XVIth INTERNATIONAL CONFERENCE
OF THE LITHUANIAN BIOCHEMICAL SOCIETY

BIOCHEMISTRY TARGETING DISEASES

Taujėnai, Lithuania,
June 28-30, 2023

PROGRAMME AND ABSTRACT BOOK

Welcome word

Dear Friends,

This is my pleasure and an honor indeed to welcome you in Taujėnai manor to the event of Lithuanian Biochemical Society. This year, the focus of annual LBS meeting is on the Medicinal Biochemistry, where passion for science directly meets the social responsibility of Life Sciences research – and a researchers. We strive to gather a knowledge and cure a diseases, taking front rows in a battle for a healthier future of people. For us to be successful, sharing of ideas is a key.

It is of special privilege to organize a long-awaited event in quite a turbulent time. A rich program on Neurobiochemistry, Cancer Research and Drug Discovery fields awaits you during this venue. It is my firm belief that science research community of Lithuania has to share with many fascinating discoveries since our last meeting unbelievably long five (!) years ago.

Welcome to XVI International Conference of Lithuanian Biochemical Society "Biochemistry Targeting Diseases"!

SERVA

Prof. Saulius Serva

Chairman of Lithuanian Biochemical Society

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Programme of the XVIth International Conference of Lithuanian Biochemical Society

BIOCHEMISTRY TARGETING DISEASES

Taujėnai, Lithuania, 2023

Wednesday, June 28	
09:00	**Registration**
10:00	**Welcome address**
Cancer research session. Moderated by *D. Jurėnas*	
10:30	*Prof. S. Jarmalaitė*. Molecular signature of renal cancer
11:15	*Dr. A. Navickas*. An mRNA processing pathway suppresses metastasis by governing translational control from the nucleus
12:00	**Lunch**
Drug discovery session. Moderated by *S. Serva*	
13:00	*Prof. J. Skiecevičienė.* We contain multitudes: gut microbiota from birth to old age
13:25	*Dr. D. Jurėnas*. Delivery and function of RHS-caged toxins
13:50	*Dr. V. Dudutienė*. Fluorine in Drug Design
14:10	*Dr. R. Žūkienė*. How to make sweet stevia sweeter: green technology of cold plasma
14:30	**Coffee break**
Neurobiochemistry session. Moderated by *V. Smirnovas*	
15:00	*Prof. A. Pivoriūnas*. Extracellular vesicles as a novel therapies against Parkinson's disease
15:30	*Dr. U. Neniškytė*. Molecular signals guiding neuronal circuit remodelling
16:00	*Dr. P. Vaitkienė*. Decoding the m6A Epitranscriptome: Exploring RNA Modifications in Brain and Glioma Tissues
16:30	*Dr. D. Širvinskas*. lncRNA: the secret code of the brain?
17:00	**Poster session 1** Coffee break at poster area
19:00	**Welcome reception**

Thursday, June 29	
08:30	**Registration**
09:00	**Opening remarks**
Neurobiochemistry session. Moderated by *V. Borutaitė*	
09:10	*Prof. G. Brown*. Cells eating cells in sickness and in health
10:00	*Dr. K. Pampuščenko*. The role of S100A9 protein in inflammation-mediated neurodegeneration
10:45	*Dr. V. Smirnovas*. The role of environment in amyloid aggregation
11:30	*Dr. A. Saudargienė*. Impaired hippocampal synaptic plasticity in Alzheimer's disease: integrating experimental data and computational modeling
12:00	**Lunch**
Cancer research session. Moderated by *A. Navickas*	
13:00	*Prof. S. Šatkauskas*. Development of antitumor therapies using reversible and irreversible electroporation
13:40	*Prof. K. Sužiedėlis*. In search for more efficient anticancer radiotherapy
14:20	*R. Sampath*. Migration of Breast Cancer cells is influenced by mutant GTPases of Rab40 and Rap2
14:35	*Dr. R. Žūkienė (FEBS representative)*. Introduction and presentation of FEBS activities
15:00	**Coffee break**
15:30	**LBS meeting**
17:00	**Poster session 2** Coffee break at poster area
19:00	**Gala Dinner & Concert**

Friday, June 30	
08:30	**Registration**
09:00	**Opening remarks & LBS medal award**
Drug discovery session. Moderated by *S. Serva*	
09:30	*Prof. V. Borutaitė*. Repurposing of drugs: neuroprotective effects of imeglimin
10:00	*Dr. V. Vengelienė*. Novel approach to treat alcohol use disorder: calpain inhibitors
10:30	*Dr. R. Aldonytė*. Phytochemicals for human respiratory health
11:00	*Dr. V. Raškevičius*. Prediction of potency of gap junction inhibitors using a quantitative structure-activity relationship model
11:20	*Dr. L. Žemaitis*. Decoding the Rise and Transmission Patterns of SARS-CoV-2 Viral Lineages in Lithuania: A Thorough Study over Two Years
12:00	**Lunch**
Awards and closing	
13:00	**Student poster presentation awards** **Conference closure speeches**

ORAL PRESENTATIONS

CANCER RESEARCH

C.1. MOLECULAR SIGNATURE OF RENAL CANCER

Sonata Jarmalaitė[1,2], Raimonda Kubiliūtė[1,2], Rasa Sabaliauskaitė[1], Algirdas Žalimas[1,2], Albertas Ulys[1]

[1]*National Cancer Institute, Vilnius, Lithuania;*
[2]*Life Sciences Center, Vilnius University, Vilnius, Lithuania*

Renal cell carcinoma (RCC) is the most lethal neoplastic disease of the urinary system accounting for 2-3% of all cancers in adults. The highest incidence of RCC is registered in Balkan and Baltic countries. Furthermore, Lithuania has the highest incidence rate globally and takes 4th place according to the mortality rate. Obesity, hypertension and tobacco smoking are the main known risk factors, while hereditary factors account for up to 10% of the cases. Most of RCC cases are asymptomatic and discovered incidentally during radiological examination frequently in late stages of the disease, thus cause a significant health burden. There is a vital need for better understanding of the molecular signature of RCC for more precise management of the disease. Aiming for this we analyzed the mutational and epigenomic signature of Lithuanian RCC.

Most frequently mutated tumor suppressor genes (TSG) in RCC are VHL, PBRM1, SETD2 and BAP1, accounting for 50-80% of the burden, but further analysis in a large list of TSG might provide a more detailed mutational signature of RCC. 58 renal tumors were analyzed by next generation sequencing: 22% of RCC were identified with known pathogenic variants and 68% with new variants of uncertain pathogenicity. Up to 14% RCC cases were identified with the CHEK2 gene mutations, previously not linked to RCC.

DNA methylation changes in TSG are early and frequent events during carcinogenesis. We performed genome-wide DNA methylation and gene expression profiling in 22 RCC and paired noncancerous renal tissues which identified 175 RCC-specific hypermethylated genetic regions. Deregulated genes were enriched among biological processes related to tumor development and progression and ten such protein-coding genes were selected for further analysis.

Significantly higher methylation frequencies for all genes were found in RCC tissues compared to noncancerous tissues. The best diagnostic performance was demonstrated for a panel of six genes showing 85% sensitivity and 96% specificity. The epigenetic test was able to diagnose RCC from liquid biopsy (urine) samples with the highest diagnostic power (AUC=0.78) calculated for the panel of two genes (ZNF677 & PCDH8). Moreover, the same set of two genes was prognostic for overall survival of RCC patients' with HR of 12.5. In cases with small renal tumors (≤ 4cm; pT1a) the urinary four-gene test showed the diagnostic power of AUC=0.74. The methylation level of some genes correlated with tumor size and tumor growth dynamics showing diagnostic and prognostic potential of this liquid biopsy test.

In conclusion, detailed molecular characterization of RCC may favor early detection and accurate personalized management of the disease. Wider application of liquid biopsy-based molecular tests in clinical practice holds a strong promise to improve overall survival of patients with RCC.

