyp17a1* and decreased circadian pattern of *Star*) and some core clock genes (increased rhythm robustness and mesor of *Per1 and Reverba*). Ten times repeated IMO also decreased and flattened oscillatory pattern of testosterone secretion and changed rhythm of steroidogenic genes transcription (decreased rhythm of *Cyp17a1,* increased and initiated cyclic pattern of *Hsd31/2*). The transcription of clock genes was also deregulated (*Bmal1, Per1*, *Cry1* and *Cry2* decreased while *Per2* and *Reverba* increased).
Conclusion: Comparing the IMO effects at different time points during the 24 hours, it was found that IMO effect on the clock genes was more pronounced in the morning. Accordingly, presented data suggest that IMO resets circadian clock and disturbed rhythm of endocrine function of Leydig cells.

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INDUCTION OF CYTOCHROME P450 1A AND 1B ACTIVITY BY CYPERMETHRIN AND ITS COMMERCIAL MIXTURES AND THEIR INFLUENCE ON EXPRESSION OF THE CORRESPONDING GENES

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Introduction: Cypermethrin is widely used synthetic pyrethroid insecticide. In mammals, transformation of pyrethroids is mediated by cytochrome P450 (CYP) isozymes and carboxylesterases.
Objectives: Testing the effects of technical cypermethrin and two cypermethrin-based plant protection products (commercial, Cipkord 20 EC and another one which is under development) on the activity of isozymes of CYP1A and 1B subfamily and the expression of several genes encoding CYP isoforms: *Cyp1a1*, *Cyp1a2*, *Cyp1b1* and *Cyp3a1*.
Methods: Experiments were performed on rat hepatoma cell line H4IIE. CYP1A1/2 and CYP1B1 inducing potential was determined using micro-EROD (ethoxyresorufin-O-deethylase activity) assay. Relative expression of the selected genes was analyzed using quantitative RT-PCR.
Results: Effects of technical cypermethrin on the EROD activity were not observed, while both tested cypermethrin-based mixtures significantly increased the activity of this enzyme. *Cyp1a1* expression was elevated after 24 h-treatment with the product under development, indicating that overexpression of this isoform is induced by some of the coformulants present in this product. Significant overexpression of *Cyp1a2* and *Cyp3a1* was induced by cypermethrin and both tested plant protection product. Most likely, overexpression of these genes is induced by active substance, and different coformulants contributed to the shown effect. Expression of *Cyp1b1* isoform was only elevated by Cipkord 20 EC, indicating that this isoform responds to some of the Cipkord 20 EC coformulants.
Conclusion: These results contribute to the understanding of mode-of-action of cypermethrin and commercial products based on this active substance on the cellular level and highlight the problem of unpredicted toxicity of commercial mixtures.

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PREDICTIVE DNA ANALYSIS: ASSESMENT OF IRISPLEX SNPs FOR EYE COLOR PREDICTION IN SERBIAN POPULATION

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Introduction: In forensic investigations, externally visible characteristics (EVCs) are important when it comes to narrowing the scope of searching for perpetrators. Therefore, in addition to studies concerning the genetic profile itself, further studies are focusing on genes associated with EVCs. One of the most distinctive EVC is human eye color, a highly polymorphic characteristic that is associated with six single nucleotide polymorphisms (SNPs) in six genes (*HERC2, OCA2, SLC24A4, SLC45A2, TYR, IRF4*). Multiplex genotyping system named IrisPlex, that targets those SNPs, so far, was found to be the most informative for predicting an eye color.
Objective: To determine association of SNP genotypes with eye color in Serbian population using IrisPlex system.
Methods: Buccal swabs were taken from 65 volonteers with informed consent, together with a digital image of each volonteer iris. Six genes of IrisPlex system were amplified in multiplex PCR and a desired SNP (rs12913832, rs1800407, rs12896399, rs16891982, rs1393350, rs12203592) in each of six genes was targeted in SnaPshot reaction. Products were separated by capillary electrophoresis and analyzed using the GeneMapper® ID-X 1.4 software.
Results: A strong difference in SNPs genotypes was found when it comes to blue and brown eyes, while intermediate eyes colour was associated with different SNP combinations. Out of the six genes correlated with eye color, *HERC2* was found to be the most informative.
Conclusion: IrisPlex system can be useful in predicting eye color in Serbian population, mostly to sort out dark and light eyes color, with SNP in *HERC2* gene as the most influential.

DNA CONSERVATION BY HESPERETIN: THE MECHANISM OF INHIBITION OF EMS-INDUCED ALKYLATION

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Introduction: Flavanone hesperetin is the major bioflavonoid present in oranges and lemons.
Methods: The genotoxicity and antigenotoxicity of hesperetin was evaluated *in vivo* in the anterior midgut of *Drosophila melanogaster* using Comet assay. Third instar larvae were treated with 1 mM hesperetin. The same concentration was combined with 1 mM ethyl methanesulfonate (EMS) for evaluation of antigenotoxicity. Molecular docking performed on either supercoiled DNA or Topoisomerase IIa, by means of AutoDock Vina, revealed the mechanisms of EMS genotoxicity or hesperetin antigenotoxicity.
Results: The results revealed that hesperetin displays no genotoxic activity. Moreover, combined treatment showed protective effect against DNA damages induced by EMS with percentage reduction of 77.8%. According to Vina, EMS genotoxicity is exerted throughout the alkylation of either guanine or thimine, on the supercoiled DNA level, upon which emerging O^6-ethylguanine and O^4-ethylthimine lesions are incorporated into the replication after the DNA double strand catenation catalyzed by Topoisomerase IIa. Hesperetin exerts antigenotoxicity by preventing the alkylation on supercoiled DNA or by prohibiting the binding of alkylated DNA to Topoisomerase IIa.
Conclusion: The present work demonstrates that tested flavanone possess protective effect against DNA damage induced by EMS. Hesperetin exerts antagonistic effect towards EMS when administered simultaneously.

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THE CORRELATION BETWEEN CD44 EXPRESSION AND SPHERES CHARACTERISTICS

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Aim: The aim of the study was to examine the correlation between CD44 expression in basal cell carcinoma cells of diferent types with sphere number and diameter.
Methods: Tumor cells were isolated from tumor tissue of recurrent and nodular BCC. Cells were grown in DMEM supplemented with 10% FBS and 100 U/mL penicillin – streptomycin and cultivated on 37 ^{0}C and 5% CO_2. After the fifth pasage RNA isolation was performed using standard phenol-chloroform procedure followed by cDNA synthesis with reverse transcriptase.The expression of tumor-associated genes CD44 was measured by real-time PCR. Cells after the fifith pasage were also plated on a low adherent 24 well plate, treated with polyhydroxyethyl methacrylate (polyHEMA), in concentration of 1x10^4 cells per well and incubated under standard conditions. Every other day the serum free medium was replaced and plates were photographed. After seven days cell spheres were analyzed using the software Scope Image 9.0.
Result: RT-PCR showed higher CD44 expresion in infiltrative BCC.The test of sphere formation showed that cells isolated from infiltrating BCC had already formed a sphere on the fifth day in 80% of cases. Diameters and number of these spheres were higher compared to the sphere of nodular BCC on the seventh day.
Conclusion: BCC cells with high CD44 expression formed spheres in vain time and larger diameter.

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ELUCIDATING *IN VIVO* ROLES OF NUCLEASES MGME1 AND DNA2 IN MITOCHONDRIAL DNA REPLICATION

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Introduction: Mitochondrial diseases encompass genetically heterogeneous group of different disorders caused by mutations in mitochondrial and/or nuclear DNA and display variability in their clinical presentation as well as tissue specificity. Mutations in genes encoding nuclease MGME1 and nuclease/helicase DNA2 were recently described to cause mitochondrial DNA maintenance disorders in patients and are suggested to play a role in mitochondrial DNA (mtDNA) replication. Although the localization of DNA2 is still unclear, there are studies indicating its role in base excision reparation of mtDNA. To study *in vivo* function of DNA2 and its potential redundant function to MGME1 we analyzed *Mgme1*$^{-/-}$ *Dna2*$^{+/-}$ knockout mice.
Methods: All the following methods are performed for different tissue samples from 3 mouse model systems we compared, *wildtype*, *Mgme1*$^{-/-}$ knockout and *Mgme1*$^{-/-}$ *Dna2*$^{+/-}$ double knockout: long extension PCR for detecting mtDNA deletions; Southern Blot analysis for assessing mtDNA and 7S DNA levels; Northern Blot analysis to assess mitochondrial transcript levels; Western Blot analysis to study levels of OXPHOS complexes.
Results: No significant changes in molecular phenotypes between *Mgme1*$^{-/-}$ *and Mgme1*$^{-/-}$ *Dna2*$^{+/-}$ mice can be observed.
Conclusion: This study brings us to two possible explanations. Either 50% of transcripts of *Dna2* in our double knockout model might be insufficient for DNA2 nuclease to demonstrate its role in mtDNA maintenance or this nuclease is involved in completely different pathway within or out of mitochondria.

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AMELIORATIVE EFFECTS OF SILICON IN IRON DEFICIENT BARLEY

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Introduction: Silicon (Si) is a beneficial element for plants, well known for its protective role in abiotic and biotic stresses; however molecular mechanisms of its ameliorative effects are poorly understood. Si-mediated alleviation of Fe deficiency has already been described only in dicotyledonous plants utilizing reduction based strategy I for iron acquisition.
Aim: The aim was to investigate whether Si also alleviates Fe deficiency in plants using phytosiderofore based strategy II and to take a closer look into the possible molecular mechanisms involved.
Methods: Barley, a graminaceous plant utilizing Fe acquisition strategy II, was grown in hydroponics in the presence or absence of FeEDTA, with or without $Si(OH)_4$. Leaves and roots were sampled at several time points and used for molecular analyses.
Results: Si successfully alleviated iron deficiency stress in barley. Chlorosis and biomass reduction of young leaves were mitigated. Si significantly increased Fe content in youngest leaves resulting in increased iron dependent antioxidative enzyme activities and decreased ROS level. Expression of a strategy II Fe acquisition gene *HvYSL1* and *HvNAS1* involved in Fe retranslocation were up-regulated in Si treated roots 5 hours after iron removal from growth medium, suggesting acceleration of iron uptake under influence of Si. *HvNAS1* as well as *HvTOM1* involved in phytosiderofore eflux were remarkably up-regulated in leaves contributing to the more efficient retranslocation and utilization of iron within plants.
Conclusion: Si alleviates Fe deficiency in barley by increasing Fe content in young leaves and modulating expression of Fe acquisition and translocation genes.

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EXPRESSION ANALYSIS OF THE GENE ENCODING DSS1 PROTEIN IN *Arabidopsis* UNDER OXIDATIVE STRESS

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Introduction: DSS1 protein has essencial role in maintance of genome integrity, with regard to its involvement in BRCA2 mediated repair via DNA recombination. Also, DSS1 takes part in protein homeostasis, participating in the proper 26S proteasome biogenesis. Two genes encoding DSS1 protein have been discovered in the *Arabidopsis thaliana* genome: DSS1 (I) and (V), which suggests its multifunctionality. Zhang Y. *et al*. have proposed potentionally new role of DSS1 protein, which refers to its ability to bind oxidized damaged proteins and thus marking them for degradation through ubiquitin-proteasome system.
Methods: Mature arabidopsis plants grown hydroponically were exposed to oxidative stress by toxic concentration of copper and hydrogen peroxide. Level of lipid preoxidation was measured as an indicator of oxidative stress. By Real time PCR and Western blot we analyzed expression profiles of DSS1 (I) and (V) genes and DSS1 protein.
Results: Increasing trend of lipid perodixidation was detected in plants exposed to mentioned stress factors. None of the tested concentrations of copper and hydrogen peroxide in the leaves of mature arabidopsis caused significant changes in the level of DSS1 expression. The increase of DSS1 (V) expression is particularly significant in roots, after treatment with 30 μM copper. In particular, dramatic DSS1 (V) expression elevation was observed in the roots treated with 5 mM or 10 mM hydrogen peroxide.
Conclusion: The results indicate that both DSS1 pottentialy have the role in oxidative stress alleviation.

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MORPHOLOGICAL AND MICROMORPHOLOGICAL IDENTIFICATION OF *CANNABIS* POLLEN AND CONFIRMATION OF MARIJUANA IN FORENSIC TRACES BY THCA SYNTHASE GENE ANALYSIS

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Introduction: Pollen of *Cannabis sativa* L. (marijuana) is one of the common traces found at the crime scene and could be very important clue in resolving criminal cases. The aim of this study was to establish methods for the detection of marijuana pollen on evidence material, which are simple, efficient and reliable enough for forensic application. This was done by the use of Light Microscopy (LM), Scanning Electron Microscopy (SEM) and molecular genetic analysis of THCA synthase gene of DNA extracted from marijuana pollen.
Methods: Ten samples from wardrobe which was used during marijuana cultivation and two negative control samples were collected. Samples (5+1) for LM and SEM were collected by adhesive tape, while samples (5+1) for DNA extraction were obtained by cotton swabs immersed in saline, from the same region on evidence material where adhesive tape samples were taken. After extraction, two different portions of THCA synthase gene were amplified in order to determine whether any pollen from marijuana was present.
Results: Pollen was found on 3 out of 5 samples and all of those samples were also positive for *marijuana* specific PCR testing.
Conclusion: LM, SEM, as well as THCA synthase gene analysis, showed high sensitivity, which make them suitable methods for forensic practice. By LM and SEM one could unequivocally mark out *Cannabis* pollen, but for the distinction of marijuana from hemp one should perform THCA synthase gene analysis.

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INHIBITION OF HMGB1 RELEASE DECREASES BOTH APOPTOTIC AND AUTOPHAGIC ACTIVITY IN THE HEPATOCYTES AND REDUCES LIVER INJURY IN STEPTOZOTOCIN TREATED RATS

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Introduction: Hepatocellular death is the main trigger of liver disease. Since diabetic patients are very prone to liver diseases there is a urgent need to identify key regulators of cell death processes. High-mobility group box protein 1 (HMGB1) is a non-histone nuclear protein with a role in apoptotic and autophagic activation when it is present in cytosol and extracellular space. The aim of this study was to elucidate HMGB1 contribution to liver injury trough activation of apoptosis and autophagy in streptozotocin (STZ)-induced diabetic rats since the role of HMGB1 in hepatic cell death during diabetes is partially known.
Methods: Diabetes was induced with a single intraperitoneal (i.p.) injection of STZ (65 mg/kg). Inhibition of HMGB1 release was achieved by ethyl pyruvate (80 mg/kg/i.p./daily). We followed changes in expression of serum and cytosolic HMGB1 and its interaction with TLR4 and RAGE and how these changes affect on apoptotic and autophagic activity and liver morphology.
Results: In the serum of diabetic rats elevated levels of HMGB1 were accompanied by increased HMGB1 interactions with TLR4 and RAGE receptors. Enhancement in these interactions led to increased activity of both apoptotic and autophagic signaling pathways resulting in altered liver morphology and acummulation of autophagosomes in hepatocytes. Inhibition of HMGB1 release caused reduction in apoptotic and autophagic activity which resulted in preservation of normal liver architecture and decreased number of autophagosomes.
Conclusion: HMGB1 causes liver damage through activation of apoptosis and autophagy, therefore it's a suitable new target for prevention of liver diseases in diabetic patients.

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ASSESSMENT OF MUTATION RATES FOR PPY23 STR LOCI IN SERBIAN FATHER-SON PAIRS

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Introduction: Y short tandem repeats (Y-STRs) are widely used in forensic casework. They provide male-specific profiles in male-female DNA mixtures, as well as clue in kinship and ancestry analyses. Y-STRs reflect paternal lineages since Y chromosome is transmitted from father to son virtually without any recombination. Nonetheless, father-son pair profiles can differ due to germline mutations what represents a need for the assessment of mutation rates of Y-STRs used.
Objective: To estimate mutation rates of 23 Y-STRs for the proper use of Y-STRs and accurate interpretation of obtained data.
Methods: DNA samples extracted from buccal swabs collected from 130 Serbian father-son pairs were analyzed using the Promega Powerplex® Y23 kit. PCR products were separated by capillary electrophoresis and analyzed using the GeneMapper® ID-X 1.4 software.
Results: In analyzed sample, 12 mutation events occurred, of which all were one-step mutations. In total, we observed mutations in 9 out of 23 STR loci: DYS389II (1), DYS19 (1), DYS391 (2), DYS635 (1) and YGATH4 (1), five loci which falls in the group of so called medium mutation rate loci, as well as in four fast mutated loci DYS533 (1), DYS570 (1), DYS439 (2) and DYS456 (2). No mutation was seen in DYS576 which is, along with DYS570, pointed out as rapidly mutated locus.
Conclusion: Generally, high mutation rates of specific Y-STR loci are associated with a better discrimination power in male forensic identification. However, they can have a negative impact in paternity testing by leading to erroneous exclusion of biological paternity.

IMMUNOMODULATORY ACTION OF *Chelidonium majus* ETHANOLIC EXTRACT: EMERGENCE OF UNCONVENTIONAL POPULATIONS OF PERIPHERAL BLOOD CELLS

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Introduction: *Chelidonium majus L* (*Papaveraceae*) is widely used in alternative medicine for treatment of various disorders. Immunomodulatory effects of purified compounds isolated from this plant have been reviewed, while studies that examine properties of the whole extract are lacking.
Aim: The aim of this study was to examine the effect of *C. majus* ethanolic extract on peripheral blood mononuclear cells (PBMNC).
Methods: Blood was obtained from healthy donors and PBMNCs were isolated by density gradient centrifugation. After 24 hours treatment with 10, 50 and 250 μg/ml extract, PBMNCs were analysed on flow cytometer.
Results: Treatment induced dose-dependent increase in proportion of monocyte/macrophages (Mo/Mf) and B cells and concomitant decrease in percentage of T cells. Importantly, the percentage ratio of both double positive CD4+CD8+ T lymphocytes and helper T cells coexpressing CD14 molecule increased with extract concentration. Even the lowest concentration of extract induced coexpression of CD4 on almost whole population of Mo/Mf cells.
Conclusion: Increase in proportion of Mo/Mf and B cells, expression of CD4 molecule on monocytes that triggers differentiation of human monocytes into functional mature macrophages, expression of CD14 molecule on helper T cells that indicates activation of lymphocytes, and coexpression of CD4 and CD8 molecules on T cells postulated to be the result of lymphocyte activation, point to presumable immunostimulatory effect of *C. majus* ethanolic extract.

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THE ABSENCE OF INSULIN AND IGF1 RECEPTORS IN STEROIDOGENIC CELLS DISTURBS TRANSCRIPTION OF MAIN MARKERS OF SEXUAL DETERMINATION AND DEVELOPMENT AS WELL AS MITOCHONDRIAL BIOGENESIS IN SEMINIFEROUS TUBULES OF PREPUBERTAL MICE

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Introduction: Although insulin receptors family (IGFs) plays unequivocally critical roles in testicular function, their specific roles in sexual development and mitochondrial biogenesis markers in seminiferous tubules have yet to be determined.
Aim: To follow the effect of insulin and IGF1 receptors absence in steroidogenic cells on the markers of sexual development and mitochondrial biogenesis in seminiferous tubules of prepubertal mice.
Methods: Model of functional genomics: prepubertal (P21) male mice with conditional deletion of *Insr* and *Igf1r* (*Insr*/*Igf1r*-DKO) in steroidogenic tissues. Outcomes: genes expressions were followed by RQ-PCR, hormones by RIA, morphological changes in seminal epithelium and mitochondrial morphology/architecture by TEM.
Results: Transcription of *Ppargc1a* and *Ppargc1b*, the key regulators of mitochondrial biogenesis and integrators of environmental signals, significantly decreased in seminiferous tubules from *Igf1r*-SKO and were not detectable in samples from *Insr*/*Igf1r*-DKO mice. The similar pattern was observed for PGC1-downstream-target *Tfam*. Transcription of *Nrf1, Pparg* as well as *Opa1* and *Mfn1* (mitochondrial architecture markers) significantly increased in both *Igf1r-SKO* and *Insr*/*Igf1r*-DKO mice. In the same samples, *Cyp19a1* transcript increased, while *Wnt4* was not detected in seminiferous tubules from *Insr*/*Igf1r*-DKO mice. Histological analysis suggested impaired growth of seminal epithelium in *Insr*/*Igf1r*-DKO mice compared with wild type. This was followed with reduced spermatogenesis (probably at the level of primary and secondary spermatocyte), absence of spermatides and many apoptotic bodies in *Insr*/*Igf1r*-DKO mice.
Conclusion: Preliminary results suggest that insulin and IGF1 receptors in steroidogenic cells probably are important for normal morphology of seminiferous tubules as well as expression of mitochondrial biogenesis/architecture markers.

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THE EXPRESSION OF KEY REGULATORS OF MITOCHONDRIAL BIOGENESIS IS DISTURBED IN STEROIDOGENIC CELLS OF PREPUBERTAL TESTES, BUT NOT OVARIES IN MICE WITH DELETION OF GENES FOR INSULIN AND IGF1 RECEPTORS IN STEROIDOGENIC TISSUES

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Introduction: Controlled changes in mitochondrial biogenesis and morphology are required for cell survival and homeostasis, but the molecular mechanisms are largely unknown.
Aim: To investigate the role of insulin and IGF1 receptors in the expression of the key regulators of mitochondrial biogenesis (*Ppargc1a, Ppargc1b, Pparg, Nrf1, Tfam*) in Leydig cells, ovaries and adrenals, as well as mitochondrial architecture (*Mfn1, Mfn2, Opa1*) in Leydig cells.
Methods: Functional genomics: prepubertal mice (P21), with *Insr* and *Igf1r* deletion in steroidogenic tissues (*Insr/Igf1r*-DKO). Gene expression was followed by RQ PCR and western blot, while hormones level was measured by RIA.
Results: The expression of PGC1, the master regulator of mitochondrial biogenesis and its downstream target *Tfam*, significantly decreased in testosterone-producing Leydig cells from *Insr/Igf1r*-DKO animals. This was followed by reduction of *Mtnd1*, the core subunit of the NADH dehydrogenase belonging to the minimal assembly required for catalysis. Transcription of mitochondrial biogenesis markers remained unchanged in ovaries. In adrenals, the pattern was similar in both sexes and for most markers opposite from Leydig cells. The transcription level of mitochondrial architecture markers (*Mfn1, Mfn2*) significantly increased in Leydig cells from *Insr/Igf1r*-DKO mice suggesting that the mitochondrial architecture and mitochondrial phase of steroidogenesis were affected in males. In the same cells, MAPK signaling, downstream mediator of insulin and IGF1 receptors and hormone productions were disturbed.
Conclusion: The insulin and IGF1 receptors are important for mitochondrial biogenesis in gonadal steroidogenic cells of prepubertal males, but not females, and regulate both steroidogenesis as well as mitochondrial biogenesis/architecture.

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CHLAMYDIA TRACHOMATIS INFECTION AND DEVELOPMENT OF EPITHELIAL MESENCHYMAL TRANSITION IN CONJUNCTIVA: POSSIBLE EPIGENETIC MECHANISMS UNVEILED

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Introduction: Trachoma is the most common cause of infectious blindness worldwide, initiated by repeated infection of the conjunctiva with *Chlamydia trachomatis* (*Ct*). The resulting chronic inflammation and formation of fibrotic tissue eventually lead to corneal damage. Based on the facts that epithelial to mesenchymal transition (EMT) plays an important role in the development of fibrosis and that EMT is epigenetically regulated process, the aims of this study were to reveal the capacity of *Ct* to induce EMT *in vitro* and to unveil potential underlying epigenetic mechanisms.
Methods: Human conjunctival epithelial (HCjE) cells were infected with 10^7 IFU of *Ct* for 72 h. EMT-inducing signaling pathways, as well as mRNA and protein expression of EMT markers (E-cadherin, fibronectin and a-SMA) were evaluated by RT-qPCR, Immunoblotting and Immunocytochemistry. DNA methylation patterns of selected regions of E-cadherin, fibronectin and a-SMA genes were examined by Methylation-Specific PCR, High Resolution Melting analysis and Bisulfite Sequencing.
Results: Infection with *Ct* was accompanied with the activation of EMT-inducing signaling pathways, downregulation of epithelial marker E-cadherin and upregulation of mesenchymal markers fibronectin and a-SMA. While DNA methylation status of E-cadherin gene promoter correlated with its expression, methylation status of fibronectin and a-SMA genes couldn't be related to their expression levels.
Conclusion: *Ct* infection of HCjE cells triggers EMT that goes along with changes in the methylation profile of the E-cadherin promoter. Sequence of events described herein could contribute to scarring process in trachoma and open up possibilities for development of new therapeutic strategies in trachoma treatment.

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FULLERENOL/IRON NANOCOMPOSITE MODULATES DOXORUBICIN-INDUCED HEPATOTOXICITY

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Introduction: Doxorubicin is the most prominent chemotherapeutic, but its clinical use is limited by its severe systemic toxicity. An iron overload aggravates anthracycline toxicity. Fullerenol in aqueous solutions is in the form of polyanionic nanoparticles, serving as a good carrier of positively charged ions, such as Fe^{2+}. Fullerenol's antioxidant activity has already been proved in different biological systems. The aim of our study was to investigate the effects of the fullerenol/iron nanocomposite on the rat liver as a pretreatment to doxorubicin application.
Methods: After the 24h-treatment, adult male Wistar rats were sacrificed and livers were collected for ultrastructural and qRT-PCR analysis. Considering the ability of doxorubicin to induce oxidative stress, and the fullerenol's capability to mitigate it, gene expression of enzymes involved in antioxidant defense was measured.
Results: Ultrastructural analysis revealed that liver tissue was mainly preserved after the nanocomposite was applied prior to doxorubicin. However, the hepatocytes of animals treated with doxorubicin, presented significantly damaged morphology. Apoptosis of hepatocytes and endothelial cells, mitochondria of irregular size and with disruption of cristae, diffuse injury of capillaries were observed. RT-PCR results have shown that treatment with doxorubicin alone significantly increase the mRNA levels of catalase ($p=0.008$) and superoxide-dismutase ($p=0.000003$), while the pretreatment with the nanocomposite prior the doxirubicine treatment, dramaticly downregulated the mRNA levels of catalase ($p=0.0004$) and superoxide-dismutase ($p=0.0001$).
Conclusion: Our results suggest that the fullerenol/iron nanocomposite applied as pretreatment to doxorubicin, demonstrated protection to the liver tissue and induced less damage to the hepatocytes in comparison to doxorubicin alone.

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HSP 70 EXPRESSION LEVEL IN SHORT- AND LONG-LIVED POPULATIONS OF THE SEED BEETLE (*Acanthoscelides obtectus* Say)

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Introduction: Positive correlation between stress resistance and longevity is widely recognized. Among many overlapping mechanisms underlying these traits, up-regulation of heat shock proteins (Hsps), molecular chaperons, is suggested to be of major significance due to their role in preventing protein damages. We tested the above issues in laboratory populations of the seed beetle.
Material and Methods: For this experiment we used short-lived and long-lived populations obtained after long-term selection for early (E) and late reproduction (L), respectively. Longevity and ageing dynamics were assessed in control beetles reared at 30°C and in beetles exposed to heat shock (40°C for 1 h). Constitutive and heat shock induced Hsp70 expression levels were determined in 1 day old females and males, using SDS polyacrylamide gel electrophoresis and Western blotting analysis.
Results: Longevity was slightly increased in heat shocked beetles regardless of selection regime or sex. With the exception of L females this trend was also followed by increased Hsp70 level. Constitutive Hsp70 expression was higher in females than males, and in L than E beetles. Also, selection for increased longevity appeared to be related to more plastic Hsp70 expression to heat shock.
Conclusion: Our results indicate that Hsp70 might be involved in stress resistance and evolution of ageing in the seed beetle.

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TRANSDIFFERENTIATION OF PANCREATIC ALPHA TO BETA CELLS USING EPI-CRISPR DIRECTED DNA METHYLATION

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Introduction: Since diabetes is characterized by impaired ability of pancreatic beta-cells to respond and/or produce insulin, new approaches for renewal and replacement of deficient beta-cells are indispensable. The aim of this study is direct pancreatic alpha- to beta-cells transdifferentiation by using a new synthetic epigenetic tool, Epi-CRISPR system. Using Epi-CRISPR system we aim to introduce targeted DNA methylation and subsequent repression of genes responsible for maintaining alpha-cell identity.
Methods: AlphaTC1-6 cells (a-cells) were transiently transfected with dCas9-Dnmt3a-Dnmt3L constructs and one or four different vectors containing guide RNA components for specific targeting the promoter region of aristaless-related homeobox gene (*Arx*). The success of a-cells transdifferentiation into insulin-producing cells was evaluated by measuring *Arx* and insulin mRNA level, amount of secreted insulin and by immunostaining of insulin/glucagon in the cells.
Results: We observed *Arx* transcriptional repression in a-cell transfected with Epi-CRISPR construct that targets the *Arx* gene promoter inducing subsequent methylation. At fifth day post-transfection the expression of *Arx* was decreased in a-cells followed by consequent increase in insulin (mRNA and protein level). At the same time, the glucagon levels remained unchanged. At twelfth day post-transfection the transfected cells start to lose glucagon while still secreting insulin.
Conclusion: This study is near to confirm Epi-CRISPR system functionality and to verify the concept of cell transdifferentiation through silencing of genes responsible for maintaining cell phenotype. The obtained results will be valuable for later Epi-CRISPRs use in mouse *in vivo* models of diabetes and eventually as a future therapy for diabetes attenuation in humans.

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COULD N-(2-nitooxyethyl)-1H-indol-2-carboxamide BECOME NOVEL ANTICANDIDAL AGENT?

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Introduction: Pathogenic yeast are gaining resistance to drugs that are currently used in clinic, also information about side effects of different drugs are more familiar so there is constant need for development of new antimicrobials.
The aim: N-(2-nitooxyethyl)-1H-indol-2-carboxamide, novel azole compound, is examined to test its potential to inhibit growth of pathogenic yeast *Candida albicans*.
Methods: Four *C. albicans* strains were isolated from oral cavities of patients. Microdilution method was used to test anticandidal effect. Impact on biofilm formation was evaluated after 24 hours treatment of cells with subinhibitory concentration of compound. Inhibition of candidal CYP51 protein was examined as possible mode of action. Ketoconazole was used as control and potential synergistic action of these compounds was examined.
Results: All tested strains were sensitive to N-(2-nitooxyethyl)-1H-indol-2-carboxamide with MIC 0.056-0.224 and MFC 0.112-0.448 mg/ml, but more sensitive to ketoconazole with MIC 0.00156-0.00312 and MFC 0.00624-0.1 mg/ml. Both compounds inhibited biofilm formation, in ½MIC N-(2-nitooxyethyl)-1H-indol-2-carboxamide inhibited formation of *C. albicans* biofilm for 61%, while ketoconazole caused 73% inhibition. Inhibition of CYP51, enzyme involved in ergosterol biosynthesis, is potential mode of action since N-(2-nitooxyethyl)-1H-indol-2-carboxamide inhibited it with K_d 4.19 μM, and showed selectivity since it didn't bound to CYP51 enzyme of human origin, while ketoconazole inhibited both enzymes of human and candidal origin with K_d<0.05 μM. Compounds didn't show synergistic, only additive effect (FIC=1.5).
Conclusion: N-(2-nitooxyethyl)-1H-indol-2-carboxamide has promising antimicrobial and antibiofilm activity although lower than ketoconazole. Possible mode of action is examined and selectivity of N- (2-nitooxyethyl)-1H-indol-2-carboxamide is great advantage.