C.2. AN MRNA PROCESSING PATHWAY SUPPRESSES METASTASIS BY GOVERNING TRANSLATIONAL CONTROL FROM THE NUCLEUS

Albertas Navickas[1,2,3,4,#,$], Hosseinali Asgharian[1,2,3,4,#], Juliane Winkler[5], Lisa Fish[1,2,3,4], Kristle Garcia[1,2,3,4], Daniel Markett[1,2,3,4], Martin Dodel[6], Bruce Culbertson[1,2,3,4], Sohit Miglani[1,2,3,4], Tanvi Joshi[1,2,3,4], Keyi Yin[1,2,3,4], Phi Nguyen[1,2,3,4], Steven Zhang[1,2,3,4], Nicholas Stevers[1,2,3,4], Hun-Way Hwang[7], Faraz Mardakheh[6], Andrei Goga[3,5,8], Hani Goodarzi[1,2,3,4]

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Cancer cells often co-opt post-transcriptional regulatory mechanisms to achieve pathologic expression of gene networks that drive metastasis. Translational control is a major regulatory hub in oncogenesis, however its effects on cancer progression remain poorly understood. To address this, we used ribosome profiling to compare genome-wide translation efficiencies of poorly and highly metastatic breast cancer cells and patient-derived xenografts. We developed novel regression-based methods to analyze ribosome profiling and alternative polyadenylation data, and identified HNRNPC as a translational controller of a specific mRNA regulon. Mechanistically, HNRNPC, in concert with PABPC4, binds near to poly(A) signals, thereby governing the alternative polyadenylation of a set of mRNAs. We found that HNRNPC and PABPC4 are downregulated in highly metastatic cells, which causes HNRNPC-bound mRNAs to undergo 3' UTR lengthening and subsequently, translational repression. We showed that modulating HNRNPC expression impacts the metastatic capacity of breast cancer cells in xenograft mouse models. We also found that a small molecule, previously shown to induce a distal-to-proximal poly(A) site switching, counteracts the HNRNPC-PABPC4 driven deregulation of alternative polyadenylation and decreases the metastatic lung colonization by breast cancer cells in vivo.

C.15 DEVELOPMENT OF ANTITUMOR THERAPIES USING REVERSIBLE AND IRREVERSIBLE ELECTROPORATION

Paulius Ruzgys, Neringa Barauskaitė, Rūta Palepšienė, Baltramiejus Jakštys, Dovilė Uždavinytė, Salvijus Vykertas, Martynas Maciulevičius, Saulius Šatkauskas

Vytautas Magnus University

Cell electroporation (EP) occurs when cells in vitro or in tissues are exposed to electric field pulses that induce overcritical transmembrane potential leading to formation of transient pores in the plasma membrane. Depending on the pulse parameters, the delivery of pulses can lead to reversible (EP) or irreversible (IRE) electroporation. Both types of electroporation have been thoroughly investigated and applied for in vivo and clinical studies as novel modalities of antitumor treatment. When reversible EP is used, to achieve antitumor responses, anticancer drugs, such as bleomycin or cisplatin, or calcium locally or systemically are administered prior to delivery of electric pulses at the tumor site. These antitumor therapies correspondingly are known as antitumor electrochemotherapy and calcium electroporation. When IRE is used, no administration of any exogenous substances is needed, since the pulses leads cell death because of complete destabilization of cellular homeostasis.

We have demonstrated that following IRE cells release various substances, like ATP, proteins, and RNA. Similarly, when REP is used in combination with anticancer substances, the dying cells also release molecules, that affect cell viability. It was hypothesized that all these molecules might have an immunoregulating effect in vivo. To evaluate the possible effects, we performed in vivo studies on BALB/c mice bearing two 4T1 tumors. To find out whether the treatments can induce an abscopal effect, one of the tumors was exposed to electrochemotherapy, calcium electroporation or both therapies, while another was left directly unexposed. Some mice were additionally treated with IL2-coding plasmid electrotransfer into tibialis cranialis muscles for possible immune response and abscopal effect enhancement. Results have shown that combined therapies induced significant or complete antitumor response on exposed tumors, but also had significant antitumor response on unexposed tumors. Boosting of IL-2 serum concentration by gene electrotransfer did not enhance the abscopal effect however, it had a significant inhibitory effect on directly exposed tumors.

C.16. IN SEARCH FOR MORE EFFICIENT ANTICANCER RADIOTHERAPY

Rimvilė Prokarenkaitė[1,2], Kristijonas Veličkevičius[1,2], Linas Kunigėnas[1,2], Vaidotas Stankevičius[3], Audrius Dulskas[1,4], Vytautė Starkuvienė-Erfle[2,5], Kęstutis Sužiedėlis[1,2]

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[5]*BioQuant, Heidelberg University, Heidelberg, Germany*

Radiotherapy to treat cancer patients is used for more than 100 years already. Nevertheless, radiobiology research to develop more efficient radiotherapy is still ongoing at cancer research institutions world-wide. Short overview of radiobiology research from the discovery of X-rays and main advances in the field resulting in the conventional and advanced technological variants or alternative radiation modalities of radiotreatment will be presented to indicate the main challenges of current radiobiology. Research models used in the Laboratory of Molecular Oncology at National Cancer Institute (Lithuania) to overcome these challenges and the discovered potential targets for the increase of the efficacy of radiotreatment will be presented as well.

C.17 MIGRATION OF BREAST CANCER CELLS IS INFLUENCED BY MUTANT GTPases OF RAB40 AND RAP2

Revathi Sampath[1], Valeryia Mikalayeva[1], Rytis Prekeris[1,2]

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The most difficult problem in cancer research is figuring out how cells behave and move through a three-dimensional extracellular matrix during metastasis and invasion (Shahbandi and Jackson 2019). Under normal circumstances, cell migration helps multicellular organisms maintain their appropriate organization. Any modifications to the system that controls cell migration would lead to the spread of cancer throughout the body and the development of secondary tumors from primary tumors. Recent research reveals that altered signaling pathways, cytoskeletal formation, and membrane trafficking all influence cancer cell motility and metastasis. (Justus et al., 2014)

It is still unclear how to coordinate and direct all metastasis-related activities to specific events. Rab40 mutants belong to a small monomeric GTPase that is a member of the Ras oncogene and is primarily involved in intracellular membrane trafficking. On the other hand, Rap mutants which also belong to the Ras superfamily of GTPases, such as Rap1 and Rap2 are also known to have similar functions in regulating cancer cell migration. It could be possible to understand more about the mechanisms behind invasion and metastasis by altering the GTPases of Rab40 and Rap2 mutants. To achieve this, Rab40abc triple KO (a, b, and c expressing different isoforms of Rab40 subsets), Rap1 KO, and Rap2 KO along with MDA-MB-231 Cas9 as control cells (expressing tetracycline-inducible Cas9) were created as knock-out lines.

To analyze the cell migration, directionality, distance, length, and speed were all evaluated simultaneously for each cell line using time-lapse migration analysis. Where directionality denotes the direction or guidance of a single cell, distance describes the cell's travel from Point A to Point B, length denotes the measurement, and speed calculates the rate of movement of the cell's travel from Point A to Point B. Images for time-lapse migration analysis were achieved using the OLYMPUS fluorescence microscope fixed in a temperature-controlled incubation chamber and examined using Excellence Trackit software.

For metrics including trajectory distance, length, speed, and persistence of movement, all mutant knockout lines with Rab40abc, Rap1, and Rap2 displayed considerable movement activity and single-cell migration. It will be helpful to find other families of Rab40 or those linked to Rab40, such as Rap1 and Rap2, whose role in controlling cell migration is not fully understood, using this method for identifying functional targets for breast cancer cell migration. Rap1 and Rap2 will be the subject of more research that will clarify their control and function during breast cancer cell migration.

Acknowledgements: This project has received funding from the Research Council of Lithuania, agreement No S-MIP-22-60.