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EXPRESSION OF *ldb3a* GENE IN MECHANICAL STRESS RESPONSE OF ZEBRAFISH (*Danio rerio*) SKELETAL MUSCLE

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Introduction: Mammalian LDB3 (ZASP, CYPHER), a member of the PDZ-LIM family, is a cytoskeletal protein localized in the sarcomeric Z-disk, playing a critical role in maintenance of cardiac structure and function. In zebrafish heart and skeletal muscle thirteen alternative spliced forms are coded by *ldb3a* gene. Knock-down experiments indicate its importance for normal somite formation and heart development. Since *LDB3* expression is downregulated in exercised human muscles, the aim of this work was to test if *ldb3a* expression is regulated by mechanical stress in zebrafish.
Methods: Adult zebrafish were subjected to force swimming test. *Ldb3a* expression level and isoform profiles in trained and control animals were determined by qRT-PCR and western blot. Intracellular localization of endogenous Ldb3a was analyzed by immunohistochemistry.
Results: Expression of *ldb3a* transcripts was unaffected in heart, but down-regulated in skeletal muscles of trained zebrafish. Using antibodies to full length human LDB3 and its domain encoded by exon 6, present only in skeletal muscle isoforms, we detected several zebrafish isoforms in skeletal muscle extracts. No significant differences in the quantity or pattern of detected isoforms were observed between skeletal muscles of trained and control animals, probably due to the sensitivity limitations of the western blot. We showed exclusive sarcomeric localization of the Ldb3a in zebrafish skeletal muscle, although we were not able to correlate them to specific isoform.
Conclusion: The role of *ldb3a*/*LDB3* in mechanical stress response is evolutionary conserved, its expression is down-regulated in trained zebrafish skeletal muscles, similarly to human muscles.

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PROFILE OF MITOCHONDRIAL BIOGENESIS MARKERS AND ACROMOSOMAL REACTION ARE DISTURBED IN SPERMATOZOA FROM STRESSED ADULT RATS

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Introduction: Although psychophysical stress is the most common stress in human society and major contributor to wide variety of pathological conditions, the molecular adaptation of spermatozoa from stressed males were not described well.
Aim: The aim was to determine the functionality and molecular adaptation of spermatozoa from stressed rat by applying *in vivo* and *in vitro* approach.
Methods: For *in vivo* experimental model, psychophysiological stress by immobilization (IMO), was performed for 3 hours in different time during the day (03^{00}h,07^{00}h,15^{00}h), for one (1xIMO) or ten (10xIMO) consecutive days. For *in vitro* approach, epididymal spermatozoa from undisturbed rats were stimulated with stress hormones adrenaline and cortisol for 30 minutes.
Results: Results showed that number of spermatozoa significantly decreased in all 10xIMO rats comparing to control. Acrosomal status (response to acrosome-reaction-inducer progesterone) significantly decreased in spermatozoa from 1xIMO and 10xIMO rats comparing to control. The same effect was observed in spermatozoa stimulated *in vitro* with stress hormones. Preliminary RQ-PCR results revealed that transcription of the main mitochondrial biogenesis markers *Nrf1*, *Ppara* and *Ppard* decreased in spermatozoa from 10xIMO rats. In the same spermatozoa samples the similar effect was registered for *Ucp2*, the mediator of regulated proton leak. Oppositely, the significant increase of *Cytc* transcription was registered in spermatozoa from 10xIMO rats. Incubation of spermatozoa with adrenaline decreased level of *Ppargc1a* and *Nrf2a* transcripts, while cortisol decreased expression of mitochondrial transcription factor TFAM.
Conclusion: Repeated psychophysical stress decreased the number and functionality of spermatozoa and disturbed transcriptional profile of their mitochondrial biogenesis markers.

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MELATONIN MODULATES CATECHOLAMINE BIOSYNTHESIS AND RE-UPTAKE IN ADRENAL MEDULLA OF RATS EXPOSED TO CHRONIC UNPREDICTABLE MILD STRESS

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Introduction: Stress is considered a determinant in the etiology of depression. The adrenal medulla plays a key role in response to stress by releasing catecholamines, which are important to maintain homeostasis. Many studies have assessed the antidepressant-like activity of the melatonin, a neurohormone synthesized in the pineal gland. Thus, in the present study, we examined the effect of chronic melatonin treatment on mRNA levels and protein content of catecholamines biosynthetic enzymes (TH, DBH and PNMT) and transporters (NET, VMAT2) in adrenal medulla of rats exposed to chronic unpredictable mild stress (CUMS).
Material and Methods: CUMS was used as an animal model of depression. Exposure of rats to CUMS and placebo or melatonin (10 mg/kg body weight, i.p.) administration started on the same day and was continued for 4 weeks. For quantifying TH, DBH, PNMT, NET and VMAT2 mRNA and protein levels we used real-time PCR and Western blot analysis.
Results: We observed that CUMS induced increased mRNA levels of catecholamine biosynthetic enzymes (TH, DBH, and PNMT), and noradrenaline transporter NET, while treatment with melatonin decreased these biosynthetic enzymes and transporter. Conversely, CUMS induced a decrease in protein content of TH and NET, while chronic melatonin treatment increased NET protein levels in both control and stressed rats. CUMS and melatonin treatment has no effect on mRNA levels and protein content of VMAT2.
Conclusion: This study suggests that the observed decrease of catecholamine biosynthesis and enhanced re-uptake in adrenal medulla of rats exposed to CUMS are connected to the beneficial effects of chronic melatonin treatment.

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HISTONE MARKS ON THE PROMOTERS OF HUMAN *OCT4* AND *NANOG* GENES IN THE EARLY PHASES OF NEURAL DIFFERENTITATION OF NT2/D1 CELLS

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Introduction: Transcription factors OCT4 and NANOG, together with SOX2, are main constituents of a complex regulatory circuitry that controls stem cell renewal, pluripotency and early lineage decisions. In the present study we investigated expression profiles of OCT4 and NANOG during the early phases of neural differentiation using NT2/D1 cells induced by RA as an *in vitro* model system of human neurogenesis. Furthermore, in the same experimental settings we analyzed selected histone marks deposited on the promoters of *OCT4* and *NANOG* genes.
Methods: OCT4 and NANOG protein expression profiles during early phases of RA-induced neural differentiation of NT2/D1 cells were analyzed using Western blot and immunocytochemistry. Selected H3 and H2B histone modifications on the promoters of *OCT4* and *NANOG* genes were investigated by ChIP-qPCR.
Results: We have demonstrated significant decrease of OCT4 and NANOG protein levels at day 2 of RA treatment, with further decline to undetectable levels during the following days of neural differentiation. We have shown decline in H3K4me3, H2BK5ac and H2BK120ac marks on both *OCT4* and *NANOG* promoters which paralleled the decrease in their expression levels. Moreover, we have detected differential enrichment of H2BK16ac on these two promoters, pointing out to differences in epigenetic regulation of *OCT4* and *NANOG* genes expression.
Conclusion: Based on the presented data, we suggest that the first response of pluripotency genes *OCT4* and *NANOG* to the differentiation-inducing stimuli is mediated by the dynamic changes in chromatin marks, which precedes DNA methylation acquired in the later stages of neurogenesis.

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ANALYSIS OF MITOCHONDRIAL DNA CONTROL REGION IN THE DOMESTIC DOG

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Introduction: Dog trace material is often encountered in forensic cases, since dogs are very present in human society. Consequently, evidence connecting suspects with victims and crime scenes can be provided by analysis of dog DNA found on a crime scene. Most frequent dog traces found are hairs, which usually contain degraded nuclear DNA, and therefore mitochondrial DNA (mtDNA), not autosomal STRs, is more suitable for further analysis. Dog discrimination can be achieved by analyzing the two hypervariable regions of the non-coding control region (CR) of mtDNA, where more than 100 single nucleotide polymorphisms (SNPs) have been described.
Objective: To determine diversity of mtDNA haplotypes in order to assess the evidential power of a mtDNA CR haplotype,
Methods: Samples of 20 dogs from 10 breeds were subjected to analysis. DNA was extracted from buccal swab samples and, subsequently, the two hypervariable regions of the non-coding control region of mtDNA were amplified by PCR. Purified PCR products were directly sequenced and analyzed on ABI 3130 Genetic analyzer.
Results: After comparing obtained sequences with dog mtDNA reference sequence, 10 different haplotypes were observed. A clear distinction was found within breeds as well as among different breeds.
Conclusion: Analyzed region was found to be useful to associate a dog hair with its suspected source. But, in order to improve mtDNA discrimination power for more precise individualization of the domestic dog, in addition to the CR analysis, analysis of whole coding region of mtDNA should be done.

GENETIC DIVERSITY ANALYSIS USING MICROSATELLITE MARKERS FOR SELECTION OF POLLINATORS IN HYBRID SUGAR BEET BREEDING

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Introduction: Heterosis is an important method of increasing yield and improving quality in crops. Identifying combination of inbred lines with strong heterosis is the most important step in developing hybrids. The genetic diversity of parental lines has been proposed as a predictor of hybrid performance and heterosis.
Methods: The genetic diversity of 12 diploid sugar beet pollinators and 2 cytoplasmic male sterile lines were analyzed using 40 SSR loci. Depending on the presence of self-fertility gene pollinators classified as self-sterile (S^s) or self-fertile (S^f).
Results: In total, 129 SSR alleles were identified, with an overall 3.1 for S^s pollinators and 3.0 for S^f pollinators. The number and percentage of polymorphic loci were the highest in NS1 pollinator and CMS1. Among tested genotypes pollinators CR10 (Na=2.0; Ne=1.6; He=0.3) and FC220 (Na=1.9; Ne=1.6; He=0.3) exhibited the lowest level of variation, whereas pollinators EL0204 (Na=2.6; Ne=2.1; He=0.5) and NS3 (Na=2.6; Ne=2.0; He=0.5) had the highest. The genetic distance between the pollinators and the CMS lines was higher in the S^f than in the S^s crosses, but it was generally low indicating that the genetic base of the investigated germplasm was narrow. Cluster and correspondence analysis grouped sugar beet pollinators according to their origin.
Conclusion: Genetic diversity analysis of sugar beet pollinators is useful for breeding programs, as it helps in selecting the appropriate genetic material for the classification of parental lines, heterotic groups and predicting hybrid performances.

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EXPRESSION OF STRESS AND ENDOCRINE DISRUPTION RELATED GENES IN LIVER OF CAGED COMMON CARP (*Cyprinus carpio* (L.), CYPRINIDAE) – ASSESSING *IN SITU* EFFECTS OF UNTREATED SEWAGE INTO THE RIVER DANUBE

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Introduction: In the city of Novi Sad, untreated sewage discharges into the River Danube. In the previous study, water samples from this spot have been chemically characterized and their toxic potency was confirmed in a battery of *in vitro* assays. However, gene expression changes in organisms exposed to chemical stress present sensitive indicators of toxicant response *in situ*.
Aim: To check if the *in vitro* observed biological effects can be seen *in situ*, through expression of selected stress and endocrine disruption related genes in liver of common carp caged at the Novi Sad hot spot locality.
Methods: Fishes were caged at three sites in the River Danube: upstream the Novi Sad major sewage discharge, 700 m and 7 km downstream the discharge. After a nine day exposure, body indices were measured, and gene expression in fish liver was analyzed using qRT-PCR.
Results: Gonadosomatic index was decreased in males caged at downstream localities. Stress representative genes for heat shock protein 70 (*hsp70),* cytochrom oxidase subunit 1 (*cox1),* and cortisol receptor (*cr*) and endocrine disruption related genes for estrogen receptor alpha (*era) and* beta (*erβ)* in liver of specimens caged at downstream localities follow the trend of overexpression in both sexes, while the gene for vitellogenin (*vtg)* was down-regulated in females and up-regulated in males.
Conclusion: The results indicate induction of adaptive stress responses and endocrine disruption, contribute to estimation of impact of chemical stress alone and comprehensive linking of identified chemical pollution to stress responses of fish *in situ*.

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LONG-TERM DIETARY RESTRICTION MODULATES INSULIN SIGNALING PATHWAY IN AGING BRAIN

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Introduction: Dietary restriction is one of the most investigated and most widely used experimental intervention in aging research. So far, little is known about interventions that may modulate normal brain aging. Considering its very specific energy demands it is important to determine the effect of diets on age-related changes in brain. Insulin is a master regulator of corporeal aging in all known species, and also plays an important role in brain aging and cognitive decline associated with pathological and normal brain aging. The largest number of insulin receptors are located in the hippocampus, a region associated with learning and memory. Here, we investigated whether dietary restriction (DR) can influence hippocampal insulin signaling pathway in aging rats.

Methods: We used adult (6-month-old), aged (18-month-old) and old (24-month-old) male Wistar rats fed ad libitum (AL) or exposed to dietary restriction (DR, 60% of AL daily intake starting from 6-months of age). The expression of insulin, insulin receptor β (IRβ), insulin receptor substrate 1 (IRS1), protein kinase B (Akt) and their phosphorylated forms were determined by Western blot analysis.

Results: We detected significant increase in protein levels of insulin, IRS1, phospho-IRS1 and Akt, and a decrease in phospho-IRβ levels in 24-month-old animals on DR compared to adult animals. In aged animals on DR, we noticed significantly decreased levels of phospho-IRβ and phospho-IRS1, compared to 6-month-old animals.

Conclusion: We demonstrated DR's ability to modify several elements of hippocampal insulin signaling pathway during physiological brain aging, still the exact mechanism underlying these changes needs further analysis.

Acknowledgements: This study was supported by the grant no. 173056 from the Ministry of Education, Science and Technological Development, Republic of Serbia.

AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION OF *Viola cornuta*

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Introduction: *Viola cornuta* is a valuable perennial ornamental plant. Development of new traits, such as new flower color with classical breeding suffers from many difficulties, which can be overcome using genetic engineering. With aim to develop protocol for *Agrobacterium*-mediated transformation of *V. cornuta,* we used *Agrobacterium tumefaciens* strain LBA4404 harbouring the superbinary vector pTOK233 carried a GUS reporter gene and hygromycin phosphotransferase selectable marker gene.
Methods: Hypocotyl explants obtained from seedlings were grown on ½MS medium supplemented with 0.1 mg/l 2,4-D and 2.0 mg/l BA for shoot induction. After two days of pre-cultivation, hypocotyl explants were inoculated in bacterial suspension for 15 min and placed on the same culture medium with addition of acetosyringone 100 μM at pH 5.2. After two days of co-cultivation, explants were transferred on shoot induction medium supplemented with cefotaxime and hygromycin B for selection. Regenerated putative transformants were analyzed by PCR for hygromycin phsphotransferase gene presence and by histochemical assay for β-glucuronidase (GUS) activity.
Results: Shoots were obtained within 8 weeks after explants were inoculated with *A. tumefaciens*, with 2.0% regeneration efficiency. PCR analysis confirmed selectable marker gene presence in twelve out of sixteen (75.0%) independently derived putatively transformed lines that were tested. Additionally, all analyzed lines exhibited a notable β-glucuronidase activity that was not present in untransformed plants.
Conclusion: This is the first report about *V. cornuta* susceptibility to *A. tumefaciens*. Presented protocol for genetic transformation can be used for further introduction of desirable traits in *V. cornuta* cultivars.

Acknowledgements: This study was supported by the grant of no. TR31019 from the Ministry of Education, Science and Technological Development, Republic of Serbia.

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF ACETONE EXTRACTS OF *Hydnum repandum* AND *Craterellus cornucopioides* MUSHROOMS

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Introduction: The aim of this study was to evaluate the biological activities (cytotoxic, antioxidative, antimicrobial, anticancer, genotoxic and antigenotoxic) of acetone extracts of *Hydnum repandum* (HR) and *Craterellus cornucopioides* (CC) mushrooms.
Methods: Polyphenolic composition was determined by HPLC. The cytotoxic activity was tested using MTT method on human epithelial carcinoma HeLa cells, human lung carcinoma A549 and human colon carcinoma LS174 lines. Antioxidative activity was evaluated by free radical scavenging, super oxide anion scavenging and reducing power. Antimicrobial activity was determined by microdilution method on ten species of microorganisms. Genotoxic effect was determined by cytokinesis block micronucleus (MN) assay on cultured human peripheral blood lymphocytes, separately and in combination with mitomycin C (MMC) for assessment of antigenotoxic effect.
Results: Both extracts contained quercetin as major flavonoid and ferulic acid as major phenolic acid. CC showed higher cytotoxic activity in all malignant cell lines, with IC_{50} values ranging 65.53-131.72 μg/mL. CC expressed higher free radical scavenging activity (IC=19.73 μg/mL), while HR had stronger superoxide anion scavenging potential (IC=19.28 μg/mL). HR showed better antimicrobial activity, with minimum inhibitory concentration values ranging 0.0097-0.0078 μg/mL. HR extract showed genotoxic effect in two highest tested concentrations (100 and 200 μg/mL), while CC did not show genotoxic effects. Combined treatment with MMC reduced MMC-induced MN in a dose dependent manner for both mushroom extracts.
Conclusion: Rich polyphenolic composition of tested mushrooms is the basis of positive biological activities which both species exhibit and that is why they should be an important supplement of human diet.

Acknowledgements: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Projects No. III41010; 173032).

ACTIVITY OF LACTATE DEHYDROGENASE AND CITRATE SYNTHASE IN THE LIFE CYCLE OF THE EUROPEAN CORN BORER *Ostrinia nubilalis* Hbn.

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Laboratory of biochemistry and molecular biology, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia.

Introduction: European corn borer, *Ostrinia nubilalis* Hbn., is a pest lepidopteran species whose larvae avoid harsh environmental winter conditions by entering a dormant state – diapause. In order to investigate metabolic changes, more precisely aerobic – anaerobic shift in metabolism, we measured activities of citrate synthase (CS) and lactate dehydrogenase (LDH) in the whole body homogenates of pupae (P), non-diapausing (ND) and diapausing larvae (D). Because of a close connection of cold hardiness and diapause in this species, diapausing larvae were also exposed to low temperatures (5°C, -3°C, and -16°C).
Methods: Activities of CS and LDH were measured spectroscopically by continuous method.
Results: The results showed that the highest activity of CS was detected in non-diapausing larvae, which reflects high level of oxidative metabolism during active life. On the other hand, CS activity of the diapausing larvae was decreased, but remained stable even after low temperature exposure, enabling low-rate mitochondrial oxidation of lipid reserves. Contrary to CS, highest LDH activity was recorded in the diapausing larvae, evidencing hypomethabolic and anaerobic-preffering conditions in dormant state. In such conditions, LDH activity is rather directed towards oxidation of lactate to pyruvate which, later on, is converted to tricarboxilic amino-acids, as well as glycerol, a major cryoprotectant of this species.
Conclusions: Results of this study indicate that the CS activity is probably developmentally regulated and refractory to low temperatures, while the activity of LDH seems to be more influenced by temperature, especially those slightly below zero (-3°C).

Acknowledgements: This study was supported by the grant no. 173014 from the Ministry of Education, Science and Technological Development, Republic of Serbia.

DIFFERENT FORMS OF MIF ARE EXPRESSED AND SECRETED BY CHORIOCARCINOMA AND NORMAL TROPHOBLAST CELLS

Aleksandra Vilotić, Milica Jovanović Krivokuća, Žanka Bojić-Trbojević, Ljiljana Vićovac

Institute for the Application of Nuclear Energy, INEP, University of Belgrade, Belgrade, Serbia.

Introduction: MIF is a multifunctional cytokine expressed by various cell types including trophoblast. Recently, MIF has been described as an important regulator of tophoblast cell invasion. We examined the expression of MIF in immortalized first trimester of pregnancy extravillous trophoblast cells HTR-8/SVneo and choriocarcinoma cell lines JAr and Jeg-3.
Methods: The expression of MIF and its receptor CD74 was investigated at mRNA level using quantitative real-time PCR. Cell-type specific levels and distribution were studied using immunocytochemistry and native electrophoresis in whole cell lysates and subcellular fractions, as well as in conditioned media.
Results: There was no significant difference in the levels of neither *MIF* mRNA nor MIF protein in the three cell types studied. CD74 mRNA expression however, was significantly higher in transformed compared to normal trophoblast - 13.5 fold for JAr and 12-fold for Jeg-3 ($p<0.01$). Native electroforesis revealed a persistent form of MIF at ~140 kDa in Jeg-3 lysates and conditioned media, which was absent from HTR-8/SVneo cell lysates, and only sporadically appeared in JAr cell lysates. Cellular fractionation showed that this band was present in cytoplasmic fraction. In all three cell lines MIF was located in nuclear chromatine fraction, and to a lesser degree in cellular membrane fraction.
Conclusion: Based on significantly higher expression of CD74 on transformed trophoblast cells and expression/secretion of specific form of MIF by Jeg-3 cells we could hypothesise that MIF might be linked to uncontrolled invasion of choriocarcinoma cells.

Acknowledgement: This work was funded through project 173004 of the Ministry of Education, Science, and Technological Development, Republic of Serbia.

Session
BIOMEDICINE
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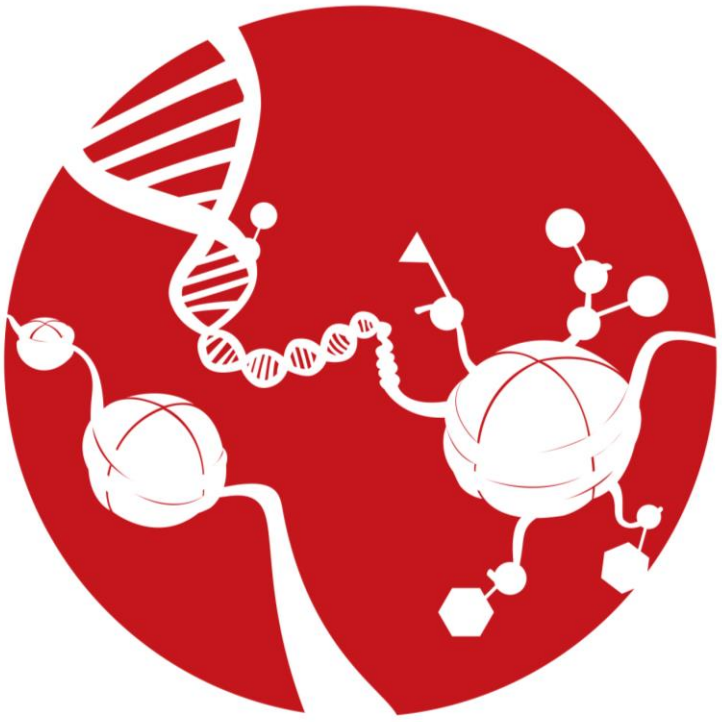
Plenary lectures



TRANSLATIONAL CONTROL OF ENERGY HOMEOSTASIS

Ivan Topisirović (Kanada / *Canada*)

Ivan Topisirović, PhD
Assistant Professor
Department of Oncology
McGill University
Montreal, Canada

Research Interests:

His laboratory is interested in studying the molecular mechanisms which underlie the role of mRNA translation in modulating growth (increase in cell volume) and proliferation (increase in cell number) of normal and malignantly transformed cells. Rates of cell growth and proliferation are modulated by signaling pathways in response to various extracellular stimuli and intracellular cues. The mammalian target of rapamycin (mTOR) pathway is a major regulator of mRNA translation, cell growth and proliferation, and it is frequently dysregulated in human diseases such as cancer, diabetes and heart disease. The main focus of his future studies will be to determine how the effects of mTOR on mRNA translation influence cell growth and proliferation. Changes in growth and proliferation rates are also characterized by alterations in gene expression. To achieve optimal growth and proliferation rates in a given environment, cells need to coordinate the expression of a subset of genes which need to be expressed at specific time points. He aims to elucidate post-transcriptional regulatory networks which play a role in coordinating the expression of genes which regulate cell growth and proliferation and investigate the mechanisms which lead to aberrant function of these networks in human disease.

Source web site: http://www.ladydavis.ca/en/ivantopisirovic/

MISLOCALIZATION AND AGGREGATION OF RNA BINDING PROTEINS IN ALS AND FTLD

Boris Rogelj (Slovenija / *Slovenia*)

Boris Rogelj, PhD
Professor
Odsek za biotehnologijo (B-3)
Institut "Jožef Stefan"
Ljubljana, Slovenija

Research Interests:

Proteins TDP-43 and FUS are major contributing factors in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). We identified neighbouring mutations in a highly conserved region of TDP-43 and FUS in sporadic and familial ALS cases. Furthermore we have observed changes in degradation and location of both proteins in temporal lobe tissues in post-mortem tissues of patients with FTD. They are both predominantly nuclear proteins that are implicated in processing and transport of RNA. We have also characterized their RNA-binding properties as well as shown disease relevance of their nuclear transport.
We discovered that p62 is a major marker for ALS/FTLD associated with newly discovered hexanucleotide repeat mutation in C9orf72 gene and shown that the hexanucleotide repeat RNA sequesters hnRNPH in neurons. In addition, we have neuropathologically characterised optineurin in the context of ALS and FTLD and in a global analysis of alternative splicing changes in aging and neurodegeneration we discovered some correlations between the two processes.

Source web site: http://bio.ijs.si/biotech/brogelj.html

Session
BIOMEDICINE
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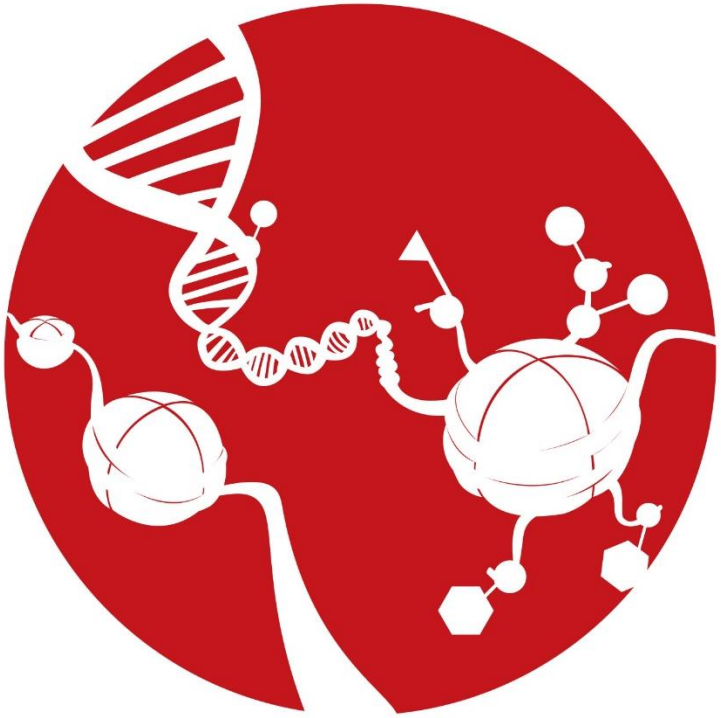
Invited lectures



GENETIC BASIS OF PROSTATE CANCER: ASSOCIATION STUDIES

Goran Brajušković[1], Zorana Nikolić[1], Ana Branković[2], Nevena Kotarac[1], Dušanka Savić-Pavićević[1]

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Prostate cancer (PCa) is the most common malignancy in men. This paper reviews the results of previous work of the study group PROSTATSERBIA. In the candidate gene association studyat the beginning of our research, we tested the association between several single nucleotide polymorphisms (SNPs) in the NOS3 gene and PCa riskand/or progression. In apopulation-based case-control study, we explored the possible association between PCa risk and seven SNPs identified by genome-wide association analyses (GWASs) in two chromosomal regions (8q24 and 17q12). For the first time in a European population, microRNA genetic variants and genetic variants in RNA-induced silencing complex (RISC) genes have been analyzed for their potential association with PCa.

Acknowledgements: Grant No. 173016, MESTD, Republic of Serbia.

ZEBRAFISH (*Danio rerio*) IN DECIPHERING MOLECULAR MECHANISMS OF HUMAN DISEASES

Snežana Kojić, Srđan Bošković, Jovana Jasnić, Nemanja Stamenković and Dragica Radojković

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11010 Belgrade, Serbia

Biomedical research increasingly exploits zebrafish (*Danio rerio*) for genetic disease modeling and medical genetic research with the main goals of deciphering and understanding disease processes, and identifying new molecular markers and therapeutic targets. Zebrafish has emerged as a high-throughput and low-cost model organism based on the advantages that include the availability and ease of generating mutations in homologous disease-causing genes, the ability of noninvasive imaging for the analysis of phenotypes of different organs in an intact animal, and the suitability of zebrafish larvae for large-scale chemical screens. Identification of causative genes in human diseases and their functional characterization is enabled by forward and reverse genetic manipulation tools including CRISPR/Cas9-based genome editing. Here we review the use of the zebrafish for biomedical research, with a focus on tumors, cardiovascular diseases and myopathies.

Acknowledgments: Zebrafish research in the Laboratory for Molecular Biology, IMGGE, is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 173008 to DR, and 451-03-01766/2014-09/3 to SK). We apologize to our colleagues whose work we were not able to cite due to space limitations.

GENOMICS AS A BASIS FOR PRECISION MEDICINE

Sonja Pavlović, Maja Stojiljković, Nataša Tošić, Branka Zukić, Milena Ugrin, Teodora Karan-Djurašević, Vesna Spasovski

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Precision medicine, also known as genome-based medicine and personalized medicine, uses knowledge of the molecular basis of adisease in order to individualize treatment for each patient. The development of novel, powerful, high-throughput technologies has enabled better insight into the genomic, epigenomic, transcriptomic and proteomic landscape of many diseases, resulting in the application of personalized medicine approaches in healthcare. Research in the field of biomedicine in Serbia has followed the modern trendsand has made a great contribution to the implementation of genomics in Serbian clinical practice. This is a review of the state of the art of scientific achievements and their application, which have paved the wayfor personalized medicine in Serbia.

Acknowledgements: This work was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. III 41004).

SOX GENES AS PROGNOSTIC MARKERS AND POTENTIAL THERAPEUTIC TARGETS IN CANCER

Marija Mojsin[1], Nataša Kovačević Grujičić[1], Jelena Marjanović Vićentić[1], Milena Milivojević[1], Isidora Petrović[1], Jelena Popović[1] and Milena Stevanović[1,2,3]

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Human *SOX* genes (SRY-related HMG-box genes) represent a family of transcription factors with essential roles in various developmental processes. They control stem cell pluripotency maintenance, cell fate determination and cell differentiation. In the past decade, the focus on *SOX* gene research changed from their roles in development to their functions in disease, particularly cancer. The growing amount of data has shown *SOX* genes to be amplified in various types of cancer. SOX proteins are involved in cancer cell functions through modulations of signaling pathways and protein-protein interactions. In this paper, we review the roles of *SOX* genes in glioblastoma, nonseminoma testicular germ cell tumors (TGCT) and cervical carcinoma, focusing on our recent findings about the roles of *SOX1*, *SOX2*, *SOX14* and *SOX18* in these cancer types. We also evaluate the potential use of these genes as diagnostic markers, indicators of metastasis and targets in new therapeutic approaches.

Acknowledgments: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 173051.

"TRANSEPIGEN-OMICS" IN CARDIOVASCULAR DISEASE RESEARCH: UNRAVELING THE GENETIC BASIS OF COMPLEX DISEASES

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The genome, methylome, and transcriptome are functional components of a comprehensive network working in the background in support of our health. Thus, an integrative approach in combining and analyzing data from different sources and of different types is necessary in order to improve the understanding of biological processes and biological systems as a whole. In the past two decades, most candidate gene association studies in cardiovascular disease (CVD) have identified genes and variants that affect lipid levels, inflammation and the biology of the vascular wall. Further, a non-candidate-driven approach has yielded the definition of both biologically explainable and novel genes without a known biological background. In summary, the genetic susceptibility to CVD is mainly described by the influence of many common single nucleotide polymorphisms (SNPs), with the small effect size supporting the common disease/common variant hypothesis. Consequently, many integrative concepts were applied in order to distinguish functionally relevant genetic variants, especially noncoding ones. Expression quantitative trait loci (eQTL) analysis refers to the widespread regulation of gene expression mostly by *cis*-acting SNPs. MicroRNAs have also become interesting targets in both research and therapy in atherosclerosis and CVD. A number of miRNAs has been shown to have a role as risk factors for atherosclerosis progression, while some share their atheroprotective effect. The complex and multidimensional nature of cardiovascular risk factors and outcomes could additionally be resolved by research into epigenetic regulation. Distinct epigenomic patterns exist in key DNA elements (promoter CpG islands, intragenic CpG islands, gene bodies and H3K36me3-enriched regions) of the cardiac genome. Moreover, differential expression of each corresponding gene correlates with differential DNA methylation in heart failure. It is clear that the aim of cardiovascular -omics in the next decade is to find better algorithms to integrate as much as possible different types of data into the biological networks underlying the disease phenotype.