DRUG DISCOVERY

D.3 WE CONTAIN MULTITUDES: GUT MICROBIOTA FROM BIRTH TO OLD AGE

Jurgita Skiecevičienė

Lithuanian University of Health Sciences, Kaunas, Lithuania

Each of us have different appearance, character, habits, and these differences are determined by just 0.1% of human genetic material. But we contain not only human cells, we also carry a separate invisible world of microorganisms within us. This world is unique in each of us (0-50% similarity between unrelated people) and as shown by research in recent years, it has a great influence not only on our physiology, but also on our behavior. The microbiota colonizes us only at birth, but the processes important for its development begin already at the fetal level. Important environmental factors such as lifestyle, nutrition, health conditions and even our genetics shape microbiota throughout our life. However, what constitutes a healthy microbiota and how we might modify it still remains a relevant area of current research in disease, personalized nutrition, healthy aging, and other areas.

D.4. DELIVERY AND FUNCTION OF RHS-CAGED TOXINS

Dukas Jurėnas, Leonardo Talachia-Rosa, Laurent Terradot, Remy Fronzes, Eric Cascales

Université Libre de Bruxelles, Institut Européen de Chimie et Biologie, Centre National de la Recherche Scientifique

Rearrangement hot spots (RHS) were discovered a few decades ago as bacterial loci with frequent recombination events. It later appeared that these loci code for large polymorphic toxins that comprise conserved central region and hyper-variable C termini. With the help of cryo-EM structure we have demonstrated that the central region constitutes large beta-roll barrel enclosing C-terminally encoded toxic domain. The barrel is closed at both ends, but it undergoes auto-proteolytic cleavages allowing the release of the toxin. The N termini of the RHS dictate their secretion pathway across the membranes of the secreting and of the target bacteria. Using detailed protein interaction mapping we could demonstrate how the RHS-caged toxins are loaded on the type 6 secretion machinery – a bacterial contractile apparatus that injects toxins upon the contact with prey cell. We have now demonstrated a number of unprecedented toxic activities encoded by C-termini of the RHS, most notably the class of ADP-ribosyltransferases that can be specific to different targets in the cell. The RHS secreting bacteria code for cognate immunity proteins that block the toxic activity and prevent intoxication of the sister cells. Bacterial competition selects for new toxic domains and immunities integrated at the RHS loci and is likely a major determinant of their hyper-variability. In addition to their role in bacterial competition in various environments, RHS presents an opportunity for drug design, allowing encapsulation and delivery of proteins.

D.5. FLUORINE IN DRUG DESIGN

Virginija Dudutienė, Asta Zubrienė, Gediminas Žvinys, Agnė Petrošiūtė, Daumantas Matulis

Vilnius University, Institute of Biotechnology

The natural abundance of fluorine as fluorite, fluoroapatite, and cryolite is considered to be almost the same as of nitrogen. However, only 12 organic compounds possesing fluorine have been found in nature. In spite of such rarity, gigantic number of synthetic fluorine-containing compounds have been widely used in a variety of medicinal chemistry fields. The incorporation of fluorine atom often produce molecules with unique properties that cannot be attained using any other element.

Our research team used fluorinated compounds as carbonic anhydrase inhibitors. Fluorination of benzensulfonamide ring increased affinity immensely. This successful approach led to obtainment of wide variety of compounds. Furthermore, particular emphasis was driven towards cancer-related isoform- carbonic anhydrase IX showing perspective in cancer management.

D.6. HOW TO MAKE SWEET STEVIA SWEETER: GREEN TECHNOLOGY OF COLD PLASMA

Rasa Žūkienė

Vytautas Magnus University

Stevia rebaudiana Bertoni is a valuable plant in the food and pharmaceutical industry due to its secondary metabolites steviol glycosides (SGs) which are widely used as natural sweeteners. Stevia extract and SGs may offer many beneficial effects on health besides the sweet taste Next to diterpenes SGs, the non-sweetener fraction is rich in phenolic compounds, giving additional health benefits to the leaf material and adding extra value to the product. Stevia extracts and SGs are associated with anti-hypertensive, anti-hyperglycemic, antioxidant, anti-inflammatory, antifungal, anti-microbial activities, and anti-cariogenic action. Due to these various beneficial attributes and the absence of side effects in long-term use, sweeteners produced from stevia plants are gaining popularity. Stevioside (Stev) and rebaudioside A (RebA) are the most abundant of about forty SGs found in stevia. The taste quality of RebA is better than that of Stev, therefore the improvement of stevia plants by applying various agricultural techniques is directed to higher Reb concentration and higher RebA/Stev ratio.

In the recent decade, seed treatment with non-thermal or cold plasma (CP) was shown to stimulate seed germination, grown plant morphometric parameters, biomass production, and disease resistance in different plant species by inducing changes in plant biochemical phenotype. Our group studies, focused on Stevia rebaudiana Bertoni plant, revealed some CP-induced changes that can be reproduced in various cultivars from different seed sources, seed storage periods or using various plasma sources. Short (2-7 min) pre-sowing stevia seed treatment with CP was shown to increase SGs amount up to 7 times and increase RebA/Stev ratio in cultivars with low RebA content. The concentrations of other bioactive compounds such as phenolics, flavonoids, and subsequent antioxidant activity were decreased or unchanged when plants were grown in soil but not in an aeroponic system. In aeroponic cultivation conditions, the CP-treated group had significantly higher concentrations of total phenolic compounds (by 43%), flavonoids (by 19%), and antioxidant activity (by 45%) compared to the control. The main economically disadvantageous property of CP-treated groups was a tendency for lower biomass which can be a result of a trade-off between growth and secondary metabolism in response to CP. The more detailed mechanism of effects interplay can be elucidated by transcriptome and epigenome analysis in the future.

D.19. NOVEL APPROACH TO TREAT ALCOHOL USE DISORDER: CALPAIN INHIBITORS

Valentina Vengelienė

Vilnius University

Preclinical studies revealed contribution of N-methyl-D-aspartate receptors (NMDAR) to a variety of neuropsychiatric diseases including alcohol use disorder. However, development of NMDAR antagonists for therapeutic use has been a challenge in part due to severe side effects. One of the key intracellular events resulting from stimulation of NMDAR is activation of calpains – calcium-dependent cysteine proteases. Here we studied whether inhibition of calpains would reproduce therapeutic effects of NMDAR antagonists but would lack NMDAR mediated side effect profile. The novel calpain inhibitor A-705253 (3-10 mg/kg) was tested in rat behavioral models of alcohol-seeking and relapse. Our results indicate that calpain inhibitors like A-705253 are capable of retaining at least some of the preclinical efficacy of NMDAR antagonists while achieving superior therapeutic safety. These data demonstrate the involvement of calpains in alcohol-related behaviors and present a good rationale for a novel pharmacological intervention that may reduce craving and relapse in alcohol dependent patients.

D.20. PHYTOCHEMICALS FOR HUMAN RESPIRATORY HEALTH

Rūta Aldonytė, Jovilė Raudoniūtė, Edvardas Bagdonas, Rimantas Venskutonis

State Research Institute Center for Innovative Medicine, Kaunas University of Technology

According to World Health Organisation, air pollution increases the risk of cardiovascular disorders, respiratory diseases, viral infections, cancer, neuro-degenerative and other diseases. It is also known that various phytochemicals may mitigate such risks. Some effects of phytochemicals, i.e., cannabidiol, mangiferin, Z-ligustilide and some others were investigated. Air pollution-exposed human lung bronchial epithelium was used as a model. Organic PAH extract was obtained from the urban fine PM with high benzo(a)pyrene content collected in an Eastern European mid-sized city during the winter heating season. Cell proliferation traits and levels of intracellular oxidative stress were examined. In addition, wound healing and barrier functions were evaluated. In sum, most of the compounds tested were able to ameliorate oxidative stress, speed up a wound-healing process and restore the proliferation rate in exposed bronchial epithelium. Such protective effects of phytochemicals in air pollution-affected airway epithelium stimulate further research on this promising topic.