Acknowledgments: The Ministry of Education, Science and Technological Development of Republic of Serbia funded the research (Grants III41028 and OI175085).

PERSONALIZED MEDICINE AND DIAGNOSTICS BASED ON WHOLE EXOME AND WHOLE GENOME NEXT GENERATION SEQUENCING

Sanja Mehandziska[1], Aleksandra Stajkovska[1], Margarita Stavrevska[1], Kristina Jakovleva[1], Zan Mitrev[1], Ivan Kungulovski[2], Mirko Spiroski[1], Goran Kungulovski[2]

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Introduction: With the advent of next-generation sequencing (NGS) technologies, the cost of sequencing whole human genomes (WGS) and whole exomes (WES) has dropped dramatically. This drop in price and the development of new and easy to use bioinformatic tools has paved the way for NGS technologies to the clinic and set the foundation for a new wave of diagnostics and therapy, as well as personalized genetics and personalized medicine.
Methods: Nowadays, WES and WGS can be used as first line tools for high-end diagnostics of rare and undiagnosed conditions. In the same vein, WES and WGS approaches are becoming the bedrock of personalized medicine and now patients in our Laboratory of Genetics and Personalized Medicine have the opportunity to act proactively and comprehensively assess their risk for developing cancer, cardiovascular diseases, neurodegenerative diseases, their response to drugs and diet, etcetera. NGS approaches have a prime role in molecular profiling of tumor tissues and serve the oncologists as a starting point for targeted cancer therapy.
Results: Herein, we present our first experiences in genetics based proactive medicine, as well as diagnostic case reports of more than a hundred symptomatic and asymptomatic patients. We also discuss the benefits and pitfalls of different approaches such as Sanger sequencing, NGS gene panels, CES, WES and WGS relating to both – genetic diagnostics and personalized medicine.
Conclusion: Comprehensive NGS approaches open a new era in personalized medicine and diagnostics, and our experience dictates that advances in technology should be complemented with proper education of staff and physicians.

Acknowledgements: The study was carried out as part of the regular diagnostic work in the Zan Mitrev Clinic.

ANNOTATION OF THE FUNCTIONAL IMPACT OF CODING GENETIC VARIANTS

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Coding genetic variants can have profound effects on protein function. Computational tools for the prediction of these effects are used to complement and guide experimental biological studies. Phylogenetic analyses that determine the evolutionary relationship among related sequences are commonly used to distinguish between functionally significant and insignificant gene variations. Here, we have reviewed applications of the non-alignment sequence analyses method for phylogenetic analyses, ISTREE. Furthermore, we assessed how an unsupervised ISTREE-d3 method based on the universal d3 measure responds to this task compared to supervised and semi-supervised ISTREE methods that were previously used in two studies. The findings presented here suggest that ISTREE-d3 can efficiently substitute for the corresponding supervised models, given that it is more suitable for automatic applications. In conclusion, the ISTREE-d3 method has a broad biological relevance and represents a promising approach in functional assessment of coding gene variations.

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OPTIMIZATION, CLASSIFICATION AND DIMENSIONALITY REDUCTION IN BIOMEDICINE AND BIOINFORMATICS

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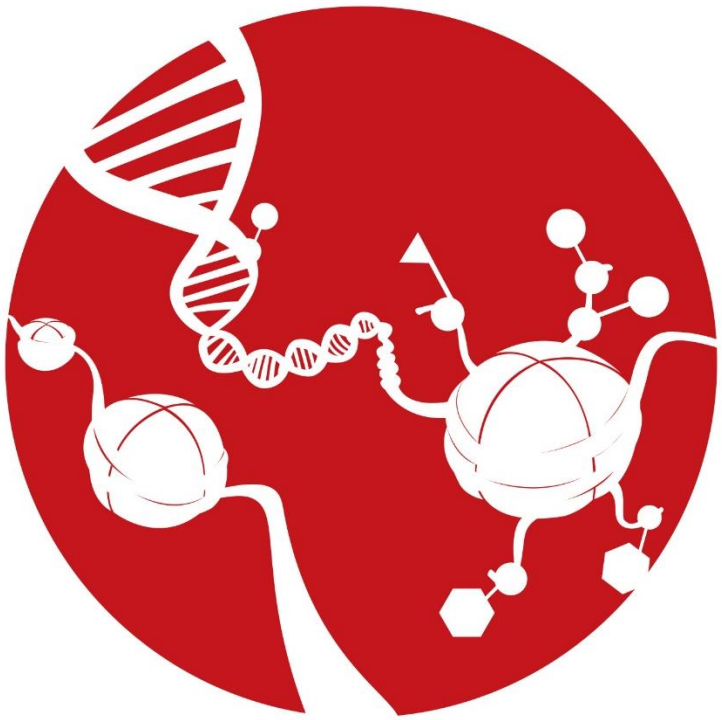
Research in biomedicine isfaced with various problems connected tohigh-throughput processing – the need to handle the high frequency of incoming data and its high-dimensionality by means of a large number of measured features. Biomedicine needs efficient methods to deal withthe enormous amount of collected data as well aseffective tools to extractmeta-data and information. It needs methods to explore data by means of classification and to evaluate data and models with respect to accuracy and reliability. Optimization methods have been successfully applied tothese problems,but the complexity of the data, i.e. varying data density, high dimensionality and model reliability, is still very challenging. This paper addresses some important issues concerning the classification of alarge amount of data: k-nearest-neighbor (kNN)-based and support vector machine (SVM)-based classification, dimensionality reduction for kNN and SVM classification, and optimal parameter settings for a SVM-based classifier. Dimensionality reduction and parameter selectionare accomplished by using an electromagnetism-likemetaheuristic(EM).The same EM is used for solving another optimization problem studied in this paper – the maximum betweenness problem (MBP). During radiation hybrid experiments, X-rays are used to fragment the chromosome. The probability that the given dose of an X-ray will break the chromosome rises with the distance between chromosomes. In thisway, markers are placed on two separate chromosomal fragments. By estimating the frequency of the breaking points, and thusthe distances between markers, it is possible to determine theirorder in a manner analogous to meiotic mapping. In this context,improvement of the radiation experiment is achieved by solving the MBP,i.e. bydetermining the total ordering of the markers that maximizes the number ofsatisfied constraints.

Acknowledgments: This research was partially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project No. 174010.

Session
BIOMEDICINE
(In memoriam Stanka Romac)

Flash presentations



COMBINED ANALYSIS OF *p16* AND *p14* METHYLATION AND VEGF EXPRESSION STATUS COULD PREDICT MORE AGGRESSIVE PHENOTYPE OF LOCALLY ADVANCED RECTAL CANCERS

Bojana Kožik[1], Nikola Kokanov[1], Slavica Knežević-Ušaj[2], Ivan Nikolić[2], Radoslav Davidović[1], Snežana Jovanović Ćupić[1], Milena Krajnović[1]

[1]University of Belgrade-Vinča Institute of Nuclear Sciences, Belgrade, Serbia; [2]University of Novi Sad-Faculty of Medicine Novi Sad, Oncology Institute of Vojvodina, Sremska Kamenica, Serbia.

Background: Preoperative chemoradiotherapy (CRT) represents the standard treatment for patients with locally advanced rectal cancer. Since only subset of patients has benefit from this preoperative treatment, development of reliable molecular biomarkers is required. In this retrospective study, we investigated methylation status of *p16* and *p14* tumor suppressor genes in locally advanced rectal cancer, in order to evaluate their potential predictive and prognostic role.
Methods: Methylation-specific PCR was used to examine methylation status of *p16* and *p14* genes in pretherapeutic and preoperative biopsy specimens of 60 patients with locally advanced rectal cancer.
Results: Aberrant methylation of *p16* and *p14* genes was detected in 43.3% (26/60) and 39.6% (23/58) of cases, respectively. In general, *p16* and *p14* methylation status did not affect the response to CRT, recurrences rate and overall survival. However, patients with simultaneous presence of either *p16* or *p14* methylation and high vascular endothelial growth factor (VEGF) expression showed significantly worse response to CRT ($p = 0.005$ and $p = 0.038$, respectively). In addition, tendency toward more frequent local recurrences and metastasis was observed in cases with concurrent presence of methylation of either *p16* or *p14* gene and high VEGF expression ($p = 0.075$ and $p = 0.072$, respectively), while patients with both of *p16* methylation and high VEGF expression had significantly shorter overall survival ($p = 0.010$).
Conclusion: Obtained results strongly suggest the importance of *p16* and *p14* methylation analyses in combination with other parameters, particularly VEGF expression, in order to better predict treatment response and patient outcome.

Acknowledgements: This study was supported by the grant no. 173049 from the Ministry of Education, Science and Technological Development, Republic of Serbia.

MOLECULAR GENETIC CHARACTERIZATION OF MYOTONIC DYSTROPHY TYPE 1 PATIENTS CARRYING VARIANT REPEATS WITHIN *DMPK* EXPANSIONS

Jovan Pešović[1], Stojan Perić[2], Miloš Brkušanin[1], Goran Brajušković[1], Vidosava Rakočević-Stojanović[2], Dušanka Savić-Pavićević[1]

[1]University of Belgrade, Faculty of Biology, Center for Human Molecular Genetics, Belgrade, Serbia; [2]University of Belgrade, School of Medicine, Neurology Clinic, Clinical Center of Serbia, Belgrade, Serbia.

Introduction: Myotonic dystrophy type 1 (DM1) is caused by an expansion of CTG repeats in the *DMPK* gene. Its huge phenotypic variability cannot be explained solely by repeat number. Recently, variant repeats within *DMPK* expansions have emerged as potential disease modifiers.
Methods: The frequency of variant expanded alleles was estimated in 242 DM1 patients from 174 Serbian families using repeat-primed PCR (RP-PCR). Patterns of variant repeats were determined by direct sequencing of RP-PCR/PCR products. PCR-based Southern blot and Small-pool-PCR was performed to get insight into meiotic and mitotic mutational dynamics of variant expanded alleles. All patients carrying variant repeats were clinically re-examined.
Results: Variant repeats were observed in 2.9% of patients, only at the 3'-end of *DMPK* expansions. The most common variant repeats (CCG) were present either as a part of CCGCTG hexamers, individual repeats, or CCG blocks. Analyses of three intergenerational transmissions revealed a considerable stability or likely a contraction of variant expanded alleles, intriguingly accompanied by a decrease in age at onset (anticipation). Variant expanded alleles were somatically unstable with repeat length distributions biased toward further expansion. However, somatic mosaicism was less pronounced than expected for the corresponding expansion sizes with pure CTG repeats. Overall, patients were characterized by a milder phenotype and/or some atypical symptoms that could be rather clinically suggestive of myotonic dystrophy type 2.
Conclusion: Variant repeats might explain a part of the phenotypic variability in a small percent of DM1 patients and likely display a stabilizing effect on meiotic and mitotic instability of *DMPK* expanded alleles.

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ANALYSIS OF CYTOCHROME P450 2D6 FUNCTIONAL VARIANTS IN SERBIAN HUMAN POPULATION

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Introduction: Predicting the drug response from individual's genotype is a main goal of pharmacogenetics. The efficacy of, and adverse reaction to more than 25% of commonly prescribed drugs depend on the inherited functionality of Cytochrome P450 2D6 monooxygenase. A dozen of *CYP2D6* haplotypes is associated with decreased or null activity of this enzyme, while multiple copies of *CYP2D6* render its activity increased. Not all alleles are encountered in every ethnic group, and allele frequency distributions differ significantly even between neighboring populations. Therefore, in designing the testing platform for *CYP2D6* genotyping population's idiosyncrasies should be addressed.
Methods: DNA was collected and purified from stratified random sample of 500 persons from general Serbian human population. Single nucleotide variants (SNV) were determined on 10 polymorphic sites in *CYP2D6* gene using Taqman Drug Metabolism Genotyping Assays. *CYP2D6* haplotypes were inferred via PHASE.1 software. Copy number variation (CNV) of *CYP2D6* was estimated by Copy Number Assay.
Results: Out of 10 analyzed sites, 5 were monomorphic and on one minor allele frequency was less than 5%. Obtained genotypes were assigned to seven haplotypes: two of which were nonfunctional, additional two with decreased function, and remaining three were fully functional. Only one functional allele (*1) was found in multiple copies per genotype. The most common metabolic phenotype was extensive metabolizer (81.2%), followed by ultrarapid (9.4%), poor (7.5%) and intermediate metabolizer (1.9%).
Conclusion: Genotyping platform based on only 5 SNVs and one CNV assay is successful in predicting all metabolic phenotypes in Serbian population.

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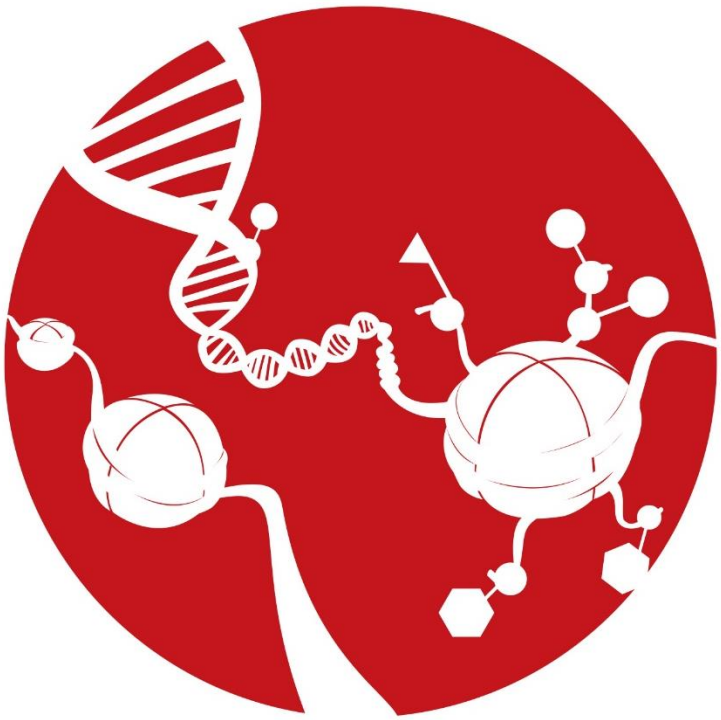
Poster Session
BIOMEDICINE



LCT c.-13910C>T (rs4988235) and c.-22018G>A (rs182549) POLYMORPHISMS LINKED TO ADULT-TYPE LACTOSE INTOLERANCE IN POLISH AND BOSNIAN SUBJECTS WITH AND WITHOUT CROHN'S DISEASE

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Introduction: The prevalence of Crohn's disease (CD) in Europe varies from 1.5 to 213 cases per $1 \cdot 10^4$ subjects. Extrapolating these numbers, it is estimated that in European population may be about 1.6 million subjects with CD. Genetic factors strongly influence on CD. The role of *LCT* c.-13910C>T and c.-22018G>A polymorphisms in CD remains unclear. The presence of genotype CC/GG is interpreted as lactose intolerance (known as lactase non-persistence), while the CT or TT genotypes and GA or AA as lactose tolerance (known as lactase persistence).
Aim: Our aim was to determine the distribution of *LCT* c.-13910C>T and c.-22018G>A polymorphisms and their association with Crohn's disease in Polish and Bosnian patients with CD and to compare with controls, without CD.
Material and Methods: We recruited 110 CD patients and 82 controls in Poland and 30 CD patients and 30 controls in Bosnia and Herzegovina. Samples of DNA, obtained from 227 participants in total, were genotyped using PCR-RFLP method. Statistical analysis was performed using R software version 3.3.3. R Core Team (2017).
Results: The CC/GG genotype, related to LNP was found in 38 (34.5%) and 25 (30.5%) Polish CD patients and controls, and in 18 (60%) and 15 (50%) Bosnian CD patients and controls, respectively. The genotypes and alleles frequencies analysis revealed no significant differences between Polish and Bosnian CD patients compared to controls ($p<0.05$).
Conclusions: In both Polish and Bosnian population, the *LCT* c.-13910C>T and c.-22018G>A polymorphisms linked to adult-type lactose intolerance do not contribute to Crohn's disease susceptibility.

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ASSOCIATION OF *HNF1B* GENE POLYMORPHISM (rs4430796) WITH ENDOMETRIAL CARCINOMA

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Introduction: Endometrial carcinoma is the most common invasive genital carcinoma among females, which incidence reaches a maximum between 50-70 years of age.Endometrial carcinoma is usually connected with obesity. Nowadays, obesity can be considered a metabolic disorder which affects homeostasis of estrogen, leading to the proliferation of endometrial cells, and creating the basis for carcinoma. The gene *HNF1B* has been associated with different carcinoma, endometrial in women and prostatic in men. As a transcription activator *HNF1B* is expressed very early in the embryo, participating in the development of mesoderm and endoderm. Common polymorphism rs4430796 G>A, located in the second intron of the gene, is shown to be associated with occurrence and prognosis of endometrial carcinoma.
Methods: Genomic DNA from 40 women with adenocarcinoma, between 50-70 years of age, and 51 women without adenocarcinoma from the general population from the same ethnic and age group, was isolated from peripheral blood. Genotyping was performed with TaqMan Assay. Data were processed with IBM SPSS ver. 21 softvare.
Results: Obesity significantly increases the risk for endometrial carcinoma (OR-3.6, p=0.011). Although A allele was more common in the patients group, our results didn't show any significant statistical relation between *HNF1B* (rs4430796) and endometrial carcinoma (p>0.05). Together *HNF1B* gene (rs4430796) and obesity didn't show any statistical significant (p>0.05).
Conclusion: Only obesity, but neither *HNF1B* genotype, nor their interaction, increases the risk for endometrial carcinoma. Effect of these factors on prognosis of the disease remains to be elucidated.

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MOLECULAR CHARACTERIZATION OF PATIENTS WITH PRIMARY CILIARY DYSKINESIA REVEALED NOVEL GENETIC VARIANT IN *DNAI1* GENE

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Introduction: Primary ciliary dyskinesia (PCD) is rare, inherited, autosomal recessive disorder that mainly affects lungs. Immotile or dysmotile respiratory cilia with or without ultrastructural defects leads to defective mucociliary clearance and causes respiratory ailments. The aim of this study was genetic profiling of clinically diagnosed PCDs and molecular characterization of novel genetic variant found in two siblings.
Methods: We analyzed 23 patients from Serbia with diagnosis of PCD according to clinical presentation using a NGS panel with 4813 genes to detect disease-causing variants. For the purpose of detailed characterization of novel genetic variant in *DNAI1* gene found in two siblings we performed RT-qPCR, *in silico* prediction of variant impact at protein level and protein analysis by Western Blot method.
Results: In 14 of 23 patients we detected disease-causing variants in PCD candidate genes. In the remaining 9 patients we detected variants responsible for other PCD like diseases. We found a novel homozygous frameshift mutation that leads to premature stop codon in *DNAI1* gene (c. 947_948insG, p. Thr318TyrfsTer11), in two siblings. Relative expression of *DNAI1* gene was 30-45% lower in patients and their parents compared to controls. In *silico* prediction showed truncated protein. However, Western Blot analysis showed full length protein in patients, but the amount was lower in comparison to parents and positive control.
Conclusion: NGS approach is mandatory for genetic characterization and classification of PCD patients due to the overlapping symptoms with other rare lung diseases. We have found a novel frameshift stop mutation which most likely causes PCD.

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ANALYSIS OF PROTEOMIC PROFILES OF BLOOD LEUKOCYTES IN RESPONSE TO DAPSONE

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Introduction: Leukocytes are main effectors of inflammation in asthma and other airways diseases. High count of neutrophils, dominant leukocytes in peripheral blood, is often observed in non-allergic asthma. These patients show improvement of clinical symptoms when treated with dapsone. The molecular mechanism underlying anti-inflammatory action of dapsone is insufficiently investigated.
Methods: Peripheral blood samples were obtained from five healthy individuals (6-18 years old). Blood leukocytes were treated *ex vivo* with dapsone and protein extracts were prepared by mechanical disruption of cells. Bottom up proteomics analysis was performed on the HPLC coupled to QExactive mass spectrometer. Data were analyzed using MaxQuant/Perseus 1.5 software package.
Results: Differences between treated and untreated samples were determined using Student's t-test. The analysis of proteomic profiles of leukocytes has shown that dapsone treatment significantly increased levels of four proteins and decreased levels of eight proteins ($p<0.01$). Down-regulated proteins are mainly involved in regulation of gene expression, particularly initiation of translation and pre-mRNA splicing, while up-regulated proteins belong to different metabolic pathways. Some of differentially expressed proteins, both up- and down-regulated, have previously been associated with abnormal lung function and/or asthma symptoms.
Conclusion: Treatment of leukocytes from healthy individuals with dapsone *ex vivo* leads to significant change in expression levels of twelve proteins. Identified proteins can potentially be used as biomarkers for follow up of response to dapsone treatment, but their clinical value should be investigated in prospective studies. The obtained results can also direct further research on molecular mechanism of dapsone action.

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POLYPHENOLS FROM RED WINES AS MODULATORS OF PROSTAGLANDIN E_2 AND THROMBOXANE A_2 PRODUCTION IN INFLAMMATION

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Introduction: Prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2) are involved in normal physiology and in inflammatory response. They are products of arachidonic acid metabolism in cyclooxygenase pathway, which involves several enzymes: phospholipase A_2 (PLA_2), cyclooxygenase-1/2 (COX-1/2), PGE_2 (mPGES-1/2, cPGES) and TXA_2 (TXAS) synthase. Impact of some natural products on their expression and activity is known, but exact molecular mechanisms are still missing. In this study, which is a part of comprehensive research on polyphenols as modulators of PGE_2 and TXA_2 production in inflammation, we evaluated correlation between inhibitory activity on production of these modulators and polyphenol profile of several red wines. Namely, red wines are rich in polyphenols, and their moderate consumption could positively affect some pathological processes associated with inflammation, such as atherosclerosis.
Methods: Human U937 monocytes were differentiated into macrophages in the presence of PMA. Inflammation was induced by LPS after pretreatment with wine samples. Analysis of wine phenolics and quantification of produced PGE_2 and TXA_2 in cell lysate was done by LC-MS/MS. Gene expression (*PLA_2*, *COX-1/2*, *mPGES-1/2*, *TXAS*) was measured by qPCR.
Results: Five examined samples of red Merlot wines showed moderate potential to inhibit PGE_2 and TXA_2 production in human U937 derived macrophages challenged with LPS. Their impact on both gene expression and enzyme activity was noticed. Correlation of this activity and phenolic profile indicated that polyphenols are involved in this inhibition.
Conclusion: Beneficial effects of moderate red wine consumption could be, at least partially, due to their influence on PGE_2 and TXA_2 production in inflammation.
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LANGUAGE AND COGNITIVE PHENOTYPE OF TURNER SYNDROME

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Introduction: Turner syndrome is very common with an incidence of 1 in 2000 newborn female. It is the result of complete or partial monosomy of the X chromosome. Beside distinctive physical characteristics (cardiovascular, craniofacial, skeletal, neurological, genitourinary and otolaryngolical defects) research in recent years focus on congenital and language development of girls with Turner syndrome.
Methods: A PubMed search of articles in the last 20 years reveal available studies which are related to the development of children with Turner syndrome. Some of the important historical studies were included as well.
Results: The review presents well-known facts and highlights new findings in the cognitive and speech and language development of children with Turner syndrome.
Conclusion: Based on the literature and new findings children with Turner syndrome are at the great risk for a nonverbal learning disability, particularly in arithmetic, visuospatial skills, and processing speed. But, girls with Turner syndrome may manifest individual characteristics as a consequence of the joining effect of various factors. The detailed assessment is needed of these girls in order to use its full potential and to provide educational support if it is needed.

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THE *NOD2* SINGLE NUCLEOTIDE POLYMORPHISM c.2104C>T (rs2066844) IN POLISH AND BOSNIAN CROHN'S DISEASE PATIENTS AND CONTROLS

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Introduction: The Crohn's disease (CD) is a chronic inflammatory disease, that can involve any part of the gastrointestinal tract. The role of *NOD2* c.2104C>T single nucleotide polymorphism in CD development remains unclear, and the data on distribution in Polish and Bosnian populations are limited.
Aim: The aim of this study was to investigate the prevalence of *NOD2* c.2104C>T in CD patients and controls from Poland and Bosnia and Herzegovina.
Material and Methods: Samples obtained from Polish and Bosnian CD patients (n=85 and n=30, respectively) and controls (n=82 and n=30, respectively) were genotyped using PCR-RFLP method. Statistical analysis was performed using χ^2 test with Yates correction or Fisher's exact test, where appropriate.
Results: The prevalence of CC and CT genotypes were 85.9% *vs*. 95.1% and 14.1% *vs*. 4.9% in Polish CD patients and controls, respectively. The prevalence of CC and CT genotypes were 100% *vs*. 93.3% and 0% *vs*. 6.7% in Bosnian CD patients and controls, respectively. In both populations there were no TT homozygotes. The frequency of alleles in CD patients and controls were, C: 92.9% *vs*. 97.6%, T: 7.1% *vs*. 2.4% in Poles and C: 100% vs. 96.7%, T: 0% vs. 3.3% in Bosnians, respectively. The genotypes and alleles analysis revealed no significant differences in both, Polish and Bosnian populations ($p>0.05$). The comparative analysis showed higher prevalence of CT genotype and T allele in Polish than Bosnian CD patients ($p=0.034$ and $p=0.039$, respectively).
Conclusions: *NOD2* c.2104C>T may not contribute to CD susceptibility in Polish and Bosnian populations.

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VITAMIN D RECEPTOR AND TOLL-LIKE RECEPTORS GENE POLYMORPHISMS IN SEPSIS

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Introduction: In intensive care units, the leading cause of death in critically ill patients is still sepsis, despite advances in treatment and clinical care. Considering that TLR2, TLR3, TLR4, and Vitamin D play very important roles in inflammatory processes, the question arises whether the presence of polymorphisms in *TLR* genes and Vitamin D receptors (*VDR*) gene is associated with susceptibility to sepsis. The aim of this study was to examine the association of *TLR2*, *TLR3, TLR4, and VDR* polymorphisms with clinical characteristics and outcome of critically ill patients.
Methods: A follow-up study was conducted on 121 critically ill Caucasian Serbian patients and 104 healthy blood donors. Polymorphisms in *TLR* genes, *TLR2* (rs5743708), *TLR3* (rs3775291, rs5743312), *TLR4* (rs4986790, rs4986791), and VDR gene, EcoRV (rs4516035), FokI (rs2228570), ApaI (rs7975232), and TaqI (rs731236) were genotyped by real-time PCR.
Results: *TLR3* rs3775291 polymorphism was associated with patient's outcome. No significant associations were observed between the other TLR and VDR gene polymorphisms and analyzed variable characteristics in the patients. Patients with *TLR3* rs3775291-mutated genotype had a higher mortality rate. Adjusted odds ratio analysis showed that heterozygous and common variant genotype of VDR FokI (rs2228570) polymorphism is associated with an elevated risk of sepsis. Multivariate regression analysis showed that age, sex and *TLR3* rs3775291 polymorphism are independent variables of the outcome of patients with sepsis.
Conclusion: *TLR3 (*rs3775291) and VDR FokI (rs2228570) polymorphism could be a useful markers to identify patients with a high risk of developing sepsis and high risk for lethal outcome.

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SYNERGISTIC INFLUENCE OF THE *SMN2* AND *SERF1A* GENE COPY NUMBER ON CHILDHOOD-ONSET SPINAL MUSCULAR ATROPHY

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Introduction: Spinal muscular atrophy (SMA) is caused by homozygous absence of the *SMN1* gene. Genetic homogeneity and broad phenotypic variability of the disease suggest the involvement of disease modifiers. 5q13 SMA locus is extremely unstable and harbours not only the disease causing *SMN1* gene, but is also enriched in other genes (*SMN2*, *SERF1A* and *NAIP)*. This leads to a high rate of unequal crossing over resulting in complex structural rearrangements and in copy number polymorphism of encompassed genes. We aimed to examine the individual and synergistic influence of the *SMN2*, *SERF1A* and *NAIP* gene copy number on phenotypic variability of childhood-onset SMA.
Methods: Multiplex Ligation-dependent Probe Amplification (MLPA) was used to assess the copy number of the *SMN2*, *SERF1A* and *NAIP* genes in 99 genetically confirmed Serbian SMA patients (23 with severe type I, 37 with intermediate type II and 39 with mild type III).
Results: Inverse correlation was found between the copy number of each individual gene and SMA type (Spearman rank test, *SMN2* p=2.2e-16, *SERF1A* p=4.264e-10, *NAIP* p=2.722e-8). Generalised linear model and backward selection, starting with a full model including the *SMN2*, *SERF1A* and *NAIP* copy number and their interactions, revealed that the best minimal model explaining phenotypic variation in childhood-onset SMA with the smallest set of variables included the *SMN2* (p<2e-16) and *SERF1A* (p<2e-16) copy number and their interaction (p=0.02628).
Conclusions: *SMN2* and *SERF1A* gene copy numbers, as a consequence of complex rearrangements in the 5q13 region, modify the childhood-onset SMA clinical outcome among Serbian patients as independent variables and sinergistically.

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POLYMORPHISM IN THE GENE FOR THE SEROTONIN TRANSPORTER (SERT OR 5-HTT) AFFECTS OR NOT THE TREATMENT OF DEPRESSION

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Introduction: Reduced serotonergic neurotransmission plays an important role in the etiology of depression, therefore serotonin transporter (SERT or 5-HTT) is the main target for different antidepressants. One of the most common treatments of depression is the use of an antidepressant from the group of selective serotonin reuptake inhibitors (SSRI).
Serotonin transporter regulates the extracellular serotonin concentration, and indirectly affects the entire serotonergic system. The aim of this study is to show whether the variant of *SERT* gene affects the response to SSRI therapy.
Method: Genomic DNA was isolated from whole blood samples. Genotyping of genes polymorphisms was detected by PCR.
Results: Two most frequent polymorphism, variable number of repeats (VNTR) in the second intron (STin2) and an insertion/deletion in the promoter region of the *SERT* gene (SERTPR or 5-HTTLPR) were analyzed.
Analysis of 186 patients with depression, and 122 healthy controls showed that there was a difference in the frequency of allele l and s in SERTPR in patients with depression. No significant difference in allele frequencies L and S in STin2 polymorphism was observed. Association between the allelic variation of the *SERT* gene and SSRI treatment response and the occurrence of side effects, in patients with depression, relived that carriers of the genotype L/L, or L allele in the promoter region have a much lower incidence of side effects after treatment with SSRI antidepressants.
Conclusion: These results are in agreement with theother studies that also supports a relationship between serotonin gene polymorphism and the efficacy of SSRI.

TP53 AND *hTERT* METHYLATION AND EXPRESSION IN ORAL CANCER

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Introduction: Although most studies associate DNA methylation of CpG islands with gene silencing, some have found a correlation with higher levels of gene expression. The aim of this study was to investigate a possible influence of methylation status of two cancer related genes (TP53 and hTERT) on their expression levels (p53 protein and telomerase enzyme) in oral squamous cell carcinoma (OSCC), and examine the association of methylation and clinicopathological features of the tumor.
Methods: The methylation status of TP53 and hTERT was analyzed using methylation specific PCR (MSP), and immunohistochemistry was performed to determine the levels of p53 protein and telomerase expression in 60 cases of OSCC.
Results: Out of 60 cases of OSCCs, TP53 and hTERT methylation was present in 43 cases (72%) in total, with 33 cases (55%) exhibiting methylation of both gene promoters. 75% of p53 positive and 80% of telomerase positive samples showed TP53 and hTERT methylation, respectively. Methylation was not associated with clinicopathological features of OSCC, with the exception of hTERT methylation which showed significantly higher frequency in advanced tumor stages (P=0.041).
Conclusion: Our results suggest positive TP53 and hTERT gene regulation by methylation in oral cancer.

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THE VARIANTS IN 3'END OF PROTHROMBIN GENE IN PATIENTS WITH SPORADIC COLON ADENOCARCINOMA

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Introduction: A growing body of evidence suggests that thrombin has an important role in cancer cell proliferation, migration and metastasis. However, mechanisms involving different expression of the gene encoding for thrombin precursor prothrombin (FII), remain unclear in cancer. Due to its non-canonical architecture, the 3'end of prothrombin gene is susceptible to gain-of-function variants that lead to increased prothrombin expression.
Aim: The aim of this study was to analyse the 3'end prothrombin gene variants in patients with sporadic colon adenocarcinoma.
Methods: The study group consisted of 48 patients (18 female/30 male; 67.6±10.38 years), all suffering from sporadic colon adenocarcinoma and selected from the Croatian Tumor Bank. The DNA samples were obtained from peripheral blood, colon tumor and adjacent normal tissue for all included patients. Last intron and exon, 3'untranslated region and flanking region of prothrombin gene were analysed by DNA sequencing and RFLP-PCR.
Results: We detected FIIA19911G and FIIC20068T prothrombin variants, both previously described and associated with increased prothrombin expression. Among patients, 20 were heterozygous, 12 were homozygous carriers for FIIA19911G variant and one patient was combined FIIA19911G/FIIC20068T heterozygous carrier, in all three tested DNA samples. Three patients displayed different tissues genotypes for FIIA19911G variant: two patients were homozygous (peripheral blood)/ heterozygous (tumor tissue and adjacent normal tissue) and one patient was heterozygous (peripheral blood)/ non-carrier (tumor tissue and adjacent normal tissue).
Conclusion: Results of our pilot study suggests potential role of prothrombin 3'end gene variants in colon cancer etiology which remains to be further investigated.

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MethSpec: A COMPUTATIONAL TOOL FOR EVALUATION OF METHYLATON SPECIFIC PCR PRIMER SPECIFICITY

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The aim: False positive detections are main drawback of methylation-specific PCR (MSP). Our aim was to develop a computational tool that can be used for assessment of a primer pair specificity before its actual use in MSP experiment.
Methods: MethSpec is a program that carries out evaluation of MSP primer specificity based on primer pair's sequences and parameters such as: primer concentration, ion concentration and annealing temperature. The software calculates two parameters, SpG and SpU that describe the ability of input primer pair to differentiate between bisulfite modified methylated and native and unmethylated alleles, respectively. Based on obtained and cutoff values for SpG and SpU input primer pair may be classified as specific, non-specific or with uncertain specificity. In order to define cutoff values 27 MSP primer pairs, which specificity is proven by independent methods, have been extracted from the literature. The software calculates SpG and SpU for each referent primer pair and sets threshold.
Results: To further demonstrate MethSpec prediction capability, six primer pairs have been selected from the previously published articles. It is suggested that these primer pairs have suboptimal performances in the experimental settings. MethSpec has been predicted subpar specificity for all tested primer pairs, except one.
Conclusion: MethSpec predicts MSP primer specificity with reasonable efficacy and could serve as valuable support to experimentalists in the process of primers selection.

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TOLEROGENIC DENDRITIC CELLS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Introduction: Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis, a chronic inflammatory demyelinating disease of the central nervous system (CNS). Dendritic cells are essential for initiation, propagation, but also for regulation of anti-CNS autoimmune response that is itself crucial for pathogenesis of multiple sclerosis and EAE. Tolerogenic dendritic cells (tolDC) have immuno-regulatory properties and they are a promising prospective therapy for multiple sclerosis. However, it is still unclear if deficiency in generation and function of tolDC is a predisposing factor for multiple sclerosis. Thus, we have been investigating tolDC in EAE-prone DA rats and EAE-resistant AO rats, as an animal parallel of multiple sclerosis patients and healthy individuals.
Methods: Dendritic cells were isolated from bone marrow of non-immunized and EAE-immunized rats and propagated towards tolDC by GM-CSF and vitamin D3. tolDC were characterized phenotypically by cytofluorimetry. mRNA expression was determined by real-time RT-PCR and cytokine generation by ELISA.
Results: AO and DA rat tolDC differed in expression of various mRNA and production of pro-inflammatory and anti-inflammatory cytokines. Also, tolDC from these strains differed in response to EAE-immunization.
Conclusion: The observed differences between tolDC of rats prone and resistant to CNS autoimmunity imply similar study in humans.

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MMP9 (-1562 C/T) GENE POLYMORPHISM IS ASSOCIATED WITH INCREASED MMP-9 LEVEL AND PAPILLARY THYROID CARCINOMA PROGRESSION

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Introduction: Papillary thyroid carcinoma (PTC), a common form of thyroid malignancy, displays significant variations in clinical features and outcome. The malignant transformation of the thyroid is accompanied by an altered expression of many matrix-modulating enzymes, including matrix metalloproteinase-9 (MMP-9). A single nucleotide polymorphism in its promotor (-1562 C/T) is suspected to cause overexpression of MMP-9, which in turn contributes to development of unfavorable phenotype.
Aim: To investigate the impact of a *MMP9* gene variant (-1562 C/T) on immunoexpression of MMP9 in PTC samples. Also, to assess the value of -1562 C/T as a possible risk factor for developing PTC as well as PTC aggressive phenotype.
Methods: This study included 88 subjects, 58 PTC patients and 30 healthy controls. The expression of MMP9 in PTC was estimated using immunohistochemistry. The genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genotype frequencies were compared between controls and patients and the association of genotypes with MMP9 expression and other clinicopathological features was analyzed.
Results: The genotype frequencies were not significantly different between the patients and controls (p=0.085). However in the PTC group, the heterozygotes displayed a significantly higher expression of MMP-9 (p=0.07). Carriers of the T allele had a larger tumor size (p=0.018) and developed extrathyroid extensions more frequently (p=0.010). No association between genotype and presence of lymph node metastasis or T stage was found.
Conclusion: Data suggests that *MMP9* variant -1562 C/T does not faciliate predisposition for PTC but favors its aggressive behavior by modulating MMP-9 expression.

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ANALYSIS OF *VDR* GENE POLYMORPHISMS IN WOMEN WITH UNEXPLAINED INFERTILITY

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Introduction: Unexplained infertility refers to the absence of a definable cause of reproductive failure. The importance of vitamin D in pregnancy recently came under scientific spotlight, partly due to the fact that Vitamin D Receptor (VDR), which acts as a transcription factor, is expressed in reproductive tissues. Also, the role of vitamin D in immune system is well established. In this study we examined possible association of the polymorphisms in *VDR* gene with the reproductive success in women.

Material and method: DNA from 117 female patients with unexplained infertility (58 with primary and 59 with secondary) and 130 fertile controls was isolated from peripheral blood and genotypes of polymorphisms in *VDR* gene (FokI, BsmI, ApaI and TaqI) were detected by PCR-RFLP. Haplotypes were determined using *Haploview* software.

Results: Our results show significant association between FokI and BsmI polymorphisms and infertility ($p<0.05$). F allele in FokI and B allele in BsmI polymorphisms were associated with reduced risk for infertility ($OR<1$, $p<0.05$). The haplotype analysis showed that BsmI, ApaI and TaqI are in strong linkage disequilibrium. Two haplotypes were associated with infertility, bAT increasing the risk for secondary infertility ($OR=2.2$, $p=.0.023$) and BAT with protective role against primary infertility ($OR=0.33$, $p=0.021$).

Conclusions: Obtained results are, to the best of our knowledge, the first evidence of significant association of *VDR* polymorphisms and haplotypes with unexplained infertility. By changing the expression and activity of *VDR* gene, these polymorphisms and haplotypes possibly could have an effect on immune system in the female reproductive tract.

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TARGETED GENE PANEL PROFILING AS A BASIS FOR INDIVIDUAL PROGRAM OF NUTRITION AND EXERCISES

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Introduction: Unhealthy diet and physical inactivity have been identified as primary determinants of the increase in the incidence of obesity among general population. A number of studies suggest an exact link between individual genome variation and intensity of specific metabolic processes (catabolism of fat, carbohydrates and proteins).

Methods: DNA was extracted from buccal swabs using Chelex® 100 of 18 individuals who voluntarily participated in this study. Pyroseqencing technology was used for molecular analysis of 13 polymorphism detected at 10 genes (APOA2, MTHFR, MCM6, PPARG, FABP2, ADRB2, ADRB3, ACTN3, ACE, FUT2). Additional personal data included baseline BMI index, information about daily fitness and workout routine and dietary practice. Biostatistical analyses of covariation were performed using PAST 3.14 software.

Results: Generated individual genotypes were evaluated for each marker separately to estimate the level of impact on personal metabolic profile of each participant separately. Based on this estimate and nutritional recommendations, a personalized training and nutrition plans were obtained for each participant of the study. After the study period of 12 months the BMI were calculated and analysed. We found that BMI values significantly decreased (paired samples t=3.382; *P*=0.006; Exact P=0.015) after personalized training and nutrition plan implementation within the group of individuals classified as overweight at the beginning of the study.

Conclusion: After implementation of personalized training and nutrition plan, our study suggests that targeted gene polymorphism analysis (i.e. SNP panel genotyping) could be a valuable tool designing individual program of nutrition and exercises with maximum efficiency.

FAMILIAL MEDITERRANEAN FEVER MOLECULAR TESTING IN REPUBLIC OF MACEDONIA

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Introduction: The Familial Mediterranean fever (FMF, MIM249100) is an autoimflammatory genetic disease characterized with recurrent painful attacks in the abdomen, chest or joints, usually accompanied with high body temperature. It is associated with mutations of the MEFV gene, and is classically inherited in an autosomal recessive manner. More than 140 mutations of the MEFV gene are defined worldwide.
The aim of our study was to summarize the results of FMF genetic testing performed at the Institute for Immunobiology and Human Genetics in Skopje during 2012-2017.
Methods: Total of 67 patients were analyzed using the reverse hybridization technique which includes 12 most frequent mutations in MEFV gene (Viennalab, Austria).
Results: We have found 1 homozygous patient for M694V mutation, 4 patients heterozygous for E148Q mutation, 1 patient was genotyped as compound heterozygote for E148Q and K695R, 1 heterozygous patient was detected for each of the following mutations: P369S, K695R and R761H, and the remaining 58 patients were tested normal.
Conclusion: Despite the progress in establishing reliable genetic tests, as much as 20% of the patients with FMF remain without a detectable mutation in the MEFV gene. This is the main reason why the diagnosis of FMF is still a clinical one, according to the Tel Hashomer criteria. We present our cohort of patients analyzed for FMF and an interesting case of a young girl with unusually inherited disease. We hope that by presenting this case we will contribute to raising the awareness of this condition in patients with characteristic symptoms.

INVESTIGATION OF EPIDERMAL GROWTH FACTOR RECEPTOR POLYMORPHISM OF LUNG CANCER PATIENTS

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Introduction: Overproduction of epidermal growth factor receptor (EGFR) is very common in different human tumors, including lung cancer. Polymorphisms in the promoter region of *EGFR* may contribute to the individual differences of EGFR expression and disease susceptibility or response to treatment.
Aim: To determine *EGFR* SNPs frequencies among lung cancer patients in the Republic of Srpska.
Methods: DNA samples were obtained from 34 lung cancer patients peripheral blood using commercial extraction kit. *EGFR* polymorphisms rs712830 (-191C>A) and rs2293347 (181946G>A) were genotyped using polymerase chain reaction – restriction fragment length polymorphism method. To detect -191C>A polymorphism, PCR products were incubated with restriction enzyme *Sac*II and *Tfi*I for 181946G>A polymorphism.
Results: Study determined the frequency of two SNPs with respect to lung cancer type, gender, age and smoking status. Statistically significant differences were not found related to cancers type. Patients with adenocarcinoma reported more frequently smoking (53,33%) compared to the patients with other tumor types (12,50%), especially those younger than 65 years of age (47,06%). Results showed that the most frequent haplotype for -191C>A is CG and for 181946G>A GG (p= 0.044, Chi Square test). In addition, similar haplotype was observed/detected in patients younger than 65 years.
Conclusion: Study confirm that *EGFR* polymorphism research is significant for determinating future cancer markers. Futher regional research is required to defined specific haplotype with respect to tumor type and risk factors, which in the future can be used as genetic marker for susceptibility and prognosis of lung cancer.

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p14 AND *p16* METHYLATION IN ORAL CANCER

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Aims: To determine the methylation status of *p14* and *p16* in cancer tissue, cancer margins and normal mucosa of patients with oral squamous cell carcinoma (OSCC) and assess the relevance of these findings in terms of diagnosis and prognosis.
Methods: The study comprised 40 patients (38.5% female and 61.5% male, aged 65.31±10.50 years) with diagnosed OSCC, treated at the Clinic of Maxillofacial Surgery, School of Dental Medicine, University of Belgrade. Three samples were taken from each patient: tumor, tumor margin and swab. Methyl-specific PCR was used to examine the *p14* and *p16* promoter methylation status.
Results: A high incidence of methylation in tumor tissues was found (p14-90%, p16-77%). A statistically significant difference in gene alteration distribution was observed between tumors, margins and swabs for the 2 analyzed genes (P=0.016 for *p14* and P<0.001 for *p16*), with tumors showing the highest prevalence of alterations and swabs the lowest. However, association could not be established neither between methylation status and clinico-pathological parameters, nor between methylation status and survival.
Conclusions: Although epigenetic silencing seems to be an important mechanism of gene regulation in oral cancer, as judged from the high incidence of methylation in tumor tissue, it does not appear to be relevant as a prognostic parameter.

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A STUDY ON THE ANTICARCINOGENIC EFFECTS OF CALCIUM FRUCTOBORATE

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Introduction: Evidences about the preventive and therapeutic effects of boron compounds on cancer have been increasing in the last years. Although calcium fructoborate (CaFB) is used as a nutritional supplement, data about its preventive and therapeutic effects on neoplastic transformations are limited.
Methods: The various concentrations of CaFB were applied to the MDA-MB-231 metastatic breast cancer cell line. First, we examined the cytotoxic effect and IC50 value of CaFB by MTT assay. For the evaluation of the DNA damage, apoptosis and metastatic potential, expression levels of ATM, pATM, PARP, p53, p-p53, caspase-3, caspase-9, and VEGF were investigated by using immunoblotting and immunohistochemical methods.
Results: Cell viability was significantly reduced at 50 μM CaFB treatment. pATM, p-p53, and caspase-9 levels increased significantly in all groups; furthermore, there was approximately 12.5-, 2.4-, and 10.7-fold increase, respectively, for 100 μM CaFB treatment. ATM and p53 levels did not change with CaFB treatment, but PARP levels significantly 2.5-fold decreased. While VEGF immunoreactivity decreased in all groups, significant increase in caspase-3 immunoreactivity was observed only in the group treated with 50 μM CaFB ($p < 0,001$).
Conclusion: Our results imply that CaFB may have therapeutic potential as well as preventive benefits in cancer.

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PHARMACOGENOMIC BIOMARKERS OF GLUCOCORTICOID SENSITIVITY AND DISEASE OUTCOME IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: The first step in childhood acute lymphoblastic leukemia (ALL) treatment is the glucocorticoid induction of remission. There have been conflicting results concerning genetics of glucocorticoid sensitivity in ALL patients. The aim of this study was to investigate the influence of the genotypes of the glucocorticoid receptor (NR3C1), the P-glycoprotein (ABCB1) and the glutathione S-transferases (GST) on treatment response in childhood ALL patients.
Methods: A sample of 122 Serbian children with ALL treated with prednisone during the induction phase were genotyped for common polymorphisms in *ABCB1* (allelic discrimination PCR and allele-specific qPCR using KASPAR assay), *NR3C1* (allelic discrimination qPCR using Taqman assay), *GSTP1* (allele-specific qPCR using KASPAR assay), *GSTM1* and *GSTT1* (PCR) genes.
Results: The carriers of *NR3C1* rs6198 C allele had increased risk for resistance (> 1000 blasts/μL) to prednisone (χ^2 test; p=0,001). For the *ABCB1* investigated polymorphisms (rs2032582 and rs1045642), we didn't find any influence on glucocorticoid sensitivity or on the outcome. The carriers of *GSTP1* rs1695 G allele had decreased risk of relapse (χ^2 test; p=0,035). *GSTP1* GC haplotype showed tendency towards decreased risk of relapse (χ^2 test; p=0,058). Neither GSTT1, nor GSTM1 genotype had any influence on the outcome. The absence of *GSTM1* null genotype shortened disease free survival (DFS) time (LogRank=8,287, p=0.004). The *GSTT1* null genotype had a much shorter DFS (LogRank=3.843, p=0.05).
Conclusion: Our results suggest an important role of *NR3C1* rs6198 in glucocorticoid sensitivity and of *GSTP1* rs1695, *GSTT1* and *GSTM1* on disease outcome.

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A LOCAL SEARCH BASED HEURISTIC FOR CLUSTERING LARGE BIOLOGICAL NETWORKS INTO HIGHLY CONNECTED COMPONENTS

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Introduction: Clustering large networks into smaller components can be of a great importance for discovering new properties of a specific structure. In this work we deal with partitioning biological networks into highly connected components by removing as few edges as possible. A network with *n* nodes is called highly connected if the degree of each node is greater than *n*/2. The mentioned problem is called Highly Connected Deletion (HCD) problem and it has several applications in biology, for example in finding complexes in protein–protein interaction (PPI) data or clustering cDNA fingerprints.
Methods: We present a local search based heuristic algorithm for solving this NP hard problem. The algorithm uses swap-based local search strategy and specific objective function which takes into the consideration the degrees of nodes in each partition. In order to enable easier analysis of the results, some solutions are graphically presented.
Results: The algorithm is applied on several moderate-size protein interaction networks. The experimental results indicate that our method is competitive with other existing methods used for solving HCD. The obtained highly connected clusters are graphically represented by a software platform for visualizing biological networks.
Conclusion: Proposed method is proven to be usable for partitioning biological networks into highly connected components in a reasonable time. The algorithm is robust and adoptable, so it can be applied on various kinds of large-size biological networks.

COMBINED ANALYSIS OF GENOME AND TRANSCRIPTOME OF SINGLE DISSEMINETED PROSTATE CANCER CELLS REVEALS UNEXPECTED TRANSCRIPTOME PLASTICITY

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Introduction: Metastasis is the cause of more than 90% of cancer-related deaths. After resection of primary tumor, remaining disseminated cancer cells (DCCs) comprise founder cells of later arising lethal metastasis. DCCs are primary targets of adjuvant therapies. However, molecular features of DCCs are unknown, which explains the modest success of targeted adjuvant therapies.
Aims: Detect, isolate, and characterize the genome and transcriptome of DCCs from the BM of prostate cancer (PC) patients.
Patients and methods: DCCs exist as solitary epithelial cells in bone marrow (BM) of cancer patients. We have analyzed BM samples of three cohorts of PC patients for presence of single cytokeratin-positive (CK^+) or $EpCAM^+$ cells. These single cells were isolated and their genome and/or transcriptome subjected to whole-genome- (WGA) and/or whole-transcriptome-amplification (WTA). WGA products were analyzed using comparative genomic hybridization (CGH) and Sanger sequencing, and WTA products were analyzed using PCR, microarray analysis, and next generation sequencing.
Results: Survival analysis showed that the presence $EpCAM^+$ cells in BM, as opposed to CK^+ cells, is associated with progression of the disease. CGH analysis revealed that $EpCAM^+$ and CK^+ cells show different levels of genome instability. Next, we focused on transcriptome analysis of $EpCAM^+$ cells. We found that $EpCAM^+$ DCCs co-express epithelial and BM-haematopoietic transcripts, thereby displaying unexpected transcriptome plasticity. Furthermore, there are at least two subpopulations of $EpCAM^+$ DCCs, differing in abundance and identity of expressed transcripts, as well as in the level of genomic instability.
Conclusion: DCCs in prostate cancer show high level of heterogeneity at genome at transcriptome levels.

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FIRST INSIGHTS INTO THE WHOLE GENOME SEQUENCING RESULTS IN PROTHROMBIN BELGRADE MUTATION CARRIERS

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Introduction: Prothrombin Belgrade (p.Arg596Gln) belongs to the group of mutations leading to inherited thrombophilia, called antithrombin resistance. Previous study, carried out in large Serbian family with prothrombin Belgrade mutation, has shown complex mechanism of this mutation manifested with impaired inhibition by antithrombin, increased thrombotic potential, and decreased prothrombin activity in all prothrombin Belgrade carriers. However, potential modulators of prothrombin Belgrade mutation mechanism, responsible for phenotype differences among carriers (symptomatic and asymptomatic), are poorly understood.
Aim: To elucidate the "missing piece of the puzzle" in the complex prothrombin Belgrade mechanism that could contribute to better understanding of phenotype differences among carriers of this mutation.
Methods: Three members of a Serbian family with prothrombin Belgrade - the asymptomatic carrier (62 years old) older than the median age of first thrombosis occurrence in symptomatic carriers (26.5 years), his brother (58 years old) and daughter (38 years old), both symptomatic carriers, were screened by whole genome sequencing.
Results: In this pilot study, 53 genes involved in coagulation and fibrinolysis were analyzed. Results revealed three novel missense variants: p.Gly37Arg and p.Ala38Pro within *ADRA2A* gene (NP_000672.3), coding for α2A adrenergic receptor and p.Arg350Cys variant in gene coding for thromboxane A2 receptor - *TBXA2R* (NP_963998.2), only in an asymptomatic carrier. Both of these receptors have a pivotal role in proper activation and aggregation of platelets.
Conclusion: Platelets might be involved in prothrombin Belgrade mechanism, but further analyses are required to assess the effect of these novel variants on platelet function and their significance as potential modulators of prothrombin Belgrade mutation.

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MODULATORY EFFECTS OF BIOFLAVONOIDS ON HALOGENATED BOROXINE TREATMENT IN CULTURED LYMPHOCYTES

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Introduction: Halogenated boroxine $K_2(B_3O_3F_4OH)$, – HB has significant anti-proliferative role in different cell types *in vitro* and *in vivo*. Cytogenetic analysis showed various effects of halogenated boroxine in higher concentration on chromosome and chromatid-types aberrations. Genotoxic effect of this compound was significantly decreased when treatment was in combination with bioflavonoids (luteolin and delphinidin). In order to test for the antioxidative effect of selected bioflavonoids, a relative expression of *HIF1A* gene was estimated and compared between different treatments.

Methods: Heparinized blood samples from five healthy volunteers were treated with bioflavonoids individually and simultaneously with halogenated boroxine and cultivated at 37°C in controlled experiment. Total RNA was isolated from treated cultures using Quick-RNA™ Mini Prep Plus kit. Gene copy number was measured using SYBR based Real-Time PCR amplification method. Normalized ratio of target (*HIF1A*) and housekeeping (*GADPH*) gene was used to estimate relative gene expression between treatments (REST®).

Results: Significant up-regulation of *HIF1A* expression (p=0,009) was found in combined halogenated boroxine and luteolin treatment.

Conclusion: Variation in *HIF1A* expression between series of treatments may indicate influence of bioflavonoids in modulation of damage-induced by higher concentration of halogenated boroxine. For precise clarification of suggested anti-oxidative effects of bioflavonoids additional models of treatment and genes should be investigated.

COMPARISON OF IgE CONCENTRATIONS AND EPICUTANEOUS AEROALLERGEN SENSITIZATION IN PATIENTS WITH ATOPIC DERMATITIS

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Introduction: The level of IgE is associated with severity of atopic dermatitis (AD) and contributed by abnormality of skin barrier. Higher total IgE concentration is a good marker for sensitization in patients with atopic dermatitis.
The aim: The aim of this study was to correlate incresead IgE concentrations and aeroallergen sensitization in patients with AD with different sex, age and duration of disease.
Methods: Sixty-one patient of both sex, between the ages of one and 80 years were included in this study. Patients were categorized regarding sex (male *vs.* female), age (<18, 19-40 and >40 years) and duration of disease (1-5 *vs.* >5 years). Allergy-testing included performing of skin prick test with respect to 10 aeroallergens and measuring of total IgE concentration in serum (RAST method).
Results: Prick tests were positive in 59% of patients for at least one of tested aeroallergenes, while remaining 41% of patients were negative for all tested aeroallergenes. Aeroallergenes that most frequently showed positive reactions in patients with AD were tree pollen (45.9%), grass pollen (41%) and house dust (39.3%), while bacteria were (11.5%) the least common. In general, male patients had higher values of total IgE in serum (n=13, 68.4%) in comparison to female patients (n=19, 45%).
Group of patients who were >40 years old demonstrated highest sensitivity to tested allergens. Patients with the disease duration between 1-5 years had less positive results compared to group of patients who had AD >5 years.
Conclusion: Absolute positive correlation was obtained between an increase of IgE concentration in serum and positive reactions to aeroallergenes.

CYTOTOXICITY EVALUATION OF *Micromeria pulegium* (*Rochel*) Benth EXTRACT IN HUMAN GR-M MELANOMA

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Introduction: Lamiaceae species are mostly aromatic and well characterized for their bioactive and condiment properties coming from high levels of various secondary metabolites, such as essential oils, terpenoid and phenolic compounds. According to estimates, around 30 Lamiaceae species in B&H are traditionally used as medicaments or spices with a large number of unexplored spices due to its endemicity. This research aimed to determine cytotoxic potential of endemic Lamiaceae species *Micromeria pulegium* (Rochel) Benth in human melanoma GR-M cells.

Methods: Trypan blue exclusion assay in human melanoma GR-M cell line in triplicates was applied. Aqueous extract of *M. pulegium* (Rochel) Benth was tested in final concentrations of 0.1; 0.2 and 0.3 mg/ml. Simple linear regression and one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison were used to test statistical significance of obtained results.

Results: Simple linear regression showed significant association between increase of *M. pulegium* (Rochel) Benth extract concentration and reduction of GR-M cell culture viability ($p=0.002$). ANOVA revealed significant decrease in GR-M cell culture viability in 0.3 mg/ml concentration ($p=0.002$).

Conclusion: Due to the significant cytotoxic effect of *Micromeria pulegium* (Rochel) Benth aqueous extract in concentration of 0.3 mg/ml on human GR-M melanoma cell line, this and higher concentrations should be object of future studies.

CONCOMITANT HIGH EXPERSSION OF β-CATENIN AND EGFR ASSOCIATE WITH UNFAVOURABLE CLINICAL FEATURES OF PAPILLARY THYROID CARCINOMA

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Introduction: β-catenin is one of the major players of Wnt/β-catenin signalling pathway which regulates cell differentiation and proliferation. Disturbances in its signalling in advanced malignancies are associated with aberrant β-catenin expression and cellular localisation. Recent studies suggested a possible crosstalk between Wnt/β-catenin pathway activation and epidermal growth factor receptor (EGFR) signalling during tumour initiation and progression.
Material and Methods: Since the expression of β-catenin and EGFR in thyroid carcinoma is poorly studied, we analysed their immunohistochemical expression profiles in archival tissue (n=104) of papillary thyroid carcinoma (PTC) in relation to clinicopathological features of the patients.
Results: High cytoplasmic/membranous levels of expression of β-catenin and EGFR, found in 39/104 (37.5%) and 58/104 (55.7%) of PTC cases, respectively, showed mutual positive correlation ($p<0.0001$). We observed a correlation of the expression of each molecule with the presence of extrathyroid invasion, lymph node metastasis, degree of neoplastic infiltration and advanced pT status ($p<0.05$). Additionally, coexpression of high cytosolic levels of β-catenin and EGFR showed correlation with adverse clinicopathological features of the patients ($p<0.05$).
Nuclear localisation of β-catenin was found in a subset of PTC patients (16/104, 15.4%) showing strong association with high EGFR expression (15/16, 93.7%) and aggressive clinical phenotype (presence of capsule invasion in 81.25% and regional lymph node metastases in 52.3%).
Conclusion: Coexpression of high levels of β-catenin and EGFR in association with clinicopathological features implicates their clinical utility in risk stratification of PTC patients, and suggests the possibility of the crosstalk between Wnt/β-catenin and EGFR signalling during the PTC progression.

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ARONIA JUICE SUPPLEMENTATION AND ITS ACUTE EFFECT ON PLATELET ACTIVATION MARKERS IN HALF-MARATHON RUNNERS

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Introduction: Regular physical exercise is beneficial as it can prevent future cardiovascular events; However, intensive aerobic activity may lead to excess ROS production, which can later increase platelet activation and cardiovascular risk. Therefore, our aim was to investigate whether polyphenol-rich Aronia juice can ameliorate the level of platelet activation as a response to oxidative imbalance in recreational half-marathon runners.
Methods: We conducted a randomized, placebo controlled crossover study with 10 male recreational runners, aged 30.8±2.3. They consumed identical breakfast with either 200 ml of polyphenol-free placebo or Aronia juice (AJ) before simulating a half-marathon race. We took blood samples before the intervention and 15 minutes, 1h and 24 hours after running on a predefined lane, and assessed platelet activation- P selectin and GPIIbIIIa expression – by whole-blood flow cytometry, with or without stimulation with platelet agonist (0.5 uM adenosinediphosphate, ADP).
Results: Comparing with placebo, AJ treatmant significantly lowered expression of both P-selectin and GPIIbIIIa with or without ADP stimulation. AJ significantly decreased: % of P-selectin positive platelets 1h and 24h after running; ADP stimulated P-selectin expression at all three post-race time points; % ofGPIIbIIIa positive platelets 1h-after race; ADP stimulatedGPIIbIIIa expression 15min- and 1h-after race. All of these changes were significant comparing with the before-race values and with placebo (a two-way repeated measures ANOVA); no significant observations were present in the placebo group.
Conclusion: Our results imply that AJ consumption could counteract undesirable effects of half-marathon race on platelet activation.

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COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS OF TUMOR AND TUMOR MARGIN IN PATIENTS WITH ORAL CANCER – A PILOT STUDY

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Aims: To compare the copy number variations in two types of tissue originating from the same patient with oral squamous cell carcinoma (OSCC): cancer tissue and cancer margin, evaluated by the pathologist as histologically "negative", i.e. tumor-free.

Methods: The study comprised 5 patients with OSCC, treated at the Clinic of Maxillofacial Surgery, School of Dental Medicine, University of Belgrade. Two samples from each patient were used for DNA extraction and further array CGH analysis. Genomic DNA from a healthy donor was used as reference.

Results: Deletions and/or duplications were observed in all samples. A strong heterogeneity was also observed: most aberrations were present in single patients. Though difference between tumor and margin were present, several variants were present both in tumor and in the corresponding margin.

Conclusions: Although the tumor margin was labeled as cancer free by the pathologist, it appears that several of the aberrations detected in the tumor were also found in a seemingly normal tissue along the resection line. Molecular changes in the margins might in part explain the propensity of OSCC to recurrences and metastasis. This supports the view that, besides histological status, molecular profile of tumor margins should also be taken into consideration for diagnostic and prognostic purposes.

THE INFLUENCE OF BASELINE VIRAL AND HOST FACTORS ON THERAPY RESPONSE IN PATIENTS WITH CHRONIC HEPATITIS C GENOTYPE 1B FROM SERBIA

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Introduction: This study was designed to describe the relationship between host and viral factors and their impact on response to therapy with pegylated interferon/ribavirin (PEG-IFN/RBV) in patients with chronic hepatitis C (HCV) genotype 1b. Also, we investigated how IL28B single nucleotide polymorphism (SNP) rs12979860 may have an impact on therapy response and stage of liver fibrosis.
Methods: Samples were collected from a total 100 patients with chronic hepatitis C and genotype 1b, before the start of PEG-IFN/RBV therapy. The SNP rs12979860 were determined by TaqMan assay.
Results: We found significant association between age and response to therapy (p=0.001). The major route of HCV transmission was unknown (48%), followed by post-blood transfusion (23%) and 21% intravenous drug users (IVDU). With respect to known route of HCV transmission, patients with positive history of blood product transfusion were significant frequently in non-responders (NR) than in patients with sustained virologic response (SVR). In addition, we found significant association with gender (p=0.004). Moreover, in group of IVDU, man were significant more common than women (18% vs. 3%), while women were more common in group with positive history of blood product transfusion (16% vs. 7%). There was significant difference between response to therapy and rs12979860 genotypes (p=0.001). Thus, patients with CC genotype of IL28B rs12979860 CC were significantly more frequent in SVR than in NR. There was no correlation between rs12979860 genotypes and stage of liver fibrosis.
Conclusion: Our results confirm the complexity of viral host interactions in determining HCV infection outcome.