D.21. PREDICTION OF POTENCY OF GAP JUNCTION INHIBITORS USING A QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP MODEL

Ramona Matusevičiūtė, Eglė Ignatavičiūtė, Rokas Mickus, Sergio Bordel, Vytenis Arvydas Skeberdis, and Vytautas Raškevičius

Institute of Cardiology, Lithuanian University of Health Sciences

Gap junctions (GJs) comprising connexin-43 (Cx43) play a vital role in transmitting electrical impulses within the heart. Modulating Cx43 activity holds promise for treating cardiac arrhythmias and other disorders. This study aimed to demonstrate the effectiveness of a Quantitative Structure-Activity Relationship (QSAR) model in accurately identifying potent inhibitors of Cx43 GJs. By utilizing the QSAR model, along with 3D-QSAR and molecular docking techniques, we evaluated known Cx43 GJ inhibitors, proposed a novel candidate, and performed experimental testing. Our molecular modeling accurately predicted the concentrations required to achieve 50% of the maximal effect (IC50) for each compound and established a correlation between the predicted (pIC50) and experimental IC50 values (eIC50). Furthermore, we identified d-limonene, a monocyclic monoterpene, as a potential Cx43 inhibitor and validated its efficacy using dual whole-cell patch-clamp techniques. The pIC50 values of d-limonene and other Cx43 GJ inhibitors analyzed using our QSAR or 3D-QSAR model exhibited a strong correlation with their eIC50 values ($R = 0.88$ and $R = 0.90$, respectively), in contrast to the pIC50s obtained from molecular docking ($R = 0.78$). To search for novel, potent, selective, and specific inhibitors of GJ channels, we propose a two-step approach: initial screening of a compound library using the QSAR model, followed by validation of the most promising candidates using patch-clamp techniques.

Acknowledgements: This research received funding from the Research Council of Lithuania, grant number S-MIP-23-105.

D.22 INSIGHTS INTO THE EMERGENCE AND SPREAD OF SARS-COV-2 VIRAL LINEAGES IN LITHUANIA: A TWO-YEAR STUDY

Lukas Zemaitis[1], Gediminas Alzbutas[2], Andrius Merkys[2]

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The emergence of the COVID -19 pandemic has highlighted the importance of whole genome sequencing (WGS) in infectious disease surveillance and control. In order to better understand the occurrence, spread, and threat of the different viral lineages in Lithuania, a two-year research study was conducted. The study included WGS of the SARS-CoV-2 virus at local and global levels, as well as bioinformatics analysis to identify genetic mutations, lineages, and their transmission. The majority of COVID -19 cases in Lithuania were caused by local transmission. However, the study also showed outbreaks of viral lineages without altered biological characteristics. Analysis of transmission clusters showed that most of the different viral lineages were imported into Lithuania from the United Kingdom, Denmark, and Norway, consistent with emigration trends. Restrictions on movement had a significant impact on the diversity of viral lineages. Two case analysis will be presented – origin and spread of the B.1.1.523 SARS-CoV-2 lineage and outbreaks in mink farms.

The COVID-19 pandemic has prompted the development of new methodologies such as wastewater-based epidemiology and variants prediction tools, which are critical for preparedness for future pandemics. Some cases of possible implementation of novel methodologies in this area will be presented.

Keywords: SARS-CoV-2 Pandemic Lithuania, Whole genome sequencing, Genetic mutations, Bioinformatics analysis, Transmission analysis, Viral lineages, Wastewater-based epidemiology, Variants prediction tools, Local transmission, Diversity of virus lineages.

NEUROBIOCHEMISTRY RESEARCH

N.7 EXTRACELLULAR VESICLES AS A NOVEL THERAPIES AGAINST PARKINSON'S DISEASE

Augustas Pivoriūnas

State Research Institute Center for Innovative Medicine

Extracellular vesicles (EVs) provide a potent tool for intercellular communication by acting as a miniature lipid membranous containers for wide array of signaling molecules. EVs have several advantages from a therapeutic perspective: (1) EVs are safer in comparison to cells, because of reduced risks associated with transplantation; (2) EVs are relatively simple, stable and controllable systems, being thus suitable for the large scale clinical manufacturing; (3) EVs can cross blood brain barrier and therefore can be effectively used for the treatment of different neurological conditions.

We and others have demonstrated that EVs can be successfuly used as a potent therapies against Parkinson's disease. During my talk I wil present our data about the therapeutic effects of the intranasal administration of EVs to the Parkinsonian rats. I will also discuss potential neuroprotective mechanisms of the EVs. Finally, I will talk about the challenges that we need to overcome in order to move EV therapies towards clinical application in humans.

N.8. MOLECULAR SIGNALS GUIDING NEURONAL CIRCUIT REMODELLING

Urtė Neniškytė

VU-EMBL Partnership Institute, Life Sciences Center, Vilnius University

The mature brain connectome emerges through synaptic pruning of superfluous connections in developing brain. Microglia have central role in this process: they refine neuronal circuitry by phagocytosis and trogocytosis of synaptic structures. Microglial interaction with neurons, their processes and synapses is mediated by antagonistic "eat-me" and "spare-me" signals. "Eat-me" signals promote the elimination of exposing structures, while "spare-me" signals actively inhibit neuron-microglia interactions and promote the preservation of labelled connections. We have found that a lipid phosphatidylserine is preferentially exposed on synaptic structures and promotes microglia-synapse interaction. Phosphatidylserine exposure is developmentally regulated and requires the activity of Xk-related protein 8 (Xkr8) – a major phospholipid scramblase, which is highly expressed immediately after birth. Conditional Xkr8 knock-out in excitatory neurons diminishes axonal bouton trogocytosis and causes insufficient elimination of excitatory synapses. Mice lacking Xkr8 show increased density of cortico-cortical and cortico-spinal projections, supporting the role of Xkr8 in developmental axon pruning. In contrast, terminal moiety on neuronal glycocalyx, namely sialic acid, limits the pruning of neuronal structures. The enzymes involved in sialic acid turnover are highly expressed during brain development. Their dysregulation leads to aberrant connectivity associated with networks disorders, such as epilepsy. Therefore, the mature circuitry emerges from developing brain through a delicate balance of pruning and maintenance of precisely selected neuronal connections.

N.9. DECODING THE m6A EPITRANSCRIPTOME: EXPLORING RNA MODIFICATIONS IN BRAIN AND GLIOMA TISSUES

Paulina Vaitkienė, Daina Skiriutė, Giedrius Steponaitis, Rytis Stakaitis

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The term "Epitrancriptome" refers to the collection of chemical modifications that occur on RNA molecules, specifically the addition or removal of various chemical groups or marks. These modifications are distinct from the genetic code carried by the DNA sequence itself. Traditionally, the focus of molecular biology has been on the role of DNA and its transcription into RNA, which is then translated into proteins. However, recent research has revealed that RNA molecules can be chemically modified in various ways, which can have significant effects on their structure, stability, and function. The N6-methyladenosine (m6A) modification is one of the most prevalent and well-studied RNA modifications in the field of epitrancriptomics. It involves the addition of a methyl group (CH3) to the sixth nitrogen atom of the adenosine base within RNA molecules. m6A modification occurs on various types of RNA, including messenger RNA (mRNA), long non-coding RNA (lncRNA), and microRNA (miRNA). It is dynamically regulated and plays crucial roles in numerous biological processes. m6A modification influences RNA stability, splicing, transport, localization, and translation. It can affect RNA structure and binding to RNA-binding proteins (RBPs), thereby modulating RNA-protein interactions and RNA processing. M6A modification is critical for normal development and cellular processes. Dysregulation of m6A modification has been linked to numerous diseases, including cancer, neurological disorders, metabolic disorders, and viral infections. At the conference, I will present the work of our team related to the detection of m6A modifications in normal brain and glioblastoma tissues.

N.10. LncRNA: THE SECRET CODE OF THE BRAIN?

Dovydas Širvinskas, Daina Skiriutė, Giedrius Steponaitis, Rytis Stakaitis, Paulina Vaitkienė

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Gliomas are responsible for about 30% of all tumors within the central nervous system and are generally known so-far to be incurable. Therefore, more information is desperately needed that could help in detection, classification, and prognosis of gliomas. Long non-coding RNA (lncRNA) are increasingly being recognized as important players in many homeostatic and pathologic processes. It is thus reasonable to assume that lncRNA could shed light on the pathogenesis of gliomas.

The aim: to identify lncRNA that could be utilized for detection, classification, and prognosis of gliomas.

Methods: Primary tumor samples were obtained by collaboration with the "Kauno Klinikos" hospital department of neurosurgery. Tumor and healthy brain control samples were sequenced by direct RNA sequencing, using Nanopore technology. LncRNA genes were extracted from sequencing data according to Genecode_v42 lncRNA annotation. PCA analysis performed on lncRNA genes that were detected in at least 3 samples (n = 28). Preliminary results were compared with the TCGA and GTEx databases.

Results: Initial results suggest Low-Grade Glioma (LGG) and Glioblastoma (GB) cluster separately between each-other, as well as from normal brain tissue. LncRNA genes, responsible for the determination were obtained for further inquiry.

N.12 THE ROLE OF S100A9 PROTEIN IN INFLAMMATION-MEDIATED NEURODEGENERATION

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S100A9 (also known as Calgranulin B or MRP-14) is pro-inflammatory protein involved in the pathogenesis of neurological disorders including acute brain injury and neurodegenerative diseases. In neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, S100A9 was found to be abundant in neuronal cells with amyloid-β plaques and Lewy bodies, as well as within reactive glia. Glial cells, in particular microglia, play a crucial role in neurodegenerative and neuroinflammatory conditions. S100A9 was shown to alter the aggregation of amyloid-β and α-synuclein leading to the formation of stable structures. However, it is unclear whether S100A9 can cause neuronal loss by itself. In this talk, K. Pampuscenko will discuss how S100A9 increases the phagocytic activity of microglia and causes the exposure of phosphatidylserine on the outer leaflet of the neuronal plasma membrane, which serves as "eat-me" signal promoting death through phagocytic uptake. Overall, obtained results indicate that S100A9 may contribute to the manifestations of neurodegenerative disorders through microglial activation, which in turn induces neuronal damage.

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N.13. THE ROLE OF ENVIRONMENT IN AMYLOID AGGREGATION

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The ability to form amyloid structures may be a generic property of polypeptides, and there are two major factors which define the probability of amyloid fibril formation – amino acid sequence of the protein/peptide and the environmental conditions. In the case of folded proteins, at least partial unfolding is necessary to trigger the amyloid formation pathway, so increased temperature, extreme pH conditions, addition of denaturants or any other changes in the environment leading to destabilization of protein structure are used in amyloid aggregation studies. Even in the case of disordered proteins, neutralization of charges or contact with hydrophobic surfaces may be necessary to induce amyloid formation. In addition to the specific conditions required for amyloid formation, changes in the environment may alter the mechanism of aggregation and lead to distinct amyloid fibril conformations. Finally, environment conditions affect the kinetics of aggregation and may alter the effect of anti-amyloid compounds.

I'd like to present recent findings of our group related to environment-dependent polymorphism of amyloid fibrils and to the role of environment in detection of anti-amyloid compounds.

N.14 IMPAIRED HIPPOCAMPAL SYNAPTIC PLASTICITY IN ALZHEIMER'S DISEASE: INTEGRATING EXPERIMENTAL DATA AND COMPUTATIONAL MODELING

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Alzheimer's disease (AD) has a long preclinical stage and, before any clinical symptoms appear, pathological processes are observed in the hippocampus. Recent experimental evidence supports the fundamental role of AD-related peptides early in the pathology: in particular the most widely studied Amyloid beta (Abeta), and the less investigated Amyloid precursor protein (APP) C-terminal peptide (AICD). The aim of this project is to understand the AD-related peptide-induced mechanisms of impaired learning and memory in hippocampal CA1 region in early pathology of AD by applying the integrated experimental and computational modelling approach.

We investigated the effects of Abeta and AICD on intrinsic excitability of hippocampal CA1 pyramidal neurons and synaptic plasticity at hippocampal CA1-CA3 synapses in early pathology of AD. We developed data-driven in silico models of the hippocampal learning in CA1 region under AD conditions, and 1) extended the experimental evidence of Abeta, AICD-related changes in the properties of hippocampal CA1 pyramidal neuron synaptic plasticity, synaptic signal integration and neuronal excitability; 2) incorporated the effects of AD-related peptides into computational models of hippocampal synaptic plasticity to determine and explain the mechanisms of altered hippocampal function that leads to impaired learning in AD; 3) assessed the potential targets for innovative treatment of AD. We used Human Brain Project Brain Simulation Platform to perform computational modeling.

The modeling results support the experimental evidence that pathological concentrations of Aβ and AICD cause long-term potentiation (LTP) impairment. Long-term depression (LTD) enhancement was observed in Abeta conditions. Synaptic plasticity was strongly dependent on GluN2B-NMDA receptor subunit functioning, and rescued by its partial blockade in AD. The modeling study provides insight into the complex interactions in AD pathophysiology, and suggests the conditions under which synaptic plasticity is restored. The inter-disciplinary analysis, bringing together experimentalists and modelers, helps to further unravel the neuronal mechanisms most affected by AD, build a biologically-plausible computational models of the hippocampal CA1 area under AD conditions, and suggest potential targets for pharmacological treatment of AD.

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POSTER PRESENTATIONS

P1. LINE-1 PROMOTER METHYLATION PATTERN AS A PREDICTION TOOL IN PRECANCEROUS AND CANCEROUS COLORECTAL LESIONS

Rytė Rynkevičienė [1], Rima Miknaitė [2], Inga Kildusienė [1,2], Kęstutis Sužiedėlis [1,2], Giedre Smailytė [1,2]

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Colorectal cancer (CRC) is the fourth most common malignancy and presents the third most commonly diagnosed form of cancer worldwide. A lot of studies show that both genetic and epigenetic alterations are involved in regulating the tumorigenesis of CRC. The first changes in intestine mucosa are notable as hyperplastic polyps. Further with an increasing amount of mutations and changes in genome methylation status conventional adenomas or serrated lesions appear. Detection of all these lesions means an increased risk of developing adenocarcinoma. However, currently, the risk of cancer development is estimated according to the size and histological features of lesions. Epigenetic biomarkers such as LINE-1 could be useful tools to predict the formation of adenocarcinomas. Hypomethylation of LINE-1 promoter leads to genome instability and thus accumulation of mutations. The aim of this study was to evaluate the status of LINE-1 promoter methylation in adenocarcinoma, tubular adenoma and serrated lesions, and corresponding health tissue. During this study, the methylation status of the LINE-1 promoter at three CpG islands has been estimated using the pyrosequencing method. The results showed statistically significant differences between all groups. Interestingly, the different levels of LINE-1 promoter methylation between males and females have been detected.

P2. RELATIONSHIP OF METALLOTHIONEIN' AND TRACE ELEMENTS WITH CASES OF ASTROCYTOMA

Šulinskienė Jurgita[1,2], Baranauskienė Dalė[1], Šimakauskienė Vaida[1], Naginienė Rima[1]

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Background. Astrocytoma is a type of brain tumour that belongs to a group of tumours called gliomas. Astrocytoma is the most common type of glioma, that develop from neuroglial cells called astrocytes and is characterized by diffuse spread and infiltration into the surrounding tissues, thus the average survival time of affected patients is extremely short and reaches only 12-15 months. Over the past 50 years, these indicators haven't changed much, so research in the field of brain tumours is extremely relevant. Heavy metals affect human health through a variety of mechanisms, including altering gene expression, promoting the formation of reactive oxygen species, thereby causing oxidative stress, and increasing the likelihood of cancer development. Cysteine-rich low molecular weight proteins metallothioneins not only play a fundamental role in heavy metal chelation, thus reducing their intracellular levels, but also act as a potent antioxidant capable of neutralizing reactive oxygen species, thus overall diminishing oxidative stress in the cell.