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BINDING OF IMMUNOGLOBULINS FROM SERA OF PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME TO HTR-8/SVNEO TROPHOBLAST CELL LINE REDUCES MEDIATORS OF CELL INVASION

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Introduction: Immunoglobulins from sera of patients with antiphospholipid syndrome (APS) were shown to decrease trophoblast cell invasion *in vitro* and alter their cytokine levels. This study explored whether antiphospholipid antibodies isolated from the sera of women with APS - aPL+ IgG, affect additional mediators of trophoblast cell invasion.
Methods: Binding of aPL+ IgG to HTR-8/SVneo cells was analyzed by flow cytometry and immunocytochemistry. Phosphorylation of p38 MAP kinase, levels of integrin subunits α1, α4, α5 and β1, and total cell and secreted galectin-1 (gal-1), were analyzed by Western blot. Metalloproteinases (MMPs) -2 and -9 were assessed by gelatin zymography.
Results: After 1h of culture 21% cells bound aPL+ IgG, as opposed to 6% in control IgG treated cells. aPL+ IgG induced phosphorylation of p38 MAP kinase after 30 min of culture. After 24h of treatment, aPL+IgG decreased protein levels of integrin subunits α1 (to 78% of control; $p<0.01$), α4 (to 65% of control, $p<0.01$), α5 (to 76% of control; $p<0.01$) and β1 (to 80% of control; $p<0.01$), and secreted gal-1 (to 68% of control; $p<0.05$). Gelatin zymography showed a decrease in gelatinolytic activity of MMP-9 (to 70% of control; $p<0.001$).
Conclusion: The results regarding engagement of p38 MAPK signaling pathway and decrease in integrin subunit α4 and secreted gal-1 in HTR-8/SVneo cells, represent a novel finding wich expands our knowledge of cellular effects of aPL+ IgG on trophoblast.

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INCIDENCE OF ORAL PATHOGENS IN BLOOD VESSELS OF PATIENTS WITH ATHEROSCLEROSIS-AGE RELATED CHANGES

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Aims: (a) To determine the presence of the most common periodontopathogens in atherosclerotic plaques of carotid and coronary arteries, abdominal aortic aneurysms, inguinal and iliac arteries and the mammary artery in patients with arterial occulsive disease; b) to examine the differences in the distribution of periodontopathogens in relation to patient age groups
Methods: The study included a total of 90 patients with periodontal disease and atherosclerosis Samples of plaque were subjected to PCR analysis in order to detect the presence of the following microorganisms: *Porphyromonas gingivalis, Actinobacilus actinomycetemcomitans, Prevotella intermedia, Tannarella forsythia and Treponema denticola*.
Results: Oral pathogens showed different distribution in cardiovascular system. The highest percentage was detected in the coronary blood vessesls (38.7%) and then in carotid blood vessesls (30%). Slightly less (12.9%) was found in abdominal aortic aneurysms. The minimum presence of periodontogens was detected in mammary (9.7%) and femoral arteries (3.2%). The most affected age group in terms of microorganism presence were patients between 60 and 70 years, followed by the age group 50-60 years. In these age groups the predominant bacteria were *T. denticola* (55%) and *A. actinomycetemcomitans* (53%).
Conclusion: Microorganisms that cause periodontal disease enter the systemic circulation causing transient bacteriemia and remain on the wall of atherosclerotic blood vessels. Periodontopathogens upon entering the systemic circulation are mainly distributed and retained in blood vessels of the heart and the blood vessels that are close to the heart. Their incidence is the highest in the vulnerable group of patients aged 50 to 70 years.

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ASSOCIATION OF SLC28A3 GENE EXPRESSION AND CYP2B6*6 ALLELE WITH THE RESPONSE TO FLUDARABINE PLUS CYCLOPHOSPHAMIDE IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Introduction: Fludarabine-cyclophosphamide (FC) chemotherapy is the treatment of choice in the majority of chronic lymphocytic leukemia (CLL) patients. Besides the functionality of p53 protein, which is the main determinant of FC responsiveness, germline polymorphisms and the expression of genes involved in FC pharmacodynamics and/or disposition also influence the treatment outcome. Cytochrome P450 isoform CYP2B6 is involved in cyclophosphamide activation. The common *CYP2B6*6* allele, defined by the presence of c.516G>T (exon 4) and c.785A>G (exon 5) of *CYP2B6* gene (NG_007929.1), has been associated with reduced response to FC in some studies. In addition, elevated expression of *SLC28A3* gene, coding for human concentrative nucleoside transporter 3, has been implicated in resistance to fludarabine monotherapy.
Aim: The aim of this study was to evaluate the predictive value of *CYP2B6*6* allele and *SLC28A3* expression for the response to FC treatment in CLL.
Patients and Methods: The study included 44 patients without *TP53* defects (deletion and/or mutations). *CYP2B6*6* genotyping was performed by direct sequencing. The expression of *SLC28A3* mRNA was measured by qRT-PCR.
Results: *SLC28A3* expression was significantly higher in patients who experienced progressive disease after FC administration, in comparison to patients who achieved complete and partial remission (p=0,036). However, the level of *SLC28A3* mRNA was not associated with progression-free survival and overall survival. *CYP2B6*6* allele was detected in 25/44 patients (56,8%), and showed no association with the treatment outcome either alone, or in combination with high *SLC28A3* levels.
Conclusion: Elevated *SLC28A3* expression is a predictor of inferior short-term response to FC chemotherapy in CLL.

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JOINT EFFECTS OF VARIANTS IN RNA EDITING AND SEROTONERGIC SYSTEM GENES AND STRESSFUL LIFE EVENTS IN PREDISPOSITION FOR SUICIDE ATTEMPT IN PSYCHIATRIC PATIENTS

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The aim: The aim of this study was to test joint effects of stressful life events and variants in RNA editing (*ADAR* and *ADARB1*), serotonin receptor 2C (*HTR2C*) and tryptophan-hydroxylase 2 (*TPH2*) genes in predisposition for suicide attempt in psychiatric patients.
Methods: The study included 165 psychiatric patients who attempted suicide and 188 who did not. Childhood abuse and acute stressful life events were evaluated using the Early Trauma Inventory Self-Report questionnaire and the List of Threatening Experiences Questionnaire, respectively. 23 *ADAR* and *ADARB1* tag variants, *HTR2C* variant rs6318 and *TPH2* variants rs7305115 and rs4290270 were genotyped. Generalized linear models and backward selection were applied to test the joint effects of examined genetic and environmental factors on suicide attempt. Psychiatric diagnoses, patients' gender and age were included as potential confounders.
Results: One best fitting minimal model included the joint effect of childhood emotional abuse, acute stressful events, *ADARB1* rs9983925 and *HTR2C* rs6318 as individual risk factors and two-way interactions of childhood general traumas with psychiatric diagnoses and with emotional abuse. Another model revealed the joint effect of childhood general traumas as individual risk factors, a two-way interaction of general traumas and *TPH2* rs4290270 and a three-way interaction of general traumas, *TPH2* rs4290270 and *ADARB1* rs4819035.
Conclusion: The results indicate that *ADARB1*, *HTR2C* and *TPH2* genes and a cumulative exposure to a variety of childhood and acute stressful events display a joint effect that contributes to predisposition for suicide attempt in psychiatric patients, primarily in those suffering from mood disorders.

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ARONIA JUICE SUPPLEMENTATION IN HALF-MARATHON RUNNERS: CAN IT ACTUALY AFFECT THE RATE OF PLATELET AGGREGATION?

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Introduction: Despite its cardioprotective role, acute intensive activity may alter platelet response and promote their aggregation with other blood cells, which is associated with prothrombotic events. Data regarding platelet aggregation and polyphenols inclusion in recreational people's diets is still inconsistent, which is why we explored the acute impact of polyphenol-rich Aronia juice consumption in recreational runners after simulation of half-marathon race.
Methods: 10 recreational male runners (30.8±2.3 y old) were involved in a single blinded, randomized, placebo controlled, crossover study. The intervention consisted of 200 ml Aronia or polyphenol-free placebo juice which was consumed after calorically identical breakfast. We assessed platelet aggregation with neutrophils and monocytes by whole-blood flow cytometry, with or without adenosine diphosphate (ADP, 0.5 uM) as agonist, in 4 time-points: baseline (before intervention), 15 minutes, 1 hour and 24 hours after the half-marathon race simulation.
Results: A two-way repeated measures ANOVA showed that time had a significant impact on basal values of both biomarkers, while there was a borderline difference between the two treatment groups for the ADP-stimulated platelet-monocyte aggregation rate only. Further investigation revealed that platelet aggregation with both cell types was changed, although not uniformly in all time points after Aronia juice consumption, and the same was present in the placebo group for platelet-neutrophil interaction.
Conclusion: Aronia juice consumption did not significantly affect platelet aggregation rate in male recreational half-marathon runners, however, most of the time-induced differences were more pronounced in the intervention group.

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MOLECULAR AND PHENOTYPIC CHARACTERISTICS OF SEVEN NOVEL MUTATIONS CAUSING BRANCHED-CHAIN ORGANIC ACIDURIAS

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Introduction: Specific mitochondrial enzymatic deficiencies in the catabolism of branched-chain amino acids cause methylmalonic aciduria (MMA), propionic acidemia (PA) and maple syrup urine disease (MSUD).
Methods: Combining Sanger sequencing and targeted next generation sequencing, we identified disease-causing mutations in all patients. Furthermore, we used *in silico* and/or eukaryotic expression studies to assess the effect of novel genetic variants identified in our patients.
Results: Disease-causing mutations were identified in ten unrelated branched-chain organic acidurias (BCOA) patients. We detected eight previously described mutations: p.Asn219Tyr, p.Arg369His p.Val553Glyfs*17 in *MUT*, p.Thr198Serfs*6 in *MMAA*, p.Ile144_Leu181del in *PCCB*, p.Gly288Valfs*11, p.Tyr438Asn in *BCKDHA* and p.Ala137Val in *BCKDHB* gene. Interestingly, we identified seven novel genetic variants: p.Leu549Pro, p.Glu564*, p.Leu641Pro in *MUT*, p.Tyr206Cys in *PCCB*, p.His194Arg, p.Val298Met in *BCKDHA* and p.Glu286_Met290del in *BCKDHB* gene. *In silico* and/or eukaryotic expression studies confirmed pathogenic effect of all novel genetic variants. Aberrant enzymes p.Leu549Pro MUT, p.Leu641Pro MUT and p.Tyr206Cys PCCB did not show residual activity in activity assays. In addition, activity of MUT enzymes was not rescued in the presence of vitamin B12 precursor *in vitro* which was in accordance with non-responsiveness or partial responsiveness of patients to vitamin B12 therapy.
Conclusion: Our study brings the first molecular genetic data and detailed phenotypic characteristics for MMA, PA and MSUD patients for Serbia and the whole South-Eastern European region. Therefore, our study contributes to the better understanding of molecular landscape of BCOA in Europe and to general knowledge on genotype–phenotype correlation for these rare diseases.

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ASSOCIATION OF ALELLIC VARIANT rs1137101 IN *LEPR* GENE WITH SUSCEPTIBILITY AND CLINICAL COURSE OF MULTIPLE SCLEROSIS – PRELIMINARY RESULTS

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Introduction: Activation of leptin receptor (LEPR) regulates Th1/Th2 balance, which is related to pathogenesis of multiple sclerosis (MS). The common alellic variant in *LEPR* gene, rs113710 (c.668A>G), is associated with its altered binding activity. In this case-control study, we aimed to investigate the association of *LEPR* rs1137101 variant with susceptibility and clinical course of MS.
Methods: We included 128 MS patients (age (mean±SD)=42,6±11,5 years, sex ratio (f/m)=1,4) and 128 control subjects (age (mean±SD)=38,4±10,2 years, sex ratio (f/m)=1,6). Patients were divided into two groups by clinical course of MS: relapsing-remitting (RRMS, N=99) and secondary progressive (SPMS, N=29). Genotyping was performed using TaqMan® technology. Statistical analysis was performed using SPSS software (SPSS 17.0).
Results: We did not find significantly different distributions of *LEPR* genotypes either between MS patients and controls (AA=32.0%, AG=50.8% GG=17,2% *vs.* AA=30.5%, AG=49.2% GG=20,3%, respectively; χ2 test, p=0.813) or between RRMS and SPMS patients (AA=30.3%, AG=48,3% GG=21.3% *vs.* AA=34.5%, AG=55.2% GG=10.3%, respectively; χ2 test, p=0.418). The GG genotype was associated with a decreased level of neurological impairment, presented with EDSS score range 1.0-4.5 (χ2 test by recessive model (GG *vs.* AA+AG), p=0.049). Time-dependent disease course, presented with MSSS score, was not significantly associated with any of *LEPR* genotypes.
Conclusion: The carriers of *LEPR* rs1137101 A alelle tend to develop severe neurological impairment, compared with carriers of the GG genotype. The present finding needs to be replicated in a larger sample group.

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MOLECULAR CHARACTERIZATION OF MATURITY-ONSET DIABETES OF THE YOUNG IN SERBIAN PEDIATRIC PATIENTS

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Introduction: Maturity-onset diabetes of the young (MODY) is a rare form of diabetes characterized by autosomal dominant inheritance, young onset hyperglycemia and non-insulin dependent diabetes. Clinically and genetically heterogeneous, it is often misdiagnosed as type 1 or type 2 diabetes, leading to inappropriate therapy. MODY is caused by a single gene mutation that affects pancreatic beta-cell function. At least 13 genes, defining 13 subtypes, have been identified to cause MODY. A correct diagnosis is important for the right therapy, prognosis and genetic counseling.
Aim: The aim of this study was to perform molecular analysis of 27 clinically characterized pediatric MODY patients and to identify, according to the genes involved, MODY subtypes.
Methods: Next generation sequencing methodology, was used to sequence 13 MODY-associated genes in a single test. Different prediction software tools and public databases were used to analyze gene variants.
Results: Our study identified genetic variants in 4 MODY-genes in 19 patients (~70%): 10 patients (~52%) had a genetic variant in *GCK* gene, and the rest of the patients had genetic variants in *HNF1A* (~15%), *HNF1B* (~15%) and *NEUROD1* (~15%) genes. Most variants were missense (89%), while two frameshifts were caused by one insertion in *HNF1A* gene and one deletion in *HNF1B* gene. All variants were heterozygous, except for one found in homozygous state in *NEUROD1* gene.
Conclusion: Our preliminary results demonstrate that MODY-GCK subtype is the most common type in our population. Genetic testing is necessary for differential diagnosis and application of right therapy depending on the MODY subtype.

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EFFECTS OF PHYSIOLOGICALLY-RELEVANT CONCENTRATIONS OF ANTHOCYANINS AND THEIR METABOLITES ON ADENOSINE DIPHOSPHATE-INDUCED PLATELET ACTIVATION AND AGGREGATION

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Introduction: A growing body of evidence suggests that anthocyanins could play an important role in the cardioprotective effects associated with consumption of anthocyanin-rich berries and derived products. These health benefits, may partly be attributed to the direct effect of anthocyanins on platelet function. However, evidences supporting these claims are still scarce. The aim of this study was to investigate the effect of physiologically-relevant concentrations of anthocyanins and their metabolites on platelet activation and platelet-leukocyte aggregation.
Methods: The whole blood samples from seven healthy volunteers were treated with anthocyanins: cyanidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-galactoside, delphinidin-3-glucoside, peonidin-3-glucoside or their metabolites: 4-hydroxybenzaldehyde, protocatechuic, vanillic, ferulic and hippuric acids at physiologically-relevant concentrations (0.1-2μM). Markers of adenosine diphosphate-induced platelet activation (P-selectin) and platelet-monocyte and neutrophil aggregation were analysed using flow-cytometry.
Results: Anthocyanins cyanidin-3-arabinoside, delphinidin-3-glucoside and peonidin-3-glucoside showed the ability to decrease agonist-induced P-selectin expression (p=0.04, 0.02 and 0.03, respectively) and affect P-selectin-mediated platelet-neutrophil aggregation (p=0.02, 0.06 and 0.08, respectively). Among tested metabolites, hippuric and protocatechuic acid inhibited P-selectin expression (p=0.04 and 0.02, respectively) and showed a tendency to decrease platelet-neutrophil aggregation (p=0.07 and 0.08, respectively), while 4-hydroxybenzaldehyde significantly affected P-selectin expression (p=0.04), platelet-neutrophil (p=0.003) and platelet-monocyte aggregation (p=0.008).
Conclusion: This study showed that anthocyanins and their metabolites at physiologically-relevant concentrations could modulate platelet activation and platelet-leukocyte aggregation, possibly contributing to the reported cardioprotective effects associated with the consumption of anthocyanin-rich foods.

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COPY NUMBER VARIATIONS AND MICRODELETIONS IN Y-CHROMOSOMAL AZOOSPERMIA FACTOR REGIONS IN PATIENTS FROM SERBIA

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Introduction: Although infertility may be caused by many factors, in about half of the cases even after detailed medical examination the cause remains elusive and it is often referred to as idiopathic. In many cases male's idiopathic infertility is associated with microdeletions of AZF regions of human Y chromosome. However, it is also believed that partial deletions as well as partial duplications of AZFc region (referred to as copy number variations, CNVs) may greatly contribute to development of male idiopathic infertility in many European populations.
Methods: DNA was isolated from blood samples of 54 men with idiopathic infertitilty and 41 fertile men as controls. Multiplex Ligation-dependent Probe Amplification (MLPA) was used to tested on presence of microdeletions in AZF regions and CNVs in the AZFc region of human Y chromosome.
Results: Microdeletions were found in three out of 54 men with idiopathic infertility. CNVs were detected in 19,61% of men with idiopathic infertility and in 9,76% of controls. Detected CNVs consisted of gr/gr duplications and b2/b4 duplications. For the first time in Serbian population, two types of complex rearangements of AZFc region were detected in men with idiopathic infertility. In one of the complex rearrangements, inversion of b2/b3 amplicons preceded g1/g3 duplication. In similar rearrangement, inversion of b2/b3 amplicons lead to g1/g3 deletion.
Conclusion: Obtained results stress out that future examinations are necessary for better elucidation of association between CNVs in AZFc region and male's idiophatic infertility in Serbian population.

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EVALUATION OF ANTI- AND PRO-APOPTOTIC GENE EXPRESSION IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES BY *Micromeria pulegium* (Rochel) Bentham EXTRACTS *IN VITRO*

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Introduction: The aim of this study was to evaluate relative gene expression of apoptosis-related genes using RT MLPA Apoptosis assay in cultivated human lymphocytes, after the treatment with *Micromeria pulegium* (Rochel) Bentham aqueous and DMSO extracts.
Methods: For aqueous and DMSO extracts preparation, plants were collected from two localities in Herzegovina. RNA was extracted by using NucleoSpin® RNA extraction kit, from cultured human peripheral blood lymphocytes treated with the plant extracts in the final concentrations of 0,05, 0,1 and 0,2 mg/mL. Positive and negative controls were set up as well. Relative expression was measured with SALSA RT-MLPA R011-C1 Apoptosis assay (MRC Holland). Electrophoresis was performed on Genetic Analyzer 3500 (Applied Biosystems).
Results: Preliminary results showed that extract concentration 0,05 mg/ml and 0,1 mg/ml H_20 solvent indicate a statistically significant upregulation of different pro-apoptotic genes (*SERPINB9, CDKN1a, BAD, GZMB, BBC3, BAX, BCL2L1*), as well as *BCL2* and *BCL2A1* genes, belonging to anti-apoptotic members of BCL-2 family. Downregulated genes were *CFLAR, BIRC1, MOAP1, APAF1* and *PRF*. All extract concentrations of DMSO solvent (0,05, 0,1 and 0,2 mg/mL) showed unanimous results with only *BNIP3L* gene upregulated, and *FAS*, *BIRC3* and *BCL2A1* downregulated genes.
Conclusion: Human lymphocytes treated with *M. pulegium* extracts, regardless of solvent or concentration applied, showed uniform upregulation of pro-apoptotic genes, which can suggest that *M.pulegium* extract has pro-apoptotic influence on normal human lymphocytes in vitro.

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MOLECULAR DIAGNOSTICS OF HIGH RISK HUMAN PAPILLOMA VIRUS

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Introduction: Human papilloma viruses of high oncogenic potential were detected in over 90 percent of all cervical carcinoma cases. Our aim was to highlight the significance of their early detection due to prevention of cervical carcinoma by applying Hybrid Capture 2 test together with standard cervical cytology, as well as comparing positive humane papilloma virus findings to their biopsy findings.
Methods: The sample was consisted of squamous epithelial cells, and the method used was molecular diagnostic (Hybrid Capture 2) with enforced signal and 96-well microplate analysis of hybridization using chemiluminescent detection of DNA of 13 high risk human papilloma virus in cervical samples.
Results: 91 out of 2955 subjects had complete biopsy as well as positive humane papillomavirus results. The infection rate with high risk human papilloma virus by histological grade was as follows: 24.17% had chronic cervical infection, 13.18% CIN I (Cervical Intraepithelial Neoplasia), 21.97% CIN II, 19.78% CIN III, carcinoma *in situ* 10.99% and invasive carcinoma 6.59%.
Conclusion: Positive findings of human papilloma virus with high oncogenic potential in the cervical cells clearly indicate the possibility of developing cervical carcinoma. Their presence in cervical cells can be proven only by using molecular diagnostic DNA tests.

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REVIEW OF RARE HEREDITARY METABOLIC DISEASES IN BOSNIA AND HERZEGOVINA

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Introduction: Central register for rare diseases, including rare hereditary metabolic diseases has not yet been established in Bosnia and Herzegovina. There are two regional policies for rare diseases but not on the country level, that should be requirement for the easier recognition, diagnosis and treatment of rare disease patient in Bosnia and Herzegovina. Registered societies for rare diseases in B&H, according to study conducted in 2013, have total of 61 registered patients, including those diagnosed with Gaucher disease, leukodystrophy, NBIA and Niemann-Pick disease.

Methods: Genetic testing for rare hereditary and metabolic diseases referred from physicians from different parts of country is usually completed in-house, or in collaboration with partner laboratories. Data regarding rare hereditary diseases was collected during period from 2005 to 2017, in Institute for genetic engineering and biotechnology. Standard statistical tests were employed for evaluation of collected data.

Results: Up to date, 15.6% of testing was for rare hereditary metabolic diseases. Majority of cases were for Wilson disease testing (41.1%). Other diseases include: Glycogen storage disease type 1A; Spastic paraplegia 35; Alpha 1 antitrypsin deficiency; Multiple endocrine neoplasia Type 2B; Canavan disease; Neurodegeneration with Brain Iron Accumulation; Acute intermittent porphyria; Leber hereditary optic neuropathy and others.

Conclusions: Available data reveal that patients diagnosed with rare diseases in B&H rarely have medical community support and most tests and treatments are inaccessible for them. As public health strategy requires detailed clinical evaluation prior to genetic testing, the high rates of the suspected cases are confirmed as familial mutation carriers.

GENETIC TESTING FOR CYSTIC FIBROSIS IN B&H

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Introduction: Cystic fibrosis (CF) is an autosomal recessive genetic disorder that most critically affects the lungs. It is characterized by abnormal transport of chloride and sodium across an epithelium leading to thick, viscous secretions and severe respiratory problems. CF is caused by a point mutation in the gene cystic fibrosis trans-membrane conductance regulator (CFTR). The most common mutation, ΔF508, is a deletion of three nucleotides that results in a loss of the amino acid phenylalanine (F) at the position 508 on the protein. Although most people have two copies (alleles) of the *CFTR* gene, one functional copy is needed to prevent cystic fibrosis; therefore, CF develops when neither allele can produce a functional CFTR protein. Thus, CF is considered an autosomal recessive trait, further emphasizing the need for genetic testing and counseling.

Material and Methods: At the Center for Genetics, we analyzed 29 CF causing *CFTR* mutations. The PCR based analysis was performed on the patients' DNA isolated from peripheral blood. We had 25 patients with suspected CF, of which in 10 patients genetic analysis confirmed CF.

Results: Here we will describe 10 confirmed patients for different detected CFTR mutations in our Center. We found two patients with two *CFTR* mutations, a girl with ΔF508 and 621+1G>T, and boy with the ΔF508 and G542X *CFTR* mutation. One patient with 621+1G>T *CFTR* mutation and seven patients with the ΔF508 mutation.

Conclusions: Timely diagnosis of CF is essential because it can greatly improve the quality and quantity of a patient's life.

MUTUAL EFFECT OF miR-21 DOWNREGULATION AND RADIOTHERAPY ON GLIOBLASTOMA CELL FATE

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Introduction: Glioblastoma (GBM) remains one of the most aggressive and deadly forms of cancer despite intensive therapeutic strategies. Literature data demonstrated that microRNA 21 (miR-21) is significantly elevated in GBM and recognized as an indicator of glioma prognosis and a prosperous target for anti-tumor therapy. One of the targets of miR-21 is *SOX2* gene which high expression is hallmark of subpopulation of cells within tumor, named glioblastoma stem cells. It has been proposed that these cells are responsible for GBM radio-resistance. Our aims were to analyze whether miR-21 downregulation could sensitize glioblastoma cells to standard irradiation treatment and to analyze *SOX2* expression after downregulation of miR-21 in irradiated and non-irradiated U87 and U251 glioblastoma cell lines.
Methods: We examined the effects of downregulation of miR-21 and irradiation in U87 and U251 cell lines on cell death, senescence, cell growth and expression of *SOX2*.
Results: We revealed that downregulation of miR-21 in U87 and U251 cell lines induced cell death, senescence and cell growth inhibition. The effect on cell growth was more pronounced when cells with miR-21 knockdown were irradiated, while there was no additional effect on senescence following irradiation. Furthermore, we revealed that *SOX2* gene expression is not altered after downregulation of miR-21, as well as after irradiation of glioblastoma cells with downregulation of miR-21 expression.
Conclusion: Presented findings indicate that downregulation of miR-21 in combination with radiation represents potent mechanism to target glioblastoma cells. Obtained data imply that detected effects are not mediated through the changes of *SOX2* expression.

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BIMODAL EFFECTS OF NEUROPEPTIDE Y ON MIGRATION CAPACITY AND INVASION POTENTIAL OF HUMAN CHORIOCARCINOMA CELL LINE JEG-3

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Introduction: The disturbance of trophoblast cell migration and invasion could result in poor placental formation and perfusion leading to several pregnancy disorders. Neuropeptide Y (NPY) is bioactive neuropeptide which has recently been shown to be involved in some pregnancy complications related to diminished trophoblastsmobility. The aim of this study was to investigated the effects of two different concentrations of NPY on migration and invasion capacity of human trophoblast cells JEG-3.
Methods: The cells were exposed to NPY in 24 h and 72 h treatments, respectively in concentrations of 0.1 nM (physiological concentration) and 1 nM (pathological concentration which correspons to NPY levels in certain pregnancy disorders). Migration index was determined by using Boyden chamber transwell migration assay. Invasion capacity was determined by assessment of metalloproteinase 9 enzyme (MMP-9) quantity and by evaluating MMP-9 gene expression using qRT-PCR protocol. The parameter values were detected spectrofotometrically using ELISA microplate reader and by gene expression software.
Results: The obtained results suggest that NPY applied in physiological concentration had no significant effect on cell mobility, but increased MMP-9 protein and iRNA levels compared to control in 72 h exposure. NPY applied in pathological concentration decreased migration index in both time treatments, and the levels of MMP-9 enzyme and iRNA were decreased after 72 h exposure.
Conclusion: The obtained results indicate that NPY exerts bimodal effects depending on applied concentration. Elevated concentration could inhibit cell migration and invasion properties potentially contributing to placental complications, but under physiological concentration NPY maintains homeostasis of trophoblasts by promoting their invasion.

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IMMUNOGLOBULIN G FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENTS INDUCES OXIDATIVE STRESS AND UPREGULATES THE ANTIOXIDATIVE SYSTEM IN THE BV-2 MICROGLIAL CELL LINE

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Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder, with no diagnostics for the presymptomatic phase and no effective treatment. Having in mind the oxidative stress in the ALS brain, and the non-cell autonomous patho-mechanisms involving glial cells, the effect of the humoral immune factor, immunoglobulin G (IgG) of ALS patients was examined on oxidative stress and antioxidative system in the BV-2 microglial cell line.
Methods: BV-2 cells were treated with IgG (0.1 mg/ml) from 9 sporadic ALS patients, 4 healthy and 3 disease controls. Gene expression of antioxidative enzymes (catalase - CAT, superoxide dismutase 2 - SOD2, and glutathione peroxidase -GPx) was analyzed by qPCR 4 h post-treatment, while activity of antioxidative enzymes (superoxide dismutase 1 - SOD1, SOD2, CAT, GPx, glutathione reductase - GR), levels of oxidative stress markers (malondialdehyde, MDA, glutathione - GSH), and NO were analyzed by biochemical assays, 24 h post-treatment.
Results: ALS IgGs induced higher production of MDA and NO than IgGs from controls, while GSH was unchanged. The activities of SOD1, SOD2, CAT and GR were higher in cells treated with ALS IgGs. Expression levels of CAT and SOD2 showed no statistical difference between ALS and controls, although an upregulation trend was observed with ALS IgGs. The activity and expression of GPx remained unchanged.
Conclusion: Results demonstrate the role of inflammatory humoral factors, IgGs, as potential triggers of microglial activation that occurs in later stages of ALS. Revealing the ALS IgG signaling cascade in microglia could offer valuable molecular biomarkers and/or potential therapeutic targets.

ALTERED *VIP* GENE EXPRESSION IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

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Introduction: Vasoactive intestinal peptide (VIP) is a member of the glucagon-secretin family that includes glucagon, secretin and gastric intestinal peptide. VIP is a very powerful relaxant of the intestinal musculature as reported in several gastrointestinal (GI) disorders. Decreased expression of the *VIP* gene can be found in the gut diarrhea predominant patients suffering from irritable bowel syndrome (IBS). The aim of our study was to determine if there is a difference in the expression of *VIP* gene in IBS patients and healthy volunteers.

Methods: Total RNA was isolated from blood (20 patients and 9 healthy volunteers) and tissue (9 patients and 7 healthy volunteers), and usesd as a source for relative gene expression profiling using real-time PCR. The relative expression between these two groups for the *VIP* gene as opposed to *β-actin* have been calculated with statistical iteration using REST.

Results: Difference in gene expression patterns was observed in biopsies as more then 3-fold down regulation (P = 0.045) in a group of patients in comparison to healthy controls. When blood and tissue were compared, we observed significantly increased expression of the *VIP* gene (P = 0.018).

Conclusion: The reduced expression of the *VIP* gene suggestsits link with changes in colon mucosal tissue in IBS patients related to its main role of relaxation of smooth muscles, stimulation of secretion of water and electrolytes in the gut.