The aim of this study was to evaluate whether there is a relationship between the concentrations of trace elements zinc, copper, cadmium, and selenium with the content of metallothioneins in the blood of a control group and patients with astrocytoma.

Methods. The study was conducted using whole blood and plasma of patients with astrocytoma (n = 54) who were taking a treatment course at LUHS hospital in Neurosurgery Clinic. Patients of LUHS hospital Clinics of Eye Disease were used as a control group (n = 66). The concentration of trace elements was determined by inductively coupled plasma mass spectrometry; metallothionein concentration was measured spectrophotometrically, according to the reaction product formed with an Ellman's reagent.

Results. Our results have shown that before a surgery the concentrations of Cu and Se in the blood of astrocytoma patients were significantly (by 20% and 16% respectively) lower, than their concentrations in the blood of control group, while the preoperative concentration of cadmium was significantly (by 83%) higher than that of a control group blood. After the surgery, levels of Cu and Se remained statistically significantly lower (20% and 21%, respectively) than in the control group. Meanwhile, the postoperative Cd concentration, although remained significantly higher (17%) than in the control group ($p<0.05$), was also significantly lower than the preoperative concentration of this element ($p<0.05$). Levels of Zn in the blood of patients with astrocytoma both before and after surgery remained at the control level. Compared with the control group, metallothionein levels in the blood of astrocytoma patients were significantly increased both preoperatively and postoperatively (52% and 31%, respectively), however 21% decrease in metallothionein after the surgery (compared to preoperative metallothionein level) also was statistically significant.

Conclusion. Although no links between zinc and metallothionein levels or astrocytoma incidence has been established, it has been observed that blood levels of copper and selenium are reduced in patients with astrocytoma. A direct positive correlation was observed between the change in cadmium concentration before and after surgery and the change in metallothionein concentration at the same time, - the decrease in cadmium coincides with the decrease in metallothioneins.

P3. miR-10a-3p AS A POTENTIAL BIOMARKER FOR NSCLC PATIENTS' SURVIVAL

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Introduction. Lung cancer is the most currently diagnosed cancer type among men and women and the most common cause of death from cancer worldwide. Poor lung cancer patients' outcomes and survival rates demand the discovery of new biomarkers for the specific, significant, and non-invasive detection of non-small cell lung cancer (NSCLC) progression. Determination of cell lines' relationship to genomic changes in tumors could be valuable for functional and therapeutic discoveries. Human cell line models with different aggressiveness status may play an important role in the investigation of NSCLC. In addition, miRNAs expression patterns that are reliable in predicting NSCLC patients' survival rates are considered to be highly promising cancer biomarkers. The aim of present study is to investigate the potential of miRNA expression as biomarkers in NSCLC.

Materials and Methods. The study was performed using different aggressiveness HBEC3, NCI-H1299, A549, Calu-1, and NCI-H23 2D cell lines 3D cultures. Total RNA from all cell lines after 6 days of growth was extracted using miRNeasy Mini Kit according to the manufacturer's instructions. Small RNA libraries were synthesized using QIAseq miRNA Library Kit for Illumina® NGS systems. The expression level of selected miRNAs was analyzed in 50 paired tissue specimens and plasma samples obtained from NSCLC patients who underwent surgical treatment at the Department of Thoracic Surgery and Oncology of the National Cancer Institute. miRNAs expression levels were determined relative to the expression of housekeeping gene RNU6B. Each sample was examined in triplicate and calculated following the 2-ΔΔCt method. The statistical analysis was performed using the data analysis software package SPSS 20.0. The overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan-Meier analysis and log-rank test. Univariate analysis and multivariate Cox regression analysis was performed to detect independent factors significantly determining PFS or OS. Significance threshold level was set at $p<0.05$.

Results. After the Next generation sequencing analysis, 8 miRNAs (let-7d-3p, miR-10a-3p, miR-28-3p, miR-28-5p, miR-100-3p, miR-182-5p, miR-190a-5p, miR-340-5p) were identified as common to cell lines of higher aggressiveness level. Following expression analysis of these 8 miRNAs was performed in NSCLC patient's tumor and plasma samples. The analysis of the obtained data demonstrates that patients with high miR-10a-3p ($p=0.008$) and miR-190a-5p ($p=0.023$) expression in plasma had longer PFS rates than those with low miRNA expression. Moreover, the prognostic factors for OS and PFS were subsequently analyzed using univariate and multivariate Cox regression analysis in NSCLC tissue and plasma samples. Our results suggest that the expression of miR-10a-3p (HR: 2.1, 95% CI: 1.2-3.7, $p=0.009$) in plasma is the statistically significant variable for NSCLC patients' progression-free survival prognosis.

Conclusions. Our findings indicate that the expression level of circulating miR-10a-3p has the potential as a non-invasive biomarker to prognose the outcome of NSCLC patients.

P4. THE USE OF miRNAS IN PREDICTING RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER (TNBC)

Domas Stitilis, Kristijonas Veličkevičius, Linas Kunigėnas, Monika Drobnienė, Eglė Strainienė and Kęstutis Sužiedėlis.

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Breast cancer is the most common malignancy in women. Triple-negative breast cancer (TNBC) is an aggressive subtype that accounts for 10-15% of all cases, and the disease is associated with high invasiveness, metastasis, and susceptibility to relapse. Due to the absence of estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (HER2) receptors in TNBC, endocrine and molecular targeted therapies are ineffective. Neoadjuvant chemotherapy, the primary treatment option, is only effective in ~50% of patients.

MicroRNAs (miRNAs) are small non-coding RNA molecules whose dysregulation can alter the expression of specific genes, affecting cancer pathogenesis. This research aimed to identify whether microRNAs can be used as noninvasive biomarkers to predict a patient's response to chemotherapy. First, bioinformatic case study analysis of the cancer genome atlas (TCGA) datasets revealed 195 differentially expressed miRNAs targeting 57 genes linked to the platinum drug resistance pathway. Analysis of patient survival data showed 13 of those to be directly related to patient survival rate. Subsequently, we selected four different miRNAs for quantitative reverse transcription PCR verification in specimens from patients with TNBC undergoing neoadjuvant chemotherapy. Finally, we conducted a statistical analysis to evaluate the candidate miRNAs' relation with the overall or progression-free survival of TNBC patients.

P5. EFFECT OF NEOADJUVANT CHEMORADIOTHERAPY ON PARP GENE EXPRESSION IN RECTAL CANCER TUMOR TISSUE

Kristijonas Veličkevičius[1,2], Rimvilė Prokarenkaitė[1,2], Linas Kunigėnas[1,2], Vaidotas Stankevičius[3], Audrius Dulskas[1,4], Kęstutis Sužiedėlis[1,2]

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Colorectal cancer is a prevalent malignancy, ranking as the second leading cause of cancer-related deaths globally. Rectal cancers account for approximately one-third of all colorectal cancer cases. Neoadjuvant chemoradiotherapy (CRT) followed by surgery is a common treatment approach for this disease. However, resistance to therapy and damage to adjacent normal tissues limit its effectiveness. Since CRT induces DNA damage in cancer cells, leading to the activation of DNA damage repair, inhibition of this process could likely increase the efficacy of the therapy. Therefore, poly (ADP-ribose) polymerase (PARP) family proteins represent a potential therapeutic target for the treatment of cancer. These proteins regulate various cellular processes, such as DNA repair and transcription, by catalyzing ADP-ribosylation reactions and through catalysis-independent mechanisms.

Previous studies have demonstrated a significant increase in the expression of a few PARP genes in colorectal cancer cells upon exposure to fractionated dose ionizing radiation. To investigate if the expression of these genes is altered in vivo, this study was conducted on clinical rectal cancer specimens obtained from a cohort of 67 patients. The findings suggest that the expression of the examined PARP genes is significantly elevated exclusively in tumor tissues following chemoradiotherapy, with no observed alterations in expression in adjacent normal tissues. Further analysis including the patients' clinical and demographic characteristics did not reveal statistically significant differences between the groups. Subsequently, survival and progression analyses were performed, which revealed an association between changes in one specific PARP gene expression and survival. Taken together, the study results validate the findings of the in vitro studies and suggest that PARP proteins play a vital role in the response mechanism to chemoradiotherapy in rectal cancer cells.

P6. GENE EXPRESSION OF HISTONE METHYLATION-MODIFYING GENES IN PROSTATE CANCER

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Prostate adenocarcinoma (PCa) is one of the leading causes of cancer-associated death in men and the most dominant male malignancy worldwide. Despite the innovative molecular tests, which are already available on the market, the critical need for more accurate prognostic tools is not met, yet. In the last decade, epigenetic code alterations (e.g., DNA modifications, histone methylation, HM) have been shown to be of great importance during prostate carcinogenesis, making them a potential source of new diagnostic and prognostic biomarkers for PCa. Therefore, analysis of the associated regulatory genes of these processes might provide new insights to discover cancer biomarkers for early detection, cancer prognosis and risk evaluation.

In the present study, expression of HM-associated genes was analysed in two independent cohorts aiming to evaluate its potential applicability as biomarkers. Next-generation sequencing data of 55 HM genes was obtained for the analysis in the PRAD cohort (N = 333) from The Cancer Genome Atlas (TCGA), while quantitative PCR-based methods were used for the quantification of the selected genes in the Lithuanian cohort (N = 121). In the TCGA cohort, 14 of 23 lysine demethylase (KDM) and 26 of 32 lysine methyltransferase (KMT) genes showed differential expression in PCa compared to noncancerous prostate tissues (NPT). Differential expression of KMT1E, KDM5A and KMT5A was observed between PCa and NPT (all $P < 0.050$), with the diagnostic specificity for PCa reaching up to 87.2 % ($P < 0.001$). Several associations were identified between the genes' expression and tumor stage, differentiation grade or TMPRSS2-ERG fusion status. Lower expression of KDM4B, KDM5A, KMT1E and KMT5A was associated with biochemical disease recurrence (BCR; all $P < 0.050$). Moreover, BCR-associated downregulation of KDM5A and KMT1E was also observed in the subgroups of early-stage (≤pT2) or well differentiated (Gleason grade group GG2) tumors, as well as in tissues expressing TMPRSS2-ERG (all $P < 0.050$). Cox regression analysis confirmed the negative effect of KDM5A and KMT1E downregulation on BCR-free survival in the Lithuanian cohort and supporting results were obtained from the TCGA PRAD analysis. Multivariate models of various combinations of the genes only were also prognostic for time-to-BCR. Besides, KDM5A expression with pT and GG could predict BCR-free survival even better than the clinicopathological parameters alone.

In conclusion, KDM5A and KMT1E expression levels in PCa emerged as independent predictors of progression-free survival, revealing their potential clinical application as prognostic PCa biomarkers. However, further validation is required in independent cohorts with patients' long-term follow-up data.

P7. ANTIOXIDANT AND ANTICANCER EFFECTS OF PROPOLIS COMPOUND CAFFEIC ACID PHENETHYL ESTER

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Propolis, a natural substance, produced from plants by honeybees, contains various chemical compounds, it possesses various biological properties, including anticancer, antibiotic, and antioxidant effects. Therefore, interest in propolis and its active compounds is constantly growing. One such compound, caffeic acid phenethyl ester (CAPE), stands out for its potent biological activities, such as its anticancer, antioxidant, and immunoregulatory properties. The occurrence of oxidative stress in cancer cells has been extensively studied and documented. It is reasonable to believe that antioxidants, such as CAPE or others, can play a significant role in reducing the incidence and progression of cancer. However, no data exists about CAPE's effect on kidney cancer development, the 14th most common cancer worldwide. Therefore, our study investigates the antioxidant and anticancer effects of CAPE, applying in vitro antioxidant activity and Caki-2 (renal cell carcinoma) models.

The radical scavenging activity of CAPE was evaluated by ABTS and DPPH methods, while reductive activity was determined by FRAP and CUPRAC methods, calculating TEAC values that indicate the antioxidant activity of the tested phenolic compound compared to Trolox. Caki-2 cells were incubated in McCoy's 5A medium supplemented with 10% fetal bovine serum at 37 °C in 5% CO2 humidity. The effect of CAPE on Caki-2 cell viability was evaluated by MTT assay at concentrations ranging from 0.25–35 μM. Then cells were treated with CAPE (IC50=7 μM) for 24 hours. The mitochondrial functions in Caki-2 cells (without and after pretreatment with CAPE) were measured using a high-resolution respirometry system Oxygraph-2k, glutamate/malate, and succinate were used as substrates.

Our results revealed that CAPE has high antioxidant activity, and reductive properties are more potent than antiradical properties, as the ratio between TEACABTS=1.10±0.04 and TEACFRAP=1.99±0.08, and TEACDPPH=1.65±0.09 and TEACCUPRAC=2.93±0.08 is less than 1. Pretreatment of Caki-2 cells with CAPE dose-dependently 22–95% reduced cell viability. Moreover, 7 μM CAPE inhibited ADP-dependent mitochondrial respiration rate in Caci-2 cells with glutamate/malate as substrates and reduced uncoupled respiration (with dinitrophenol) rate.

In conclusion, our study shows that CAPE possesses antioxidant activity, suppresses Caci-2 cell viability, and affects mitochondria function. These findings contribute novel insights into the potential therapeutic implications of CAPE, particularly in pharmacological investigations targeting kidney cancer. This research expands our understanding of the potential health benefits associated with CAPE.

P8. FIREWEED (*CHAMERION ANGUSTIFOLIUM L.*) LEAF EXTRACT SUPPRESSES MITOCHONDRIAL FUNCTION AND CELL VIABILITY IN COLORECTAL CACO-2 CANCER CELLS

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Extracts from fireweed leaves are rich in various biologically active compounds such as flavonoids, ellagitannins, phenolic acids, triterpenoids and others, and display anti-inflammatory, antioxidant, imunomodulatory and anti-proliferative activity [1]. In this study the effect of aqueous fireweed (Chamerion angustifolium L.) extract from fermented (F) and non-fermented (NF) leaves on colorectal adenocarcinoma cancer (Caco-2) cell viability and mitochondrial function was investigated.

Caco-2 cells were incubated for 24 h in RPMI 1640 medium supplemented with 10% of fetal bovine serum and 1% penicillin/streptomycin at 37 °C in 5% CO2 humidity. Then cells were treated by fireweed (fermented (F) and non-fermented (NF)) leaves aqueous extracts for 48 hours (the solid-phase fermentation of leaves at 30 °C for 48 h). Mitochondrial function was measured using an Oxygraph-2k at 37 °C with glutamate/malate and succinate [2].

We revealed, that the viability of Caco-2 cells decreased by 53%,76%, 89%, 91%, $p<0.05$, after pretreatment of cells with 1.0-3 mg/ml fireweed extract from NF leaves. The viability of Caco-2 cells decreased by 20%; 50%, 72% and 75% after pretreatment of cells with fireweed extract from F leaves. The mitochondrial ADP-dependent respiration rate after pre-treatment with fireweed (F and NF) extract decreased by 40% and 52% (glutamate/malate) and by 27% and 35% (succinate), respectively. The leak respiration rate increased by 62% and 73%, $p<0.05$, showing the damage of inner mitochondrial membrane. The cytochrome c effect was also slightly increased after pre-treatment of cells with fireweed extract from NF leaves showing the damage of mitochondrial outer membrane.