NEUROCAN UP-REGULATION IN THE SPINAL CORD OF ALS TRANSGENIC RATS

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Aim: The aim of the present study was to identify differentially expressed genes (DEGs) in non-transgenic (NTG), pre-symptomatic (PRE-ALS) and symptomatic ALS (amyotrophic lateral sclerosis) SOD1G93A transgenic rats.
Methods: A total of 166 DEGs (152 upregulated and 14 downregulated) were previously identified when transcriptomes of NTG and ALS rats were compared (Bernardini et al., unpublished). Validation of 10 selected DEGs was performed in samples from three NTG, three PRE-ALS and three ALS rats. RNA was isolated from the spinal cord and RT-qPCR was performed.
Results: Out of 10 selected genes (from the top upregulated in transcriptomes and based on their gene onthology), only 5 exhibited a significant change in expression levels. The most significant increase of expression, both at pre-symptomatic and symptomatic stage of the disease, was observed for neurocan: 16-fold ($p<0.001$), while for the remaining genes (Apobec1, Blnk, Axl and Cp1) it ranged between 3-fold and 5-fold ($p<0.05$). Between PRE-ALS and ALS groups, difference in expression was significant only for neurocan (15-fold, $p=0.001$) and Apobec1 (3-fold, $p=0.042$).
Conclusion: Our findings support the involvement of extracellular matrix in ALS, since overexpression of neurocan, a chondroitin sulfate proteoglycan, may create a nonpermissive microenvironment for neural regeneration. Our results also suggest that chronic inflammation and ceruloplasmin specific oxidative activity additionally contribute to motor neuron death.

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PANK2 GENE MUTATION SPECTRUM IN SERBIAN PATIENTS WITH NEURODEGENERATION WITH BRAIN IRON ACCUMULATION

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Introduction: Neurodegeneration with brain iron accumulation (NBIA) is rare autosomal recessive disorder characterized by dystonia, parkinsonism, cognitive and visual impairment, and iron accumulation in brain. Majority cases of NBIAN result from mutations in the *PANK2* gene that encodes pantothenate kinase 2, a key regulatory enzyme in the biosynthesis of coenzyme A.
Methods and Results: Over the last 10 years we have analyzed 16 Serbian patients with clinically suggestive NBIA, using direct sequencing of the *PANK2* gene. Pathogenic changes were detected in 12 patients all of whom had c.1583C>T mutation (p.T528M) either in homozygous or in heterozygous state. In all of four heterozygous examinees the remaining mutation was deletion c.1418del7**.** Clinical findings in our patients were markedly similar, characterised by early onset and fast progression of symptoms with particular speech affection. In order to analyze a possible founder effect of c.1583C>T substitution we performed the analysis of linkage disequilibrium (LD) and organization in haplotypes of 23 single nucleotide polymorphisms (SNPs) adjacent to *PANK2* gene in 6 patients and their parents, as well as in 30 healthy child-parents trios originating from the same geographical region. Different LD structure between patients and controls is revealed, and *PANK2* 1583T allele was significantly associated with particular haplotype.
Conclusion: Only two different *PANK2* mutations in Serbian patients with NBIA were detected with the common haplotype for the more frequent one.

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ANTITUMOR PROPERTIES OF CARBORANE BASED 5-LOX INHIBITORS TESTED ON A MOUSE MODEL OF COLORECTAL CARCINOMA

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Introduction: Lipoxygenases (LOXs) are a family of enzymes that convert polyunsaturated fatty acids into biologically active metabolites such as inflammatory mediators – prostaglandins and leukotrienes. The inhibition of lipoxygenases is increasingly employed in treatment of cancer. We evaluated the anticancer potential of two novel 5-LOX inhibitors, namely 1 and 2.
Aim: 1 and 2 were synthesised as analogues of a 5-LOX inhibitor named Rev-5901. Our aim was to test the antitumor potential of novel inhibitors, and to compare their activity to Rev-5901.
Methods: The in vitro segment of this study was done on a mouse colorectal carcinoma cell line – CT26CL25. For an in vivo model we induced tumors in BALBc mice by implantation of CT26CL25 cells, and treated the animals with the potential inhibitors.
Results: Treatment of CT26CL25 cells for 48 h resulted in diminished cell viability. Calculated IC50 values were 25 μM, 15 μM and 30 μM for 1, 2 and Rev-5901, respectively. Detailed analysis of their mechanism revealed induction of caspase dependent apoptosis. Both substances induced autophagy in CT26CL25 cells. In the presence of chloroquine, an autophagy inhibitor, we observed increased mortality of cells, implying that autophagy possesses a cytoprotective role. In vivo experiment reports a significant reduction of tumor volume in animals treated with 2. Compounds 1 and Rev-5901 lacked in vivo efficacy.
Conclusion: Results presented in this study display a strong effect of compound 2 on malignant cell growth. Having in mind the important role of inflammation in cancer development these results might have significant impact and are worthy of further evaluation.

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MUTATIONAL PROFILING OF CHILDHOOD AND ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS USING TARGETED NEXT GENERATION SEQUENCING

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Introduction and aims: Acute lymphoblastic leukemia (ALL) is the most common cancer in children, whereas it is less common in adults. The aim of the present study was to assess mutational profile of both childhood (cALL) and adult ALL (aALL) patients, by applying targeted next generation sequencing (NGS) on MiSeq System.
Methodology: We analyzed DNA samples from 34 de novo ALL patients (17 cALL and 17 aALL) using TruSeq Amplicon – Cancer Panel (TSACP) that targets mutational hotspots in 48 cancer related genes.
Results: We identified a total of 331 variants in the coding regions and 429 variants in the non-coding regions. Overall, a total of 98 variants (median per patient: 2.8, range: 1–6) were potentially protein-changing, including nonsense, frameshift, and missense (NFM) mutations. There were no significant differences in the number of NFM mutations between cALL and aALL patients. Observed in individual patients detected mutations predominantly disrupted Ras/RTK pathway (*STK11*, *KIT*, *MET*, *NRAS*, *KRAS*, *PTEN*). Additionally, we identified 5 patients with the same mutation in *HNF1A* gene, disrupting both Wnt and Notch signaling pathway. In two patients we detected variants in *NOTCH1* gene. *HNF1A* and *NOTCH1* variants were mutually exclusive, while genes involved in Ras/RTK pathway exhibit a tendency of mutation accumulation.
Conclusions: Our targeted NGS study showed low number of recurrent mutations in both cALL and aALL patients. Detected mutations affect few key signaling pathways, primarily Ras/RTK and Notch pathways. This study contributes to knowledge of ALL mutational landscape, leading to better understanding of molecular basis of ALL.

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DEVELOPMENT OF A PROTOCOL FOR ENZYMATIC ASSAY, DOCKING AND CRYSTALLISATION OF ALDO-KETO REDUCTASE 1 C2 AND C3 (AKR1C2 AND C3) WITH SPECIFIC INHIBITORS

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Introduction: Aldo-keto reductase family 1 members C2 and C3 are important enzymes in steroidogenesis and redox homeostasis in humans. Overexpression of AKR1C3 contributes to tumor development and C2 is associated with disease progression in prostate cancer, and inhibition of AKR1C3 and C2 activity represents a promising target for development of new therapies. The PDB (Protein Data Bank) contains several crystal structures of AKR1C2 and C3 with different inhibitors, however there is a need for identification of more specific inhibitors, given the sequence similarity between AKR1C isoforms. To understand how specific inhibitors bind to AKR1C enzymes, we are preparing recombinant AKR1C2 and C3 proteins for crystallization in complex with new ligands.
Methods: Computational prediction of the potential binding affinity of new inhibitors is followed by induction and expression of recombinant AKR1C2 and C3 and purification of the proteins. An optimized protocol for induction, expression and purification of recombinant AKR1C2 and C3 has been developed in our lab. Using docking results as a guide, we are optimizing AKR enzymatic assays with promising inhibitors, and developing a protocol for testing new substrates.
Results: SDS-PAGE shows the recombinant proteins are >90% pure, and enzymatic assays show appropriate respone of proteins to specific substrate and inhibitor.
Conclusion: Based on results of enzymatic assays we are planning preparation of crystallization trials in complex with various ligands for structure determination to guide design of more specific inhibitors.

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STUDY OF PLATINUM DISTRIBUTION IN HUMAN NEURONS *IN VITRO*

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Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) is one of the common side effects of platinum (Pt) based chemotherapeutic drugs and a frequent cause for discontinuation of treatments, affecting both the efficacy of cancer therapy and the quality of life of cancer patients. Therefore**,** it is urgent to develop a human *in vitro* model of neuronal cells for study Pt induced neuronal damage.

Methods: NT2/D1, pluripotent embryonal carcinoma and SK-N-SH neuroblastoma cells differentiate upon treatment with retinoic acid into mature neuronal cells which can be a good model to elucidate Pt-induced neurotoxicity. The aim of this study was to treat neurons in cell culture with cisplatin and to analyze Pt distribution and subcellular localization using X ray fluorescence spectroscopy and microscopy (XFM). In order to prepare the samples for XFM we have used Si_4N_4 windows. The windows were treated with different coating agents, in order to provide the anchoring for cell adhesion. Upon successful cell attachment Si_4N_4 windows were treated with cisplatin and vitrified in liquid ethane. Prepared samples were used for XFM at the Advanced Photon Source, USA.

Results: As expected, platinum signals obtained on "Bionanoprobe" showed its localization within the nucleus of the cell, but also in the cytoplasm. At the same time it is revealed that the concentration of cisplatin should be much above level of IC_{50} value in order to be detectable with XFM.

Conclusion: Optimized conditions for XFM analysis of Pt distribution in human neurons will enable detailed analysis of mechanisms that cause CIPN.

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IMMUNOMODULATORY ACTION OF *Chelidonium majus* ETHANOLIC EXTRACT: EMERGENCE OF UNCONVENTIONAL POPULATIONS OF PERIPHERAL BLOOD CELLS

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Introduction: *Chelidonium majus L* (*Papaveraceae*) is widely used in alternative medicine for treatment of various disorders. Immunomodulatory effect of purified compounds isolated from this plant have been reviewed, while studies that examine properties of the whole extract lacks.
Aim: The aim of this study was to examine the effect of *C. majus* ethanolic extract on peripheral blood mononuclear cells (PBMNCs).
Methods: Blood was obtained from healthy donors and PBMNCs were isolated by density gradient centrifugation. After 24 hours treatment with 10, 50 and 250μg/ml extract, PBMNCs were analysed on flow cytometer.
Results: Treatment induced dose-dependent increase in proportion of monocyte/macrophages (Mo/Mf) and B cells and concomitant decrease in percentage of T cells. Importantly, the percentage ratio of both double positive CD4+CD8+ T lymphocytes and helper T cells coexpressing CD14 molecule increased with extract concentration. Even the lowest concentration of extract induced coexpression of CD4 on almost whole population of Mo/Mf cells.
Conclusion: Increase in proportion of Mo/Mf and B cells, expression of CD4 molecule on monocytes that triggers differentiation of human monocytes into functional mature macrophages, expression of CD14 molecule on helper T cells that indicates activation of lymphocytes, and coexpression of CD4 and CD8 molecules on T cells postulated to be the result of lymphocyte activation, point to presumable immunostimulatory effect of *C. majus* ethanolic extract.

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VARIANT *GSTO1* AND *GSTO2* GENOTYPES AS RISK DETERMINANTS OF CLEAR CELL RENAL CELL CARCINOMA

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Introduction: Clear cell renal cell carcinoma (ccRCC) is the most common and, probably, the most aggressive RCC subtype, characterized by the highest rate of local invasion, metastasis and mortality. Although glutathione S-transferases, GSTO1 and GSTO2, exhibit a unique range of different activities, involved in regulation of inflammation, apoptosis and cellular redox homeostasis, no study has explored association between *GSTO1* and *GSTO2* polymorphisms and ccRCC, as yet.
Aim: The aim of this study was to investigate the role of GSTO polymorphisms in susceptibility to ccRCC.
Methods: *GSTO1* (rs4925) and *GSTO2* (rs156697, rs2297235) genotyping was performed in 239 ccRCC patients and 350 age- and gender-matched controls by Taqman based real time-PCR.
Results: We found that neither of GSTO polymorphisms contributed independently towards the risk of ccRCC. However, when we combined variant *GSTO1**AspAsp genotype with either variant *GSTO2**AspAsp (rs156697) genotype or variant *GSTO2**G allele (rs2297235), homo- or heterozygous, we found 2.8-fold and 2.1-fold increased ccRCC risk, respectively, compared to wild type genotype combination (p=0.035 and p=0.050, respectively). Moreover, significant modifying effect on ccRCC risk conferred by smoking, as recognized risk factor, had been found in smokers with *GSTO2**AspAsp (rs156697) genotype (OR=2.5, p=0.042), as well as aforementioned risk genotype combination *GSTO1**AspAsp/*GSTO2**AspAsp (rs156697) (OR=3.9, p=0.036).
Conclusion: Based on our findings regarding association of combined gene variants of *GSTO1* and *GSTO2* with ccRCC risk, it can be assumed that, these polymorphisms might modulate risk for ccRCC development.

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ARTICULATION SKILLS, ORAL PRAXIS AND COGNITIVE MATURITY OF CHILDREN WITH 22q11.2 DELETION SYNDROME AND CHILDREN WITH PHENOTYPE RESEMBLING 22q11.2 DELETION SYNDROME BUT WITHOUT MICRODELETION

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Introduction: The 22q11.2 Deletion Syndrome (22q11.2DS) is the most common microdeletion syndrome in humans with an incidence of approximately 1/4000 per live births. This syndrome is among the most clinically variable syndromes. More than 180 features are associated with 22q11.2DS, but the most common are congenital heart malformations, facial dysmorphism, thymic hypoplasia, cleft palate/velopharyngeal insufficiency, hypoparathyroidism with hypocalcaemia, developmental delay and speech and language delay.Our aim was to determine a communication profile of children-monolingual native speakers of Serbian language with 22q11.2DS.
Methods: *We compared* articulation skills, oral praxis, average age of first functional, spoken word with meaning and cognitive maturity of children with 22q11.2 microdeletion and age matched children having a phenotype resembling 22q11.2DS but without microdeletion.*The presence or absence of 22q11.2 microdeletion* was revealed by fluorescence in situ hybridization and/or multiplex ligation-dependent probe amplification.
Results: The obtained results revealed that children with 22q11.2 microdeletion have a delay of language milestones and more difficulties in development of speech and language abilities compared to children with the phenotypic features of 22q11.2DS but without themicrodeletion.
Conclusion: This is the first study of speech and language abilities of monolingual native speakers of Serbian language with 22q11.2DS. Also, to the best of our knowledge, this is the first such study among native speakers of South-Slavic languages. Based on obtained results we assume that children with 22q11.2DS should be considered at risk and should be included in early stimulation and treatment in order to improve speech, language and cognitive abilities.

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PRELIMINARY RESULTS FROM MLPA ANALYSIS IN PATIENTS WITH AUTISTIC SPECTRUM DISORDERS FROM REPUBLIC OF MACEDONIA

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Introduction: Autism is a neurodevelopmental disorder with a range of variation in symptoms. Epidemiologic studies show important role of genetic factors, and many different loci have been implicated in development of at least some cases of autism.
The aim of our study was to summarize the preliminary results of the analysis using commercial set SALSA Multiplex Ligation dependent Probe Amplification (MLPA, MRC Holland).
Methods: Total of 25 patients were analyzed for deletions and duplications in 49 sequences located in specific regions of chromosomes 15, 16 and 22 along with 9 reference probes, detecting 9 different autosomal chromosomal regions. All patients were referred at the Institute for Immunobiology and Human Genetics in Skopje during 2016-2017.
Results: Although several studies report copy number variations in different cohorts of patients with autism ranging from 3-20% especially in chromosomes 16 and 20, we didn't detect any of these abnormalities in our patients. Reasons could be poorly defined criteria for selection of patients, and of course, the small number of patients.
Conclusion: The MLPA analysis is a straight forward and relatively rapid method for detection of imbalances in clinically characterized patients with disorders from the autistic spectrum. The downsides of this method that should be noted include impossibility to detect point mutations in selected chromosomal regions and the fact that not all deletions/duplications detected by MLPA will be pathogenic.

PRECISION MOLECULAR DIAGNOSTIC OF HEPATIC GSD REVEALED UNEXPECTEDLY HIGH INCIDENCE OF GSD Ib IN SERBIAN POPULATION AND THREE NOVEL VARIANTS IN THE *SLC37A4* GENE

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Background: Hepatic glycogen storage diseases (GSD) have partially overlapping phenotypes. Precise molecular diagnosis is essential for optimal medical treatment of each patient and proper genetic counselling.
Methods: We analyzed 38 patients with clinical suspicion of GSD I using Sanger and next-generation sequencing (NGS). Pathogenicity of novel variants was determined based on expressional, computational studies and/or their phenotypic effect in patients.
Results: We identified 28 GSD Ib and five Ia patients. Also, NGS detected patients with GSD III, VI, IX, cholesteryl ester storage disease and Shwachman-Diamond syndrome. Incidence of GSD Ia in Serbia was estimated at 1:172,746 and GSD Ib at 1:60,461 live births. Two variants were identified in *G6PC* gene: p.Arg83Cys and p.Leu173Pro. In *SLC37A4* gene, six variants were detected. Three previously reported variants p.Asn27Lys, p.Ser54Arg and p.Leu348Valfs*53 accounted for 87% of all analyzed alleles. Computational, expressional studies and/or clinical presentation in patients confirmed pathogenic effect of three novel variants: c.248G<A, c.404G<A and c.785G<A. In the cohort, hepatomegaly, hypoglycaemia and failure to thrive were the most frequent presenting signs of GSD Ia, while hepatomegaly and recurrent bacterial infection were main clinical signs in GSD Ib. All GSD Ib patients developed neutropenia within three years after birth. Inflammatory bowel disease was verified in 20,6% GSD Ib patients with age at onset ranging from 2 to 10 years.
Conclusion: Our study revealed the highest worldwide incidence of GSD Ib and supported usefulness of NGS for correct diagnostics of hepatic GSD. Furthermore, description of three novel variants will facilitate medical genetic practice.

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VARIATION OF A SKIN GENE EXPRESSION PROFILE IN A RAT MODEL OF IMIQUIMOD INDUCED PSORIASIS

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Introduction: Psoriasis is a chronic inflammatory dermatosis and is considered to have key genetic underpinnings. It is characterized by excessive growth and aberrant differentiation of keratinocytes. Studies show that imiquimod-induced dermatitis in mice closely resembles human psoriasis lesions in terms of the phenotypic and histological characteristics. In this work, we present the establishment of rat model of induced skin inflammation as an *in vivo* example of psoriasis.

Material and Methods: Wistar rats were housed under specific pathogen-free conditions and treated according to the Animal Welfare Regulations. All experiments were approved by the Animal Ethics Committee. Rats at 9 to 12 weeks of age received a daily topical dose of 125 mg imiquimod cream on the shaved back for 5 consecutive days. After induced inflammatory dermatosis in rats the psoriasis area and severity index (PASI) values variations were observed for the intensity of redness, scaling, and thickness from none (0) to very severe (4).

Results: Gene expression profiles of the imiquimod-treated tissue were compared to untreated tissue using REST software. We observed a significantly increased expression of the following genes: *Ccl17, Ccr2*, *Ccr5*, *Tnfsf10* and *Tnfsf13b*. None of the tested genes showed decrease in relative expression to normal (untreated) tissue type.

Conclusion: We have successfully induced inflammatory dermatosis in rat model which is adequate for the assessment of treatment effects of a topical application of investigated compound. Furthermore gene expression profiling is a comprehensive model for screening and measurement of therapeutic and dosage effect of novel compounds for psoriasis treatment.

IN VITRO PROPAGATED ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS USED IN THE TREATMENT OF KNEE OSTEOARTHRITIS – MID TERM RESULTS

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Introduction: Adipose tissue derived mesenchymal stem cells (AT-MSCs) have showed significant success in the treatment of joint lesions. We present a series of patients with knee osteoarthritis, treated with *in vitro* propagated AT-MSCs, with 18 months of follow-up.
Methods: We studied a series of 11 cases, with clinical, x-ray and MRI signs of knee osteoarthritis, Outerbridge stage II-III. A 5ml sample of subcutaneous fat tissue was taken by small incision. Stem cell isolation and propagation until therapeutic dose was perofrmed (1-2x10^7), followed by injection in affected knee joint.
Patients were evaluated by four clinical scores (Hospital for Special Surgery Knee Score, Knee Society Score, Tenger-Lysholm Score, VAS of pain), x-ray and MRI in 6 months interval. During follow-up, all the patients were instructed to have regular daily activities, and no physical therapy or pain medications were allowed.
Results: We observed highly significant improvement of all scores after 6 months: HSS-KS from 59±12.68 to 92.9±5.26; KSS from 42.1±15.71 to 88.2±2.23; TLS from 46.7±20.5 to 97±2.4; VAS pain from 5.4±1.65 na 0.9±0.65. These results are maintained throughout the 18 month follow-up period, without further narrowing of joint space. Significant clinical improvement of joint function in our series was not accompanied by detectable radiographic improvements. This confirms the contemporary view that stem cells, besides structural regeneration, also have imunomodulatory and paracrine mode of action.
Conclusion: Adipose tissue derived mesenchymal stem cells are efficient and safe method of knee osteoarthritis treatment. The duration of therapeutic effect has yet to be estimated in further follow-up.

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IMPROVEMENT OF THERAPEUTIC POTENTIAL OF CANINE ADIPOSE MESENCHYMAL STEM CELLS BY GENETIC MODIFICATIONS

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Introduction: Mesenchymal stem cells (MSCs) have the ability to differentiate in many cell types, they are immunoprivileged, exhibit intense immunosuppressive activity that contributes to the reparative process and show no detectable teratoma formation. Because of these unique characteristics MSCs hold enormous potential for cell-based therapy in the treatment of various diseases.
Aim: In the present report, the genetic manipulation of canine MSCs isolated from adipose tissue has been investigated, with the aim of enhancing their therapeutic potential.
Methods: Transient transfection conditions were optimized by the use of *LacZ* reporter vector and *in situ* β-galactosidase staining. Next, MSCs were transfected with pCIneoIL-10 plasmid that contains the cDNA of human IL-10 under the optimized conditions. The level of expressed human IL-10 was quantified from the supernatant of transfected canine MSCs using an ELISA assay.
Results: Since MSCs are regarded as hard-to-transfect, we initially optimized the transient transfection efficiency of these cells. By applying the optimized conditions we transfected MSCs with plasmid carrying the human *IL-10*, the potent immunosuppressive cytokine that is not produced by MSCs at a significant level. The successful expression of human *IL-10* transgene has been detected from the genetically modified canine MSCs.
Conclusion: The obtained results provide a working platform for further studies related to the reinforcement of MSCs-mediated therapeutic impact and the targeted delivery of various biological agents to disease sites.

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INSIGHTS INTO THE MODES OF ANTICANDIDAL AND CYTOTOXIC ACTIONS OF Apigenin-7-*O*-Glucoside COMPARED TO APIGENIN

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Introduction: Apigenin has received considerable attention due to its significant anticancer, antiviral, antibacterial and antioxidant effects. We analyzed apigenin-7-O-glucoside, a derivate of apigenin, through its antifungal activity and anticancer potential.
Aims: The aim was to bring new insight into bioactive potential of apigenin-7-O-glucoside related to its antifungal effect on Candida spp and cytotoxic potential on colon cancer cells and compare it with bioactive potential of apigenin.
Methods: Antifungal effect was tested on 14 different isolates of Candida spp. using membrane permeability assay, measuring inhibition of reactive oxidative species and inhibition of CYP51 Candida albicans enzyme. Cytotoxic activity of apigenin-7-O-glucoside was tested on colon cancer HCT116 cells by measuring cell viability, apoptosis rate and apoptosis- and colon cancer -related gene expression.
Results: Obtained results indicated considerable antifungal activity of apigenin-7-O-glucoside towards all Candida isolates compared to apigenin. We observed that reactive oxidative species inhibition could be a mechanism of antifungal action of tested compounds. On the contrary, none of the compounds exhibited binding affinity to Candida albicans CYP51 protein. Treatment with both compounds is accompanied with cytotoxic effects on HCT116 cells. We showed that compared to apigenin, apigenin-7-O-glucoside was more effective in reduction of cell's viability and induction of cell death through chromatin condensation, apoptotic bodies formation and induction of TP53 and Bax expression in HCT116 cells.
Conclusions: Both apigenin and apigenin-7-O-glucoside exhibited antifungal activity and citotoxic effect on colon carcinoma cells in vitro but apigenin-7-O-glucoside had showed more potent activity compared to apigenin in all the assays used.

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THE ROLE OF DECREASED *TSPAN14* EXPRESSION IN THE PATHOGENESIS OF NON-SMALL CELL LUNG CARCINOMA

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Introduction: Tetraspanins are group of 33 transmembrane proteins with high structural similarity that emerged as key players in human malignancies. They are involved in cell signaling, morphology, adhesion and motility. Details regarding expression and functional contribution in carcinogenesis for most of them are poorly explored.
Aim: In this study we investigated the expression and the potential role of tetraspanin 14 (TSPAN14) in non-small cell lung carcinoma (NSCLC).
Methods: We analyzed TSPAN14 expression by RT-qPCR in 25 NSCLC patients' samples and paired normal lung tissue. Obtained results were statistically evaluated in relation to histopathological parameters, survival and previously detected PTEN gene alterations (loss of heterozygosity and promotor hypermethylation). Moreover, TSPAN14 expression was assessed by RT-qPCR in 3 NSCLC cell lines (NCI-H460, A549 and HTB183). Also their invasive potential was assessed by gelatin degradation and matrigel invasion assay.
Results: TSPAN14 expression was significantly decreased in NSCLC samples compared with normal lung samples. Specifically, TSPAN14 expression was downregulated in 17 out of 25 tumor samples (68%), but did not associate with histopathological parameters nor survival. However, decreased TSPAN14 expression significantly coincided with PTEN gene alterations. Moreover, A549 and HTB183 cell lines showed decreased expression of TSPAN14 compared to NCI-H460 cells that might be associated with their increased invasive potential.
Conclusion: Downregulated TSPAN14 is significant characteristic of NSCLCs implying its important role for NSCLC pathogenesis. It is also potentially related to the invasive behavior of this type of cancer.

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MicroRNA-30A-3P EXPRESSION IN PAPILLARY THYROID CANCER

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Introduction: MicroRNAs (miRNAs) are short non-coding RNAs regulating gene expression in many biological processes including proliferation, migration and invasiveness. The deregulation of miRNA expression is believed to have an important role in the development and progression of thyroid cancer. One of the microRNAs frequently deregulated in cancers is miR-30a-3p. The aim of the present study was to investigate the expression of miR-30a-3p in papillary thyroid cancer (PTC) and its association with clinicopathological features.
Methods: Real time quantitative PCR was used to determine the relative miR-30a-3p expression levels in 22 pairs of PTC and matched non-tumor thyroid tissues in the preliminary phase of the study.
Results: The expression level of miR-30a-3p was significantly decreased in all tumors compared with matched non-tumor tissues ($p<0.0001$, Wilcoxon matched pairs test). Furthermore, the expression in tumor tissue was negatively correlated with patient's age at diagnosis ($p=0.02$, $r= -0.507$, Spearman's correlation test). We also found that higher decrease in tumor tissue compared to non-tumor tissue was associated with tumor multifocality ($p=0.01$, Mann-Whitney test; $p=0.01$, $r= -0.515$, Spearman's correlation test) and with the presence of vascular invasion ($p=0.04$, Mann Whitney test; $p=0.02$. $r= -0.520$, Spearman's correlation test). There was no association of miR-30a-3p expression level with other clinico-pathological characteristics such as gender, histological variant, tumor size, pT category and the presence of tumor capsule.
Conclusion: The obtained results suggest that miR-30a-3p may be involved in the pathogenesis of PTC and that it might be associated with more aggressive cancer features.

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PHENOTYPE OF PROTHROMBOTIC MUTATIONS CARRIERS – DATA FROM THE LARGE SERBIAN COHORT STUDY

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Introduction: Thrombosis is multifactorial disease with genetic and acquired risk factors in its etiology. The most frequently used in genetic tests are FVLeiden and FIIG20210A mutations, resulting in wide range of patients' phenotypes.
Aim: We aimed to determine average phenotype model for FVLeiden and FIIG20210A carriers in comparison to symptomatic non-carriers of these mutations (WT) in Serbian population.
Methods: Using our large database of Serbian thrombotic patients we selected 1394 patients and determined patients' distribution according to the mutation gene status and thrombotic phenotype.
Results: Patients mainly suffered from deep vein thrombosis no matter of mutation status: FVLeiden carriers - 65.7%, FIIG20210A carriers - 52.6%, WT- 50.10%. Pulmonary embolism was diagnosed in 20.2% of FIIG20210A carriers and 15.3% of WT patients, while only in 6.4% of FVLeiden carriers. Less than 6% of mutation carriers (FVLeiden - 5.6%, FIIG20210A - 3.5%) and 11.3% of WT patients suffered from stroke. Average age of the first thrombotic events did not differ between mutation carriers and non-carriers (34-36 years). There was no difference in thrombosis recurrence in the first year after thrombotic event in all three groups of patients (approximately 30%).
Conclusion: Deep vein thrombosis is the most frequent thrombotic event for all prothrombotic gene statuses, with lower prevalence of pulmonary embolism in FVLeiden carriers and stroke in FIIG20210A mutation carriers. Age of the first thrombotic event is similar for all prothrombotic gene statuses, as well as recurrence risk in the first year after thrombotic event.

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EFFECT OF DIODE LASER IRRADIATION ON OSTEOGENIC DIFFERENTIATION OF STEM CELLS FROM APICAL PAPILLA

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The Aim: To assess effects of diode laser irradiation on relative gene expression of osteogenic markers during differentiation of stem cells from apical papilla (*SCAP*).
Materials and Methods: Cells were isolated by outgrowth method. At fifth passage, cells were characterized by flow cytometry, with CD 34, CD 73, CD 90, CD 45 and CD 105 membrane markers. A diode laser, 940 nm, with the output powers of 0.5 W, 1 W, 1.5 W, and 2 W was used. At fifth passage, *SCAP* were irradiated for 5 seconds in four cycles, and further cultured in osteogenic medium. Twenty-one days after irradiation the total RNA was isolated by using the standard phenol-chloroform procedure. The RNA concentrations were measured, and one microgram of total RNA was reverse-transcribed into cDNA. The relative target gene expression of osteogenic markers *ALP*, *COL1A2, DSPP* and *DMP1*, and housekeeping gene *GAPDH*, were determined by real-time PCR.
Results: 98 – 99% of the cells were positive for mesenchymal stem cells markers, and 0% were positive for hematopoietic stem cell markers. Relative gene expression for *ALP* was increased in all irradiation groups when compared with control ($p < .05$), with the highest statistical significance in 1 W and 1.5 W group ($p < .01$). The similar trend continued in the expression of other osteogenic markers, in a power dose-dependent manner.
Conclusion: Diode laser irradiation influenced higher relative gene expression of osteogenic markers in *SCAP*. Our results indicate that expression is dependent on irradiation output power.

Acknowledgments: This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Grant no. 175075

GENETIC MODIFIERS OF β-THALASSEMIA: A RISE OF A NOVEL THERAPY APPROACHES

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Introduction: Hereditary persistence of fetal hemoglobin is characterized by persistent high levels of fetal hemoglobin (HbF) in adults which could ameliorate the severity of the symptoms caused by β-thalassemia. Several genetic factors that control HbF levels, thus representing gene modifiers of β-thalassemias, have been identified within (*HBG*), as well as outside globin gene loci (*KLF1*).
Aim: The aim of this study was to determine the effect of *HBG1*:c.-225_-222delAGCA (GenBank:M91036.1) and novel *KLF1*:c.-148G>A (NG_013087.1) promoter variant on the expression of these genes.
Methods: Constructs containing the wild type (*wt) KLF1* and *HBG1* promoter fragments, as well as *KLF1* and *HBG1* variant bearing promoter fragments, were transfected into K562 cells and the promoter activity was measured as chloramphenicol acetyltransferase (CAT) activity.
Results: The results of the functional analysis showed significantly decreased activity ($p<0.01$) of the *KLF1* promoter bearing c.-148A variant, compared to the *wt* promoter. On the other hand, *HBG1*:c.-225_-222 deletion was shown to result in drastically reduced CAT reporter gene expression compared to the *wt* sequence ($p<0.05$) but only under the conditions of erythropoietic stress, mimicked by introduction of erythropoietin into cell culture.
Conclusion: These data suggests that *KLF1*:c.-148G>A variant decreases *KLF1* promoter activity which could lead to increased HbF production. The results also indicate the possible presence of regulatory element within *HBG1*:c.-225_-222 region, which is inducible under the conditions of erythropoietic stress characteristic for β-thalassemia patients. Understanding these mechanisms that govern HbF production will bring us closer to gene manipulation as a novel therapy approach for β-thalassemias.