In conclusion, fireweed leaf aqueous extract (from both, non-fermented and fermented leaves) diminishes significantly the cell viability and mitochondrial function in Caco-2 cells and might be promissing therapeutic agent in cancer cells.

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P9. DOES POSTTRANSCRIPTIONAL m6A lncRNA LANDSCAPE IN GLIOBLASTOMA MATTER?

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Background. Posttranscriptional modifications of RNAs - epitranscriptomics, emerged in recent years as a new layer to gene regulation in normal and cancer cell biology. N6-methyladenosine (m6A) is the most abundant RNA modification, which decorates mRNAs, miRNAs, long-non coding RNAs (lncRNAs), ribosomal, and spliceosome RNAs [1]. The m6A residues on lncRNAs were reported to be important for their expression and functions, such as RNA-RNA, RNA-protein interactions, and chromatin remodeling [2]. However, the profiles and distribution patterns of m6A across human tissues are poorly characterized. The study of m6A epitranscriptome in brain tumor glioma is a young one, as the first publication describing the involvement of m6A mRNA methylation in glioblastoma (GB) growth was published in 2017 [3]. However, up to now there is no evidence on lncRNA landscape impact on glioma pathogenesis. Here, our aim was to investigate lncRNA m6A distribution pattern and to calculate m6A level in glioblastomas, as compared to low grade gliomas, and evaluate clinical relevance of epitranscriptomic biomarkers. Methods. To understand the global distribution of m6A marks, we performed epitranscripotome-wide m6A lncRNA modification analysis in gliomas applying nanopore-based direct RNA-sequencing (dRNA-seq) technology (Oxford Nanopore Technologies). Analysis encompassed lncRNA transcripts marked by m6A within RRACH motifs in 17 GB (glioblastomas, grade 4), and 9 LGG (low grade gliomas, grade 2). Sequencing data were processed using "nf-core/nanoseq" (r 3.0.0) pipeline and m6A modification prediction was calculated with "m6Anet" (v-1.1.0) and Epinano (v-1.2.0) algorithms within 5-mer RRACH motifs. Results. Epitranscriptome-wide study revealed on average 132/1250 lncRNAs containing methylated adenines in GB, and 161/1007 lncRNAs in LGG. On average 15.84 % of all RRACH motifs in lncRNAs were modified in GB, while m6A frequency in LGG group reached 23.73 %. Unsupervised clustering incorporating all GB and LGG specimens 19 lncRNAs m6A methylation status categorized glioma patients into three different clusters (C1-C3) of which patients within clusters of lower levels of m6A modifications tend to survive shorter. Conclusions. Here we conclude, that lncRNAs are highly modified in LGG while multiple epi-marks found in low grade gliomas, are absent in GB tissues. Glioma m6A lncRNA based clustering revealed m6A marked transcripts which likely contribute to glioma pathology.

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P10. EXPRESSION ANALYSIS OF PROTEINS' AFFECTING ncRNA MODIFICATIONS IN GLIOMA

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In recent years, epigenetic modifications of non-coding RNAs (ncRNAs) have received increasing attention. Various new modifications are discovered, as well as the proteins that write, read, and erase them, so-called "writers" (enzymes that deposit modifications), "erasers" (enzymes that remove modifications) and "readers" (proteins that recognize and bind epigenetic modifications). Epigenetic regulation of genes involved in cell proliferation, survival, and differentiation is believed to be involved, at least in part, in the initiation, development, and malignancy of various types of tumors. Gliomas - quite common, malignant brain tumors with poor prognosis - are no exception. Numerous publications have discussed the role of N6-methyladenosine (m6A) and 5-methylcytosine (m5C) modifications and their regulatory proteins in mentioned brain tumors. However, data on various other modifications, e.g., pseudouridine (Ψ), N7-methylguanosine (m7G), 5-hydroxymethylcytosine (hm5C), 5-N1-methyladenosine (m1A) and others, as well as their regulatory proteins in gliomas are rarely found. Since the link between these modifications and the proteins that regulate them in other types of tumors have been at least partially described, the aim of this research was to investigate expression differences of the coding genes of various "writers", "readers" and "erasers", influencing ncRNA modifications in different malignancy grade glioma patients' tumor samples.

Several ncRNA modifications were selected for the analysis: pseudouridine (Ψ), N7-methylguanosine (m7G), 5-hydroxymethylcytosine (hm5C), 5-N1-methyladenosine (m1A). Twelve coding genes' of the proteins regulating these modifications – FTO, ALKBH3, ALKBH5, BUD23, METTL1, DKC1, TET1, TET2, TET3, TRMT6, YTHDF1, YTHDC1 - as well as two reference genes' - GAPDH and βActin - expression were evaluated using qRT-PCR method. The study group consisted of 54 patients, diagnosed with different malignancy grade astrocytoma. Results were also compared with reference human brain (RHB) samples.

In the majority of analyzed cases, gene expression was related to the degree of tumor malignancy and overall survival was significantly longer in astrocytoma patients with higher than median gene expression of analyzed genes.

P11. THE EFFECTS OF HUMAN α-LACTALBUMIN AND OLEIC ACID COMPLEX (HAMLET) ON MITOCHONDRIAL FUNCTION IN COLON CANCER CELLS

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Colon cancer is one of the most common malignant tumors in the world. There is evidence that human milk protein α-lactalbumin and oleic acid complex (HAMLET - English: Human Alpha-lactalbumin Made LEthal to Tumor cells) is a protein-lipid complex that can kill a wide range of tumour cells while leaving healthy, differentiated cells unaffected. [Svanborg et al. Advances in Cancer Research (2003)]. It was demonstrated that HAMLET can activate mitochondria-dependent apoptosis and might interfere with mitochondrial function.

The aim of the study was o evaluate the effects of HAMLET on mitochondrial functions in colon cancer cells.

Mitochondrial respiration rate was recorded in three different colon cancer cell lines (HCT-116, HT-29 and WiDr) by high-resolution respirometry system Oxygraph-2k (OROBOROS Instruments, Innsbruck, Austria). Cells were permeabilized by digitonin. Datlab 5 software (Oroboros Instruments) was used for real-time data acquisition and data analysis. Oxygen consumption was related to cell number (pmol/s/1 mln cells).

The effect of HAMLET (5 μM) on mitochondrial bioenergetics was evaluated by measuring mitochondrial respiration rate with glutamate/malate and succinate as substrates. Our result showed that HAMLET had no statistically significant effect on non-phosphorylating (V0) respiration rate (substrate glutamate/malate) in all cell lines. Mitochondrial State 3 (VADP) respiration rate was reduced by 13%, 17% in HCT-116 and HT-29 cell lines, respectively ($p<0.05$), but had no statistically significant effect on mitochondrial State 3 respiration rate in WiDr cell line.

The data with succinate as substrate showed that HAMLET decreased mitochondrial respiration (Vsucc) by 33 % and 17 % in HCT-116 and HT-29 cell lines respectively but had no statistically significant effect on mitochondrial respiration rate in WiDr cell line compared to untreated cells ($p<0.05$). HAMLET had no effect on the permeability of mitochondrial outer membrane in all cell line as the cytochrome c effect ratio after addition of cytochrome c in State 3 remained unchanged as compared to untreated group.

Thus, our study showed that HAMLET caused the decrease mitochondrial complexes I and II dependent respiration in colon cancer HCT-116 and HT-29 cell lines, but had no effect on the rate of mitochondrial respiration in WiDr cells. HAMLET had no effect on mitochondrial outer and inner membrane permeability in all cell lines.

P12. SYNERGISTIC EFFECT OF VIRAL RNA-MIMETICS WITH ALPHA-SYNUCLEIN

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Parkinson's disease (PD