Acknowledgement: This work was supported by Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. III41004).

THE EFFECT OF FORMALIN FIXATION ON DNA EXTRACTION AND MOLECULAR ANALYSIS OF NON-TUMORAL BRAIN TISSUE EXCLUDED FROM AUTOPSY

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Introduction: The usage of formalin-fixed tissues for obtaining DNA for molecular analyses is limited by crosslinking and fragmentation of DNA.
Methods: The aim of this study was to evaluate the DNA extracted from formalin-fixed non-tumoral brain tissue, obtained about 24h post mortem from autopsy of persons who died a violent death. The parts of tissue were fixed in 10% neutral buffered formalin and in 4% unbuffered formalin at room temperature. The DNA was isolated 6h, 1-7 days (every 24h), 10, 14, 28 and 60 days after fixation using two different methods (phenol-chloroform-isoamylalcohol isolation as well as isolation with PureLink Genomic DNA Kit). The PCR of the *GAPDH* (150bp), *ß-actin* (262bp) and *RPL4* (600bp) genes was performed to evaluate the effectiveness of the methods of DNA extraction.
Results: The *GAPDH, ß-actin* and *RPL4* genes were amplified from DNA isolated from brain tissue using phenol-chloroform-isoamylalcohol which fixed in unbuffered formalin 28 days, 14 days and 48h respectively. The *GAPDH* and *ß-actin* genes were detected after phenol-chloroform-isoamylalcohol isolation up to 5 days of fixation in buffered formalin, while *RPL4* gene was detected up to 48h fixation in buffered formalin. Isolation with PureLink Genomic DNA Kit was allowed amplification only *ß-actin* and *GAPDH* genes from brain tissue fixed in unbuffered formalin up to 48h.
Conclusions: Preserving period, fixative and extracting method of DNA are important factors for successfully PCR amplification. For autopsy tissue samples fixation in 4% unbuffered formalin and phenol-chloroform-isoamylalcohol isolation is recommended.

Acknowledgements: This study was supported by grant (project JP: 05/13) from the Faculty of Medical Sciences, University of Kragujevac, Serbia.

EXPRESSION OF *TLR7*, *TLR9*, *JAK2* AND *STAT3* GENES IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH SYSTEMIC SCLEROSIS

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Introduction: Systemic sclerosis (SSc) is a rare, chronic, multisystem autoimmune disease clinically characterized by progressive fibrosis of the skin and internal organs. The basic mechanism appears to involve endothelial cell injury, overproduction of extracellular matrix proteins, and aberrant immune activation leading to formation of autoantibodies. So far, there were a few attempts to find circulating genetic biomarkers for monitoring disease activity or for correlation with certain symptoms. The aim of this study was discovering of such biomarkers within three signal pathways involved in different processes in the cell, including pathogen recognition, inflammation, and fibrosis.

Methods: Relative expression of *TLR7*, *TLR9*, *JAK2* and *STAT3* genes in peripheral blood mononuclear cells PBMNC of 50 SSc patients and 13 healthy controls were analyzed using RQ-PCR technique.

Results: Our results showed significant upregulation of *TLR7* gene expression in PBMNC of SSc patients compared to controls. Moreover, expression level of *TLR7* gene was significantly increased in the group of patients with late form of disease, in group of patients with limited SSc, in group of patients with active SSc and patients with renal crisis compared to healthy controls. The expression level of *TLR9* and *JAK2* genes was decreased in our patient cohort in comparison to healthy control group. The expression level of *STAT3* gene was not different in patients compared to control group.

Conclusion: Our results show that *TLR7*, *TLR9* and *JAK2* genes are potential biomarkers for SSc. This study could contribute to better classification, monitoring and outcome prediction of the patients with SSc based on genetics.

Acknowledgements: This work was supported by Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. III41004).

MICROARRAY TRANSCRIPTOME PROFILING IN MYOCARDIAL INFARCTION REGARDING PTPLAD2 rs2275888 eQTL: A DATA SCOUTING APPROACH

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Introduction: Recently, rs2275888 eQTL in PTPLAD2 gene has been associated with expression of several loci, during inflammatory stimulation in monocytes. Myocardial infarction (MI) triggers an intense inflammatory response that is essential for cardiac repair. We aimed to perform data scouting in appropriate rs2275888 genotype model to identify differentially expressed genes (DEGs), their biological meaning, and key miRs potentially associated with rs2275888 eQTL in peripheral blood mononuclear leucocytes (PBML) of MI patients 6 months after first MI.
Methods: Transcriptome data was obtained from PBMLs of 21 patients, who suffered ischemic MI, by employing Illumina iScan microarray technology. Genotyping for rs2275888 was conducted with real-time PCR, using TaqMan® assay. Preprocessing and identification of DEGs was done using *limma* package of R/Bioconductor software. The online tool DAVID v6.8 was employed for functional enrichment analysis. Most important miRs were selected using NetworkAnalyst web tool, based on the degree centrality value.
Results: Transcriptome analysis in recessive model TT+TC (n=19) vs. CC (n=2) identified 68 DEGs. Top significant biological processes involving DEGs cover vascular physiology, cell growth and signaling. Pathway analysis associated DEGs with adherens junction, Rap1 and Ras signaling. Network analysis identified hsa-miR-335-5p, -26b-5p, -93-5p, -16-5p, -124-3p, -20b-5p, -17-5p and -218-5p as miRs with top centrality degree in our DEGs list.
Conclusion: Seven of eight detected miRs have already been described in MI pathology or suggested as potential biomarkers for MI. Our result suggest the importance of integration of eQTLs, biological processes and pathway analysis coupled with miR activity for further research in MI pathology.

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Session
MOLECULAR BIOLOGY OF MICROORGANISMS

Plenary lecture

BACTERIAL RESPONSE TO PLASMA MEMBRANE STRESS: PATHOGENESIS AND ANTIBIOTIC RESISTANCE

Goran Jovanović (Velika Britanija / *Great Britain*)

Goran Jovanović, PhD
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Department of Life Sciences
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Research Interests:

Dr. Jovanović's research interest is related to the Phage shock protein (Psp) bacterial inner membrane stress response which is implicated in maintaining the membrane integrity, membrane potential, proton motive force and the energy use under stress. The studies of this classical extracytoplasmic stress system, recognized as of being of major importance in numerous stress conditions including protein translocation, biofilm formation, and development of antibiotic resistance and multi-drug resistant persister cells, have the clear implications for such systems in other organisms. His goal is to describe how sensory events at the membrane upon stress are connected to enhancer-dependent gene control on the chromosome and the effector function of the PspA and its homologues on the cytoplasmic membrane and how PspA transforms stress signals into distinct dynamic response according to the strength of stimulus and growth conditions (log vs stationary phase and stasis). From evolution point of view, the Psp system offers paradigm on the partial occupancy of target psp promoters that lead to non simultaneous promoter firing, and so asynchronous gene product formation. He studies the implications of this phenomenon in terms of the consequences of the elaboration of regulons, and a potential source of phenotypic variance between genetically identical cells. Dr. Jovanović is a Research Fellow at the Imperial College London. He received his undergraduate, graduate and postdoctoral education in Belgrade, New York, and Geneva. He joined the Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, in 2003.

Source web site: https://www.imperial.ac.uk/people/g.jovanovic

BACTERIAL RESPONSE TO PLASMA MEMBRANE STRESS: PATHOGENESIS AND ANTIBIOTIC RESISTANCE

Goran Jovanović

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All cell types need to maintain the integrity of their membrane systems to secure viability and robustness to a wide range of environmental stresses. Numerous cellular processes occur on membrane systems, and the integrity of membranes is critical to these processes. The cells have mechanisms to maintain the integrity of their membrane systems, however, little is known about the nature of the molecular interactions underpinning the control of membrane integrity. The goal of this presentation is to shed some light in deciphering how one pervasive bacterial membrane maintenance system perceives membrane stress and then manages to overcome the stressed state of the membrane. The bacterial Phage shock protein (Psp) system is an exemplar for studying the maintenance of a plasma membrane because of its experimental tractability and the important role it plays in numerous processes in a very wide range of bacteria allowing adaptation to a variety of abiotic and biotic stresses impacting on membrane integrity.

The Psp system is widely distributed in Proteobacteria[1] and protects cells against numerous extracytoplasmic stresses that cause a loss of inner membrane (IM) integrity and hence lead to a dissipation and drop in proton motive force (pmf)[2] (see Fig. 1A). In Gram-negative bacteria, the Psp system response is induced upon bacterial infection of phagocytic cells and is implicated in protein translocation, secretin production, virulence, biofilm formation, and resistance of pathogenic enterobacteria to antimicrobials such as ampicillin, kanamycin, verapamil and dibucaine and survival of multi-drug resistant persisters[2-5]. The central and conserved component of the Psp system is peripheral IM binding □-helical coiled-coil protein PspA which belongs to the IM30 family of proteins found in many organisms. PspA is an effector which as a high order oligomer associates with the IM and ameliorates the stress. In *Micobacterium tuberculosis* (MTB) the PspA ortholog Rv2744c confers the virulence and continued MTB existence in macrophages[6] and is involved in lipid homeostasis and survival of persisters in anaerobicaly-induced dormancy[7]. In Gram-positive bacteria the PspA homologue LiaH is induced by and protects the cells against antimicrobials that target cell wall and lipid II (nisin, ramoplanin, vancomycin, bacitracin) or reorganise the membrane architecture (e.g. daptomycin)[2,4]. In archaea, a PspA-like protein protects the plasma membrane integrity under severe environmental conditions and conserves the pmf[2]. The PspA homologue Vipp1 (Vesicle inducing protein in plastids) which has an additional C-terminal domain is implicated in thylakoid membrane biogenesis and protection, and supports membrane protein assembly and photosynthesis in cyanobacteria, as well as in green algae and higher plants[8]. All these PspA and PspA homolog-ortholog proteins are directly responsible for plasma/thylakoid membrane maintenance under stress conditions and conservation of pmf. Apparently, PspA in enterobacteria is a dual function protein that besides being major effector also negatively regulates its own expression via interaction with the AAA+ ATPase PspF transcription activator[1].

In enterobacteria, the core Psp system consists of sigma54-controled *pspABC* operon and sigma70-dependent divergently transcribed *pspF*. Under non-stress conditions PspA as a low order oligomer binds the *pspABC* transcription activator, bacterial enhancer binding protein PspF (active as a hexamer), and negatively controls PspF ATPase activity repressing PspF function to keep *pspABC* genes expression in check[9-11] (Fig. 1A). This interaction requires the presence of PspA N-terminal Amphipathic Helix b (AHb)[10] (Fig. 1A, B) which associates with a hydrophobic patch of PspF[5,11]. Upon IM stress caused by direct membrane interactions of the stressor agents, e.g. the misslocalisation of the outer membrane secretin pIV into the IM[2], PspA then interacts with the IM leading to release of the PspA-PspF inhibitory complex and induction of the Psp response[9,10] (see Fig. 1A).

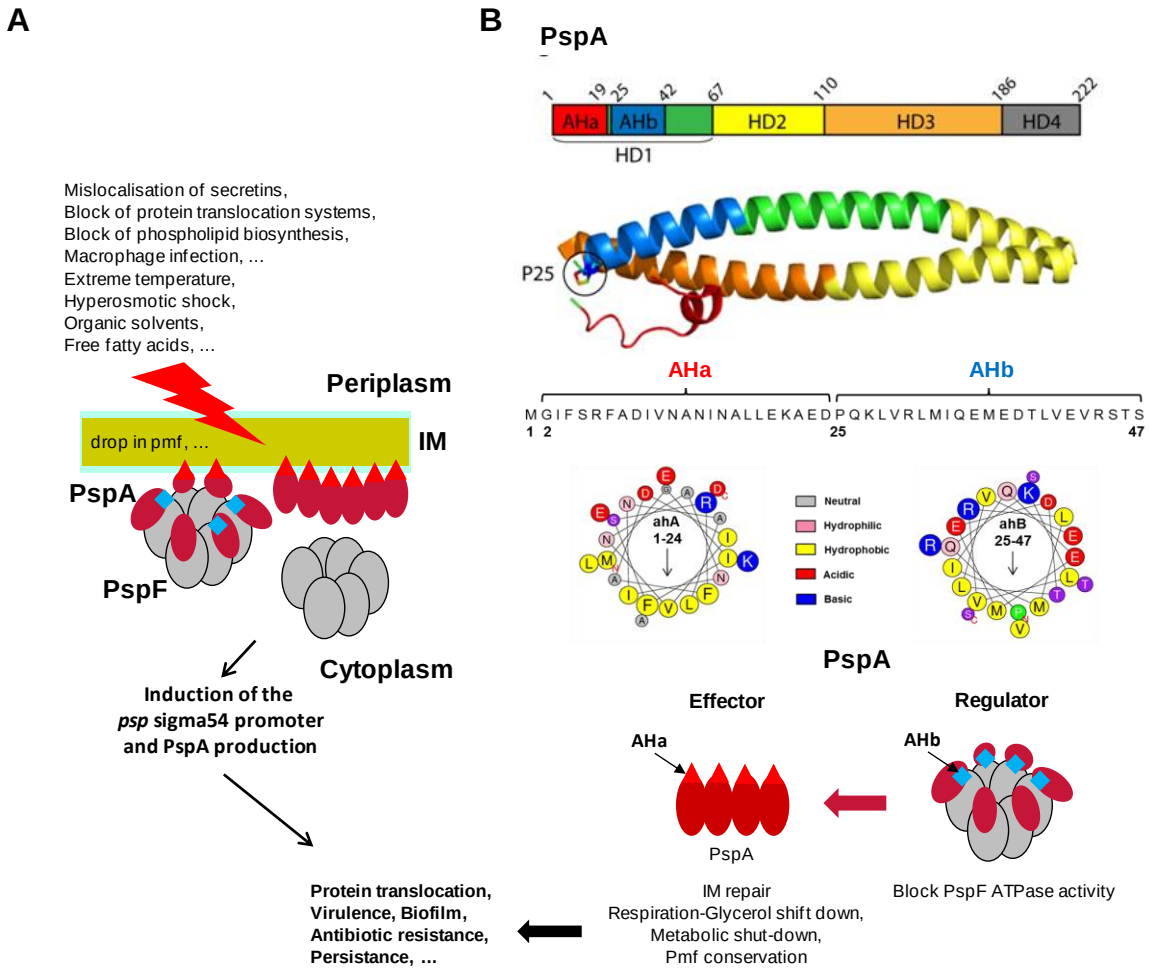

Fig. 1. A) Simplified presentation of Psp response (see text for detail). B) Schematic presentation of PspA alpha-helical coiled-coil protein domains (HD1-4) with N-terminal amphipathic helixes AHa and AHb, their sequence and function; The X-ray structure of PspA monomer lacking HD4 domain. PspA HD1-3 imposes active negative control over PspF *in vivo* and *in vitro*. This form of PspA interacts with PspF via PspA AHb (blue) and has unstructured AHa (red). (Adapted from [5] and [12])

Depending on the strength of stress, the PspB and PspC IM sensors may transduce the signal to inhibitory complex and assist PspA binding to the IM leading to release of PspF[13]. Direct membrane binding of PspA depends on its N-terminal Amphipathic Helix a (AHa) (adjacent to AHb and separated by conserved P25) (Fig. 1A, B) and

following resolution of the PspA-PspF complex, the PspA then acts as a high-order oligomer IM-bound assembly to repair the membrane damage[10,13]. Therefore, the PspA mutually exclusive use of AHb or AHa leads to switch in its function from the monomeric negative regulator to the high-order oligomeric membrane bound effector. Our genetic and biochemical background in understanding the operation of the Psp response in enteric bacteria[2] allowed us to use this system to work out how its membrane interactions are mounted and can both signal membrane stress to the gene expression apparatus as well as function to overcome membrane stress. Here the major challenge was in linking experimental observations possible with purified Psp proteins and user defined artificial membranes that are capable of accurately mimicking the properties of their *in vivo* counterparts to those events occurring *in vivo* in live cells to obtain a plausible mechanistic model explaining how membrane stress is perceived and managed.

Changes in membrane potential, anionic phospholipids and membrane curvature have all been proposed to play roles in IM stress signaling and IM repair *in vivo* and *in vitro*[1,5]. However, the molecular nature of signal(s) and the mechanism of PspA action in IM damage repair remained elusive. To progress this problem, we have established that purified PspA and its homologue Vipp1 can bind to defined membrane vesicles prepared from purified phospholipids, in a manner dependent on the presence of AHa sequence. Importantly, AHa and P25 (see Fig. 1B) are structurally conserved in all PspA/homologues/orthologues and AHa alone is responsible for recruitment of PspA to the IM upon stress *in vivo*[10,13,14]. Use of purified proteins and phospholipid vesicles of controlled size and composition (Fig. 2) established that direct membrane binding of *E. coli* PspA and *Synechocystis* Vipp1 via AHa is modulated via anionic lipid content and stored curvature elastic (SCE) stress within the membrane[14] (Fig. 3A). Increasing anionic lipid content facilitates binding and is attributed to electrostatic interactions. SCE stress and lipid packing defects strongly illicit vesicle binding relating a physical membrane state to the physiological membrane stress *in vivo*. In agreement, the IM stress causes increase and spreading of ordered membrane anionic-lipid rich micro-domains that correspond closely with localisation of PspA effectors[13]. In addition, the vesicle binding by PspA under conditions of SCE stress leads to activation of transcription by PspF through loss of the repressive PspA-PspF complex, suggesting that the primary membrane stress signal is the accumulation of SCE stress caused by agents that impair the cells IM.

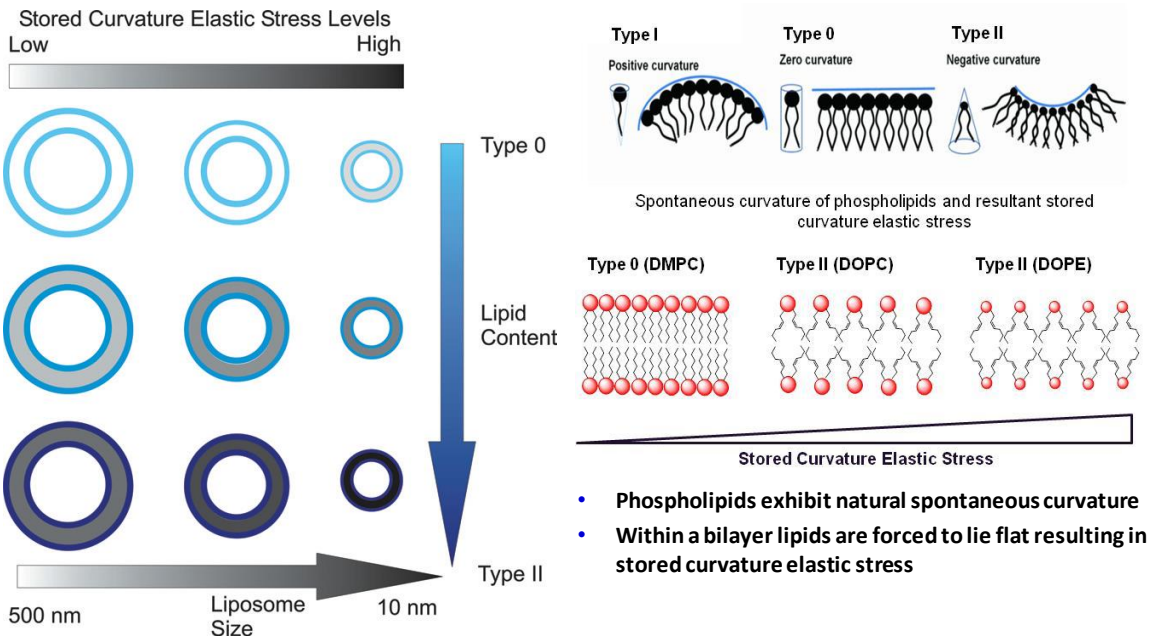

Fig. 2. The phospholipid vesicles of controlled size and lipid composition that bear SCE stress.

The somewhat different specificity of binding shown by PspA and Vipp1 *in vitro* does not depend on phospholipids' composition but rather depends on anionic lipid content and a bulk physical property of the membrane suggesting the mechanism for their effector function *in vivo* for repairing the stressed membrane. The lipid packing defects and accumulation of the SCE stress can specifically influence the structure and activity of peripheral membrane proteins (such as PspA and Vipp1) by providing the energy for membrane binding and drive changes in conformation and the oligomeric state of a protein[15]. In addition, peripheral (as opposed to integral) membrane binding proteins can (i) sense the membrane curvature and (ii) insertion into a lipid bilayer mediated by □-helical AHs can result in a decrease of SCE stress[15,16]. Consistent with this, we established that purified short AHa peptides do in isolation directly and similarly to the full length PspA and Vipp1 proteins, depending on anionic lipids content and SCE stress, interact with lipid vesicles and upon binding became structured helices and impact on membrane stability[12]. A direct interaction between AHa and the lipid bilayer and an accompanying change in AHa secondary structure from random coil (see unstructured N-terminus -in red- in X-ray structure, Fig. 1B) to alpha helical upon membrane association was determined (Fig. 3B), a structural transition likely needed for effector function. Likewise, a direct specific interaction between AHb and PspF is sufficient for the effective negative regulatory function of PspA[12]. These findings provided new insight into the membrane binding and effector functions of peripheral IM proteins responsible for maintaining membrane integrity and establish synthetic peptides can probe PspA structure-function and target regulation of the Psp response, which could be of interest for controlling pathogens where Psp response is important and conserved.

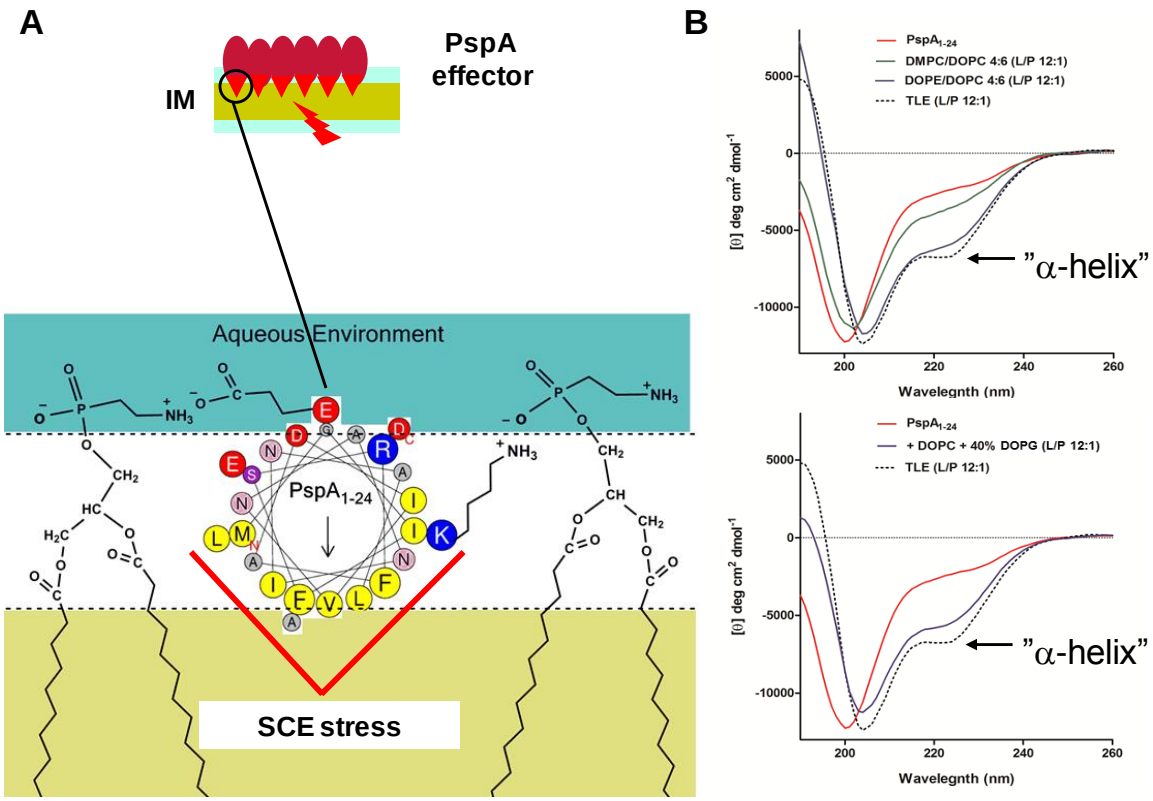

Fig. 3. A) PspA AHa in the context of high order oligomeric effector associates with the IM via electrostatic and hydrophobic interactions ameliorating the SCE stress. B) Circular Dichroism spectroscopy of PspA AHa peptide showing its transition from unstructured to alpha helical in the presence of SCE stress or anionic lipids. (Adapted from [12])

Considering these findings, our central hypothesis is that many if not all of the agents that induce the Psp response in enteric bacteria cause a change in the state of the cells IM. We propose that stress in the IM is mitigated by PspA, the major effector protein of the Psp system, and in cyanobacteria by its homologue Vipp1. To do so, these effectors recognise the unilateral signal at the stressed membrane and as high order oligomers then bind to the altered membrane via an N-terminal amphipathic helix (AHa). The IM changes caused by stress relate to increases in SCE stress and IM binding of the effectors then relaxes the SCE stress by multiple IM contacts/insertions of AHa (Fig. 3A) ameliorating the impacts of stress upon IM function and conserving the pmf. As a proof of concept, purified AHa peptides out of the PspA oligomeric context impair the membrane integrity *in vitro* and dissipate pmf and induce Psp response *in vivo*.

As stated above, the PspA and Psp system plays important roles in bacterial infections and pathogenesis, in particular in enterobacteria when type three, two and four secretion systems (T3SS, T2SS, T4SS) are active via the deployment of their specific secretins enabling virulence (Darwin, 2013). Incidentally, actions of the PspA effectors e.g. via cytoskeleton actin-like protein MreB targeting IM micro-domains containing lipid II and machinery for peptidoglycan biosynthesis[13] lead to associated antibiotic resistance. The world-wide antimicrobial resistance crisis has led to increased need for the research community to focus on the development of novel antimicrobial drugs. Specifically, new insight into the underlying mechanisms of

persistence could have important implications for interpreting antibiotic pharmacodynamics and optimising dosing regimens. Evidence suggests that a metabolically quiescent state that can be induced by Psp response and alteration of aerobic respiration and glycerol shift leading to metabolic shut-down[17] allows persisters to escape from antibiotic killing. Therefore, increasing our knowledge of Psp and similar systems that underpin virulence and antibiotic resistance will facilitate the design of strategies to reduce persistence and its negative impacts on antimicrobial therapies. Notably, the treatments taking dormant persisting bacteria into a drug sensitive state by the addition of metabolites occurred not through stimulating their growth but through energising the bacterial membrane at the level of increasing the pmf for the uptake of antibiotics. The Psp response functions to maintain the cells pmf, thus, the Psp system may poise persistent cells for establishing an energised membrane. The membrane stress is manifested at least in part through changes in lipid packing as caused by membrane perturbing agents and we now know that the PspA protein via its AHa can read out the state of the cell's cytoplasmic membrane and mediate the stress[3,10,12,13]. This underlines a strategic importance of studying PspA/homologues–AHa-lipids interactions and the outcomes may be of importance in biomedicine and development of new approaches for antimicrobial chemotherapy as well as in industrial biotechnology.

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Session
MOLECULAR BIOLOGY OF MICROORGANISMS

Invited lectures

MOLECULAR BIOLOGY OF CLASS 1 MOBILE INTEGRONS

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The rapid spread of antibiotic resistance has significantly limited treatment options, increased mortality rates of infections and has become a major clinical and public health problem. Integrons are genetic platforms carried by plasmids or contained within a transposon, and their role in the dissemination of resistance genes among bacteria has been well established and documented. Mobile integrons of class 1 are the most ubiquitous and have been the most commonly reported among clinical bacteria and are predominantly associated with Gram-negative bacterial pathogens. Although there is a number of currently available studies of class 1 integrons, only a limited number of processes crucial to the understanding of integron biology have been elucidated. Among these processes are the molecular mechanism of integrase gene expression as well as gene cassette expression in different bacterial pathogens.

Acknowledgments: This work was supported by grant No. 173019 from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

MECHANISMS OF BACTERIOCIN-RECEPTOR INTERACTION AS A KEY FOR BACTERIOCIN FUNCTION ELUCIDATION AND APPLICATION

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Introduction: Bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria, which kill other related (narrow spectrum) or non-related (broad spectrum) bacteria as one of the inherent defense system weapons of bacteria, but producer protects itself by synthesis of specific immunity proteins. In recent years, two important needs have emerged: the increasing consumer demand for natural food preservatives and the demand for new antimicrobial compounds in response to the continuing emergence of antibiotic-resistant bacteria, in the face of declining numbers of new antibiotics. Bacteriocins due to the specific characteristics, large diversity of structure and function, natural resource, and heat stability are recognized as a very promising source of antimicrobials for safe use as a food preservative and instead of or amendments to antibiotics.
Methods: Cosmid library construction of LsbB-sensitive strain. Heterologous expression of genes. Site directed mutagenesis of *lsbB* and *yvjB* genes.
Results: New receptor (YvjB) for LsbB bacteriocin was cloned. It was showed that certain amino acids and the length of LsbB are important for the bacteriocin activity; the essential amino acids in LsbB are tryptophan (Trp25) and terminal alanine (Ala30), and also distance between them. The crucial region in YvjB for the interaction with LsbB is the beginning of the third transmembrane helix, particularly amino acids tyrosine (Tyr356) and alanine (Ala353).
Conclusion: Membrane-located bacteriocin receptors have essential primary functions, and it seems that interaction with bacteriocins is suicidal for cells. Changes in the receptor interaction domain lead to a resistance, which is unfavorable for their use.

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HOST-MICROBE CROSSTALK IN THE GUT-BRAIN AXIS

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Introduction: The importance of gut microbiota in influencing the gut-brain axis has been strongly supported by recent research. It appears that the interaction between gut microbiota and gut-brain axis is bidirectional and takes place through signaling by neural, endocrine, immune, and humoral links.
Methods: In order to decipher the role of gut microbiota in gut-brain axis, the analysis of gut microbiota composition and the production of short chain fatty acids (SCFAs) in humans suffering from autism spectrum disorder, as well as in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis was performed. The immunomodulatory and probiotic potential of natural lactobacilli isolates and their effect on gut brain axis was evaluated.
Results: Diversity of common commensal bacteria, particularly *Bifidobacterium* sp. and butyrate producing bacteria *Eubacterium rectale, Faecalibacterium prausnitzii* and *Butyricicoccus pullicaecorum* were significantly decreased in the group of autism patients comparing to healthy children. Interestingly, *Turicibacter* sp. and the members of *Lachnospiraceae* family were exclusively found in healthy rats, while some members of *Firmicutes* and *Proteobacteria* (*Undibacterium oligocarboniphilum*) were detected only in faeces of DA rats after EAE induction, at the peak of the disease. Moreover, a significant decrease in the abundance of SCFAs in antibiotic treated DA rats was observed. Finally, the production of γ-amino butyric acid, the major inhibitory neurotransmitter in mammalian nervous system, was detected in natural lactobacilli isolates.
Conclusion: Our data contribute to the idea that gut microbiota and thier related metabolites considerably influence the gut brain axis through immune and metabolic pathways.

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MOLECULAR CHARACTERIZATION OF *Bacillus* spp. BIOCONTROL STRAINS

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The aim of this study was identification and genotyping of collection of 205 *Bacillus* spp. isolates, from samples of soil, manure, and straw gathered from across Serbia, using PFGE combined with sequencing of *tuf* gene, one of the housekeeping genes. In addition, we performed a screening for the presence of biosynthetic genes for the antimicrobial lipopeptides iturin, surfactin, fengycin and bacillomycin D in order to identify the isolates with the capacity for application in biological control.

Many species of the genus *Bacillus* produce lipopeptides with antagonistic activity against bacteria and fungi. Antimicrobial lipopeptides are synthesized in a nonribosomal manner. The strains that produce them have established their place in the biological control of plant pathogenic bacteria and fungi.

The PFGE analysis with *Not*I enzyme was used to determine phylogenetic relationships of isolates and referent strains. Four large groups of *Bacillus* spp. were distinguishable: cereus, subtilis, pumilus and megaterium and within, the enormous genetic diversity. The determination of *tuf* gene recommends itself to be an adequate and sufficient analysis for obtaining very clear and unambiguous results, with high resolution of separation of *Bacillus* species.

The results of the screening for biosynthetic genes for the antimicrobial lipopeptides showed that the majority of tested isolates had more than one biosynthetic operon, since 81% possessed the genes for bacillomycin D production, 54% for surfactin, 38% for iturin and 25% for fengycin production. At least five of them proved to be an excellent biocontrol strains *in vitro* and *in situ*.

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BIOCATALYTIC POTENTIAL OF BACTERIA

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Introduction: Biocatalysis is enzyme-based synthesis of organic chemicals which is established as environmentally friendly alternative to traditional metallo- and organocatalysis. Advantage of enzymes as catalysts lays in high stability, substrate specificity, renewability, biodegradability and activity at mild conditions.
Aims: The aim of the study was to determine biocatalytic potentials of *Streptomyces* sp. and *Pseudomonas* sp. isolates from Laboratory collection and to develop novel functional biocatalysts.
Methods: We screened bacterial Labortory collection by enyzmatic activity assays and/or by PCR amplification of selected enzyme coding genes. Detected enzymes were used as whole-cell biocatalysts, either in wild type strains or heterologously expressed in *Esherichia coli*. Best performing biocatalysts were further improved by site directed mutagenesis, directed evolution or whole-cell immobilization.
Results: Approximately 120 bacterial isolates were screened for 12 different industrially important enzymes. The most abundant enzymes were those involved in the biomass degradation, as the activities of lignin-peroxidase, cellulase and lacasse were detected in 48%, 40% and 27% of the isolates, respectively. Heterologously expressed lipases, laccases and 4-oxalocrotonate tautomerase were further improved toward longer reusability and broader substrate range.
Discussion: Majority of bacteria in our collection were isolated from soil and associated with plant or fungi, therefore, it was not supprising that enzymes for biomass degradation were the most frequently detected ones. Our results support the idea of revisiting bacterial collections in search for greener solutions, since microorganisms are still an untapped reservoir of biodiversity for bioprospecting.

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THE PROTEIN FOLDING PROBLEM

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The protein folding problem is the most important unsolved problem in structural biochemistry. The problem consists of three related puzzles: i) what is the physical folding code? ii) what is the folding mechanism? and iii) can we predict the 3D structure from the amino acid sequences of proteins? Bearing in mind the importance of protein folding, misfolding, aggregation and assembly in many different disciplines, from biophysics to biomedicine, finding solutions that would be generally applicable is of the utmost importance in biosciences.

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MODELING AND BIOINFORMATICS OF BACTERIAL IMMUNE SYSTEMS: UNDERSTANDING REGULATION OF CRISPR/Cas AND RESTRICTION-MODIFICATION SYSTEMS

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Bacterial immune systems protect bacterial cells from foreign DNA, such as viruses and plasmids. They also critically affect bacterial pathogenicity by reducing the flow of genes between bacteria. Two such major systems are restriction-modification and the recently discovered CRISPR/Cas systems. Here we review our work on understanding gene expression regulation in these systems, which takes a systems biology approach, combining modeling, bioinformatics and data analysis from quantitative experiments. Specifically, we address the following: (i) modeling gene expression regulation during restriction-modification system establishment in a naïve bacterial host, (ii) modeling the dynamics of CRISPR/Cas activation, in particular, how the features characterizing system transcription regulation and transcript processing affect the dynamics, (iii) predictions of transcription start sites for alternative σ factors that have been poorly studied up-to-now, but are important as CRISPR/Cas likely responds to bacterial cell envelope stress, (iv) our preliminary results on predictions of different CRISPR/Cas components, in particular, small RNAs associated with the systems, which likely have a key role in their regulation.

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Session
MOLECULAR BIOLOGY OF MICROORGANISMS

Flash presentations

Lactobacillus fermentum BGHV110 POSTBIOTICS ALLEVIATE ACETAMINOPHEN-INDUCED HEPATOTOXICITY BY PROMOTING PROTECTIVE AUTOPHAGY RESPONSE

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Introduction: Novel trends in probiotic supplementation are oriented towards replacement of live bacteria with non-viable bacterial extracts and metabolic by-products (postbiotics), which can modulate different cellular pathways producing desired response in the host. The aim of this study was to investigate potential of postbiotics produced by *Lactobacillus fermentum* BGHV110 strain (HV110) to induce protective autophagy in HepG2 cells and to counteract acetaminophen (APAP)-induced hepatotoxicity through autophagy induction.
Methods: In order to obtain postbiotics, overnight bacterial culture was homogenized in a French press and lyophilized. Cell viability was assessed using MTT and LDH assays, while autophagy was monitored by qPCR analysis of *BECN1*, *ATG5*, *p62/SQSTM1* and *PINK1* mRNA expression and by Western blot analysis of p62/SQSTM1 and lipidated LC3 accumulation.
Results: Our results showed that selected dose of HV110 caused an increased conversion of LC3 protein, as well as upregulation of *BECN1*, *p62/SQSTM* and *PINK1* mRNA levels. Also, the detrimental effect of APAP on cell viability was suppressed in the presence of HV110 which was linked with increased conversion of LC3 protein and p62/SQSTM1 protein degradation. Treatment with chloroquine, an autophagy inhibitor, finally confirmed the role of autophagy in alleviation of APAP-induced hepatotoxicity and revealed involvement of additional protective mechanism(s). Additionally, higher *p62/SQSTM1* and *PINK1* mRNA transcription were noticed in cells co-treated with APAP/HV110, simultaneously.
Conclusion: This study suggests that HV110 enhances activation of PINK1-dependent autophagy in HepG2 cells and its eventual co-supplementation with APAP could be potentially used for alleviation of hepatotoxic side effects caused by APAP overdose.

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QUANTITATIVE MODELING OF GENE EXPRESSION REGULATION IN BACTERIAL RESTRICTION-MODIFICATION SYSTEMS

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Introduction: Restriction-modification systems (RMS) are non-adaptive bacterial immune systems, which defend the host cell from foreign DNA. Their core components are a restriction enzyme which cuts specific DNA sequences, and a methyltransferase, which protects the same sequences. Tight regulation of RMS genes is of crucial importance during system establishment in a naive cell, in order to timely methylate only the host genome.
Methods: Transcription was thermodynamically modeled in RMS with various regulatory features (e.g. RMS Esp1396I includes a specialized transcription factor, Kpn2I employs overlapping promoters and a roadblock effect, while Cfr9I relies on antisense RNAs), which we use as an input for dynamical modeling of protein expression. Our collaborators performed the first single-cell measurements of RMS enzymes dynamics (for Esp1396I) which enabled us to compare our modeling predictions with the experimental data.
Results: Our model of Esp1396I system regulation can successfully explain the main proposed qualitative properties of RMS expression dynamics, required for prevention of autoimmunity: a large initial accumulation of methyltransferase and a delayed expression of restriction enzyme. On the other hand, it underlined a potentially important role of cell population dynamics in shaping the RMS expression dynamics in individual cells. Modeling of Kpn2I system with significantly different regulation leads to the dynamics characterized by the same qualitative properties.
Conclusion: Various designs of RMS seem to be optimized to achieve few common dynamical properties imposed by their immune function. Understanding the roles of their regulatory features provides a set of building blocks for constructing synthetic gene circuits.

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CHARACTERIZATION OF BACTERIOPHAGES ACTIVE AGAINST NOSOCOMIAL MULTIDRUG-RESISTANT STRAINS OF *Klebsiella*, *Acinetobacter, Escherichia* AND *Pseudomonas* sp., ISOLATED FROM BELGRADE WASTEWATERS.

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Introduction: Emergence and dissemination of antibiotic resistance among hospital acquired bacteria is a global health-threatening problem. In the lack of new and efficient antibiotics, recent years saw more attention being put to bacteriophages, viruses capable of infecting and killing bacteria, and their possible application for control of resistant infections.
Methods: Bacterial strains were isolated from patients in different Serbian hospitals and belonged to the following species: *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa*. All strains were carbapenem resistant, while *K. pneumoniae* strain carried colistin resistance, in addition. Phages active against these bacteria were isolated by enrichment method from various Belgrade wastewaters. Only the lytic phages, producing clear plaques were pursued. Phages were purified by isopycnic centrifugation through CsCl gradient and further used for TEM imaging, DNA isolation and sequencing, as wel as for SDS PAGE.
Results: Seven different lytic phages were isolated and characterized. Among these, three manifested unusually broad host range. Bacteriophage NOVI was shown capable of lysing both *A. baumannii* and *K. pneumoniae*, while phage LST4 was active against *E. coli* and *A. baumannii*. Moreover, phage ISTD efficiently lysed strains of *A. baumannii, K. pneumoniae* and *E. coli.*
Conclusion: Lytic activities of isolated phages recommend them as potential candidates for phage therapy. However, these findings suggest "out-of-order" host range, given that *Acinetobacter* and other used bacteria belong to different bacterial orders. This kind of polyvalence in phages has not yet been documented and challenges the perception of their narrow host range nature.

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Poster Session
MOLECULAR BIOLOGY OF MICROORGANISMS

CONTENT OF PHENOLIC COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF *Helleborus atrorubens* Waldst. & Kit. AND *Helleborus odorus* Waldst. & Kit. Ex Willd. EXTRACTS

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Introduction: Hellebore species have been widely used as analgesics, anti-inflammatory medicines and remedies for infectious animal diseases. These plants are a source of many bioactive compounds with potential health effects. However, only a few studies concerning antimicrobial activity have been published. The aim of our study was to test antimicrobial activity as well as to determine content of phenolic compounds in flower, leaf and root extracts of *Helleborus atrorubens* Waldst. & Kit. and *Helleborus odorus* Waldst. & Kit. Ex Willd.
Methods: Methanol, ethanol and acetone extracts of flowers, leaves and roots were prepared. The concentrations of total phenolics, phenolic acids and flavonoids were determined using spectrophotometric methods. Antimicrobial activity was tested using agar-disk diffusion method. One-way analysis of variance (ANOVA) was used to test statistical significance.
Results: Total phenolics determined in *H. atrorubens* leaf extracts and methanol and acetone flower extracts, are significantly higher in comparison to root extracts and ethanol flower extract. Leaf extracts of *H. odorus* show significantly higher concentration of total phenols in comparison to flower and root extracts. Flavonoids and phenolic acids are significantly lower in root extracts of both analysed species, regardless solvent used, when compared to flower and leaf extracts. *H. atrorubens* acetone extract has shown significant antimicrobial activity against *Enterococcus faecalis.*
Conclusion: Leaf extract of both tested species have the highest level of total phenolics, phenolic acids and flavonoids, thus in further research leaf extracts should be tested for bioactive potential. Both analysed species do not exhibit remarkable antimicrobial activity.

FROM ECF σ FACTORS TO COMMON TRANSCRIPTION INITIATION MECHANISM IN σ^{70} FAMILY

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Introduction: In distinction to well-studied house-keeping (RpoD) σ factors, that control bacterial transcription under standard conditions, alternative σ factors transcribe specialized regulons during stress, and are largely unexplored. Consequently, a common model of promoter recognition in σ^{70} family is currently lacking. To that end, we analyze ECF group, gathering alternative σ factors that are most numerous, diverse, and distantly related to house-keeping σs, in search for unifying promoter recognition paradigm.
Methods: Extensive computational comparison of ECF σs and their promoters, to infer DNA-protein interaction motifs that initiate transcription was employed. Additionally, we use biophysical modeling to investigate if mix-and-matching mechanism - well established in house-keeping, but considered absent for alternative σs - also applies to ECF group.
Results: We found classical mix-and-matching signature in ECF promoters (-35 element absence compensated with long -10 extensions), along with non-canonical σ-promoter interactions in spacer region, implying substantial promoter recognition flexibility. Accordingly, the observed extent of mix-and-matching in ECF σs is significantly stronger compared to RpoD group, which we propose is due to selection pressure, that forces promoters near the non-specific binding boundary to arrive at threshold transcription activity through promoter element complementation; note that smaller σ regulon corresponds to larger fraction of boundary promoters.
Conclusion: Overall, our results indicate that mix-and-match might act as a unifying framework of promoter recognition by diverse σ factors. Additionally, the obtained knowledge on ECF functioning could enable deeper understanding of transcription regulation in CRISPR/Cas systems (our current interest), which likely respond to cell envelope stress.

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NOVEL QUORUM SENSING INHIBITORS FROM *Achromobacter* spp. CLINICAL ISOLATES

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Introduction: Inhibition of quorum sensing represents one of the most promising strategies for antivirulence therapy of bacterial infections. Opportunistic pathogens as *Achromobacter* spp. are mostly unexplored as a source of quorum sensing inhibitors.
Methods: The 69 clinical isolates of *Achromobacter* spp. that were collected (2012-2015) from patients with cystic fibrosis (n=32) and patients receiving care for other health conditions (n=37) in Serbian CF center were tested for *Pseudomonas aeruginosa* PAO1 quorum quenching activity. Quorum sensing inhibition assay for initial screening of potential quorum quenching strains was performed on Petri dishes using indicator strain *Chromobacterium violaceum* CV026. Biochemical properties of discovered active metabolite were assessed by functional assays. Cosmid library of *Achromobacter xylosoxidans* 11304 genome was constructed in pLAFR3 cosmid. Cosmid coding for quorum quenching metabolite was sequenced and analyzed.
Results: Among analyzed *Achromobacter* spp. isolates only *A. xylosoxidans* 11304 was positive for production of quorum sensing inhibitors. Quorum quenching molecule was shown to be stable, small molecule which stayed active after treatment with protein inhibitors. Cosmid gene library of 11304 genome was screened for cosmids coding for quorum quenching molecule, and after the selection identified cosmid was sequenced by Illumina next generation sequencing. Sequenced cosmid carried genomic region which shared 97% of identity with genome of *Delftia sp*. This result indicates horizontal transfer of genetic determinants coding for quorum quenching molecule.
Conclusion: *Achromobacter* spp. were shown to be a potential source of molecules for antivirulent therapy of infections caused by *Pseudomonas aeruginosa*.

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AggLr IS A NOVEL AGGREGATION FACTOR FROM *Lactococcus raffinolactis* BGTRK10-1

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Introduction: The ability of lactococci to form multicellular aggregates is an important property for colonization of the oral cavity, human gastrointestinal or urogenital tract. Since the interest in aggregation of lactococci is related to their probiotic function, *Lactococcus raffinolactis* BGTRK10-1 was selected due to the expression of a unique auto-aggregation phenotype.
Methods: Construction of *L. raffinolactis* BGTRK10-1 cosmid library, DNA sequencing and protein analysis, functional assays of AggLr protein in heterologous hosts *Lactococcus lactis* subsp. *cremoris* MG7284 and *Enterococcus faecalis* BGZLS10-27.
Results: The novel *aggLr* gene coding for the auto-aggregation promoting protein (AggLr) of *L. raffinolactis* TRK10-1 was cloned and functionally analysed. AggLr is the largest known cell-surface protein in lactococci, consisting of 1774 aa (193.7 kDa). It contains an N-terminus leader peptide, followed by three successive collagen binding domains, six successive repeats (CnaB-like domains) and an LPXTG sorting signal at the C-terminus for cell wall anchoring. Heterologous expression of the *aggLr* gene confirmed crucial role of AggLr protein in forming large cell aggregates (auto-aggregation), collagen and fibronectin binding. These results indicate promising probiotic function of AggLr due to enhancing of effective host tissue colonization and potent prevention of pathogen colonization.
Conclusion: Heterologous expression of AggLr provided autoaggregation, collagen and fibronectin binding of MG7284 and BGZLS10-27 and demonstrated that the *aggLr* gene is sufficient for all three phenotypes.

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NEW INSIGHTS INTO DIVERSITY OF CarO AND Omp33-36 kDa PORINS FROM *Acinetobacter* spp.

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Introduction: Carbapenem-resistant *Acinetobacter* spp. has been recognized as a challenge in clinical microbiology. One of the main determinants of carbapenem resistance in *Acinetobacter* spp. are the outer membrane proteins (OMPs) CarO and Omp33-36 kDa. The aim of this study was to investigate diversity of CarO and Omp33-36 kDa proteins in order to establish conservative regions which could be used for development of vaccines in future infection therapy.
Methods: Protein sequences were aligned by ClustalW 1.7. The MEGA 6.0. was a tool for construction of the maximum likelihood (ML) phylogenetic trees. Transmembrane topology predictions of different OMP isoforms were performed by PRED-TM, while PROVEAN analysis was used for prediction of impact of amino acid changes to biological function of OMPs.
Results: ML phylogenetic analysis of CarO and Omp33-36 kDa proteins separated both in three distinct groups and defined existence of four different variants for both, CarO and OMP33-36 kDa proteins. Each CarO group contained two variable, two hypervariable regions and four conserved regions at the same positions. Omp33-36 Group I and II contained four variable and five conserved regions while group III contained six variable and six conserved regions. Detected amino acid substitutions and/or deletions in different CarO and Omp 33-36 kDa groups could influence biological function of this OMP.
Conclusion: The discovery of conservative CarO and Omp33-36 protein regions are of great importance since they could enable development of vaccines for therapy of infections caused by *Acinetobacter* spp.

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATED FROM THE RHIZOSPHERE OF THE SELECTED WEED SPECIES

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Introduction: Studying of rhizosphere microbiota of wild plants and identification and description of plant growth promoting (PGP) bacteria represents an important step for the reconstruction of natural environment for growing crops. The aim of this study was to isolate and identify bacteria with potential PGP activity from the rhizosphere of the selected weed plants, and evaluate their effect on seed germination of pepper (*Capsicum annuum* L.).
Methods: Microbiological, biochemical and molecular methods were used for isolation, characterization and PGP potential evaluation of selected isolates.
Results: Out of 69 rhizosphere isolates, seven showed PGP attributes. Sequencing of the 16S rRNA gene identified isolates as: *Bacillus aryabhattai, Pseudomonas* sp., *P. plecoglossicida, Arthrobacter globiformis, Shewanella amazonensis, P. putida and Bacillus* sp. Analysis of extracellular enzyme production showed that each isolate produced at least two of the tested enzymes, none of them solubilized inorganic phosphates and two of the isolates produced exopolysaccharides. *Bacillus aryabhattai* was selected for the treatment of pepper seeds, according to its high indole 3- acetic acid production and abiotic stress resistance. The results show that there is 10% increase in total number of germinated seeds inoculated with selected strain. However, in the presence of 130 mM NaCl, the control group had 20% more germinated seeds comparing to the inoculated group.
Conclusions: We conclude that rhizosphere of selected weed plants offers a variety of bacterial species that exhibit PGP traits, and that selected strain *Bacillus aryabhattai* has a beneficial effect on pepper seed germination in normal conditions.

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AGEING AND ORAL BACTERIA IN HIV-INFECTED PATIENTS

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Background: The need for understanding the composition of oral microbiota in HIV-infected patients has been recognized for many years, but no studies so far have dealt with age-related changes in periodontal pathogens occurrence in HIV+ individuals. The aim of the present study was to assess and compare temporal changes of seven periodontal pathogens frequency in younger (≤35 years) and older (≥50 years) HIV-infected and non-infected individuals.
Methods: Bacterial DNA was isolated from buccal swabs of 30 younger and 30 older subjects in both HIV+ and HIV-groups. Using PCR the following microorganisms have been detected: *Aggregatibacter actinomycetemcomitans* (Aa), *Eikenella corrodens* (Ec), *Peptostreptococcus micros* (Pm), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf) and *Treponema denticola* (Td). Intraoral soft tissue inspection and measurement of periodontal parameters were also done in both groups of patients.
Results: The prevalence of microorganisms was significantly higher in HIV+ patients compared to controls, and their distribution showed a notable shift-the decreasing incidence in HIV- subjects was: Pi>Pm>Pg>Aa>Ec>Tf>Td whilst in HIV+ it was: Pi>Pm>Ec>Pg>Tf>Aa>Td. Oral manifestations of HIV infection were noted in 57% of younger patients and 90% of older patients. All measured values of clinical periodontal parameters were significantly higher in older compared to younger HIV+ patients. Also, an important rise of bacterial load was observed in older HIV+ patients compared to the same age group of HIV- patients.
Conclusion: Ageing in HIV+ subjects is accompanied with a substantial increase and rearrangements of periodontal microbiota, potentially aggravating oral and systemic health.

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TANNASE AND GALLATE DECARBOXYLASE FROM *Lactobacillus* spp.

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Introduction: Tannin acyl hydrolase (E.C. 3.1.1.20) commonly known as tannase is a serine esterase that catalyzes the hydrolysis of ester and depside bonds present in gallotannins, complex tannins, and gallic acid esters yielding useful derivatives, such as gallic acid and oligomeric tannins. Tannase is one of the most versatile biocatalysts and plays an important role in a wide range of bioconversion reactions under protein-precipitating conditions, which is of great importance for food, feed, beverage, pharmaceutical, and chemical industries. Gallate decarboxylase is another enzyme which participates in tannin degradation catalyzing decarboxylation of gallic acid yielding pirogallol as end product.
Methods: Large scale screening of bacterial collection for tannase activity was performed by spectroscopic method using methyl gallate as a substrate. Gallate decarboxylase activity was determined by detection of formed pyrogallol using thin layer chromatography. To discriminate selected strains for ability to hydrolyze natural tannin molecules tannic acid was used as substrate and degradation products were identified by thin layer chromatography and high performance liquid chromatography.
Results: Among analyzed *Lactobacillus* spp. three strains were found to possess strong tannase activity *L. paraplantarum* B23115, *L. plantarum* LMG9208 and *L. plantarum* LMG9212. Estimated tannase activity for all three strains was over 10 mU per 3 x 10^8 CFU. On the other hand strain *L. plantarum* LMG18012, which was characterized by low tannase activity, showed remarkable gallate decarboxylase activity, approximately ten times higher, compared to other selected strains.
Conclusion: *Lactobacillus* spp. were shown to be a promising source of tannase and gallate decarboxylase for industrial applications.

Acknowledgements: This study was supported by the grants no. 173019 and 173005 from the Ministry of Education, Science and Technological Development, Republic of Serbia.

THE ABILITY OF LACTOBACILLI ISOLATED FROM ARTISANAL ZLATAR CHEESE TO PRODUCE GAMMA-AMINOBUTYRIC ACID

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Introduction: A wide range of traditional foods produced by microbial fermentation contain γ-aminobutyric acid (GABA), nonprotein amino acid with several physiological functions. GABA is synthesized from glutamate by the activity of glutamic acid decarboxylase (GAD). The aim of this study was to identify strains from artisanal Zlatar cheese with high GABA-producing ability.
Methods: *In silico* analysis and conserved CoreF/CoreR primers were used to screen the presence of the glutamate decarboxylase gene (*gadB*). Qualitative and quantitative estimation of GABA in supernatants was performed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The pH of the culture medium was monitored for 48 hours. The cell line HT-29 was used to determine cytotoxic effect of selected strain's supernatants by Real time cell analizer.
Results: The 67% of lactobacilli were positive for the presence of the *gadB* gene. TLC assay showed that 7 isolates can produce GABA. Strain *Lactobacillus brevis* BGZLS10-17 was the most efficient with a 97% GABA conversion rate in the culture medium containing 60 mM monosodium glutamate (MSG). The change of pH in MRS medium suplemented with MSG during growth of strain BGZLS10-17 sugested that GABA could be involved in pH regulation of lactobacilli. Results showed that supernatant of BGZLS10-17 containing GABA was not toxic for the cells in confluence state, but was toxic for the cells in proliferation.
Conclusion: This study suggests that TLC combined with HPLC was an efficient method for selection of high GABA-producing strains that can be used in formulation of innovative GABA-enriched foods.

Acknowledgements: This study was supported by the grant no. 173019 from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Session
CONGRESS PLENARY LECTURES

part two

CRISPR/Cas9 METHODOLOGY FOR EPIGENETIC EDITING

Vlatka Zoldoš (Hrvatska / *Croatia*)

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Research interests:

Epigenetics, Protein glycosylation, Epigenetics in human complex diseases, Chromatin, Plant biology.
Prof. Vlatka Zoldoš was working for 15 years in the field of plant biology where she achieved considerable recognition of the international scientific community. In 2008, she established the Laboratory for Epigenetics at the Division of Molecular Biology, Department of Biology, Faculty of Science University of Zagreb, and shifted her main research interest to epigenetics of protein glycosylation. Today, she is a group leader of a team consisting of 11 researchers. She has introduced several techniques at the Division of Molecular Biology in the fields of Molecular cytogenetics and Epigenetics: fluorescent *in situ* hybridization (FISH) on chromosomes and extended DNA fibers; microdissection using a laser capture microdissector; bisulphite pyrosequencing for DNA methylation analysis; chromatin immunoprecipitation methods (ChIP-qPCR and ChIP-seq). Her pioneering efforts in the field of epigenetics of glycosylation has resulted with publication of several important papers in the field.

Source web site: http://zoldos.biol.pmf.hr/vlatka-zoldos-group-leader/

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ELTA 90 Medical Science je najmlađi član ELTA 90 grupacije, regionalnog lidera u distribuciji medicinskih sredstava, laboratorijske opreme i potrošnog materijala. Sa više od 15 godina iskustva u regionu, ELTA 90 je gradila svoju reputaciju uspešne kompanije, kojoj su partneri na prvom mestu.
Veliki broj renomiranih svetskih kompaniija koje zastupamo svedoči o našem poslovanju koje se bazira na stalnoj edukaciji, unapređenju kvaliteta i brzine usluge, inovacijama, društvenoj odgovornosti i građenju poverenja i partnerskih odnosa sa našim klijentima. Profesionalne potrebe i razvoj naših klijenata su bile i ostale naš prioritet.

Kompanija **Illumina** je osnovana 1998. godine u San Dijegu, Kalifornija. Trenutno ima preko 6000 zaposlenih i predstavlja globalnog lidera u genetici, polju koje povezuje biologiju i tehnologiju. Nakon Projekta humanog genoma, kada je prvi put odgonetnuta kompletna primarna sekvenca molekula DNK i kada je identifikovano i mapirano preko 20 000 gena, istraživanja su prešla na još veću skalu. Ovaj naučni proboj doprineo je da molekularna genetika i studije na čitavom humanom genomu postanu fundamentalan deo medicine i brige o zdravlju ljudi. Polje kliničke molekularne dijagnostike se u poslednjoj deceniji značajno proširilo, pre svega zahvaljujući razvoju nauke i tehnologije. Tehnologija sekvenciranja nove generacije (eng. New Generation Sequencing, NGS) predstavlja novu i revolucionarnu metodu kojom je moguće analizirati hiljade gena u samo jednom koraku. Kompanija Illumina je pionir u razvoju ove metodologije, a nedavno je proslavljajući svoj 18. rođendan, potvrdila svoj položaj lidera u ovoj oblasti. Osim dijagnostičkih analiza, koje su postale rutinske na Illumininim aparatima poput MiniSeq-a i MiSeq-a, omogućavamo istraživačima da idu još dalje. Vođeni željom za novim saznanjima, kako bi pronašli odgovore na sva pitanja koja intrigiraju naučnu javnost i javnost uopšte, svakoga dana se razvijamo. Pristup Ilumina grupi ne samo da omogućava razmenu iskustva i znanja, već pruža naučnicima mogućnost da osete pokretačku enegriju koju nosi rad na sekvenatorima nove generacije. Omogućava im da budu prvi i da pomeraju granice. Pozivamo i Vas da napravite razliku, pridružite se Illumininoj zajednici.

Uz Illuminu, budućnost je sada.

Ono što izdvaja Illuminu od drugih proizvođača jeste posebna tehnika sekvenciranja sintezom – ***SBS (Sequencing by synthesis)*** čija je osnovna prednost velika osetljivost i smanjenje mogućnosti greške, kao i ***"Pair-end sequencing"*** koji omogućava dodatnu proveru sekvenciranja.

MiniSeq - nova super zvezda Illumine. Sva snaga u malom, pristupačnom i jednostavnom za upotrebu instrumentu najnovije generacije.

MiSeq – fokus na snazi i neograničenim mogućnostima. Ciljano sekvenciranje gena, metagenomika, sekvenciranje malih genoma, ciljana ekspresija gena, sekvenciranje amplikona, HLA tipizacija uz Output do 15Gb!

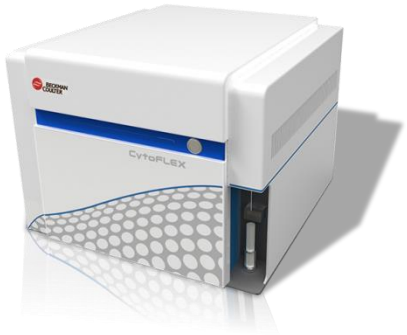
NextSeq 500 **-** najjači desktop sekvenator, objedinjuje veliku snagu, fleksibilnost i jednostavnost u radu, kako bi Vam osigurao što jednostavniji pristup u izučavanju kompletnog genoma, epigenoma i transkriptoma.

HiSeq sistemi **-** neprikosnoveni sistemi prepoznatljive snage, brzine i efikasnosti koji su postali prva opcija za sve veće genetičke centre širom sveta.

Kompanija **Beckman Coulter** je osnovana pre 80 godina, a do danas ima predstavništvo u preko 130 zemalja sveta i broji preko 11 800 zaposlenih. Beckman Coulter je predano posvećen unapređivanju i optimizovanju aparata i reagenasa u laboratorijama. Tokom godina razvijeno je veliko poverenje između klijenata i ove kompanije, tako da se za ime Beckman Coulter pre svega vezuje reč "kvalitet". Glavni fokus zaposlenih u Beckman Coulter-u je inovacija, pouzdanost i efikasnost, zbog čega se njihov uspeh na tržištu laboratorijske opreme veoma opravdano povećava iz godine u godinu.

Beckman Coulter Life Sciences

Beckman Coulter Life Sciences pruža inovativna i pouzdana rešenja za laboratorije sa najrazličitijim potrebama širom sveta. Njihovu opremu ćete videti na univerzitetima, u vladinom sektoru, u biotehnološkim i farmaceutskim kompanijama, bolnicama i komercijalnim laboratorijama. Svojim najsavremenijim pristupom Beckman Coulter Life Sciences preispituje konvencionalne metode kako bi razvio najbolje moguće proizvode koji su globalno prepoznati kao izuzetno pouzdani, a samim tim su i veoma rado i sa ponosom korišćeni u laboratorijama.



Iz Beckman Coulter Life Sciences portfolia izdvaja se program za protočnu citometriju, koji je posebno prilagođen jedinstvenim potrebama istraživača (**CytoFLEX aparat**) kao i širok dijapazon centrifuga (Allegra, Avanti i Optima serije).

Beckman Coulter Industry

Svaki korak u industrijskoj proizvodnji iziskuje precizno merenje, te samim tim istraživanje, razvoj i brza proizvodnja direktno zavise od opreme koja se koristi u datoj kompaniji. Beckman Coulter Industry pruža potpuno integrisane, lake za upotrebu, automatizovane sisteme sa najrazličitijom primenom u kontroli kvaliteta – merenje čestica, praćenje njihove distribucije i zapremine koje zauzimaju kao i kompletna analiza na ćelijskom nivou. Svi Beckman Coulter sistemi mogu da se konfigurišu tako da izađu u susret specifičnim potrebama svih zaposlenih u industriji i pružaju efikasan proces automatizacije za najrazličitije korake u trgovini.

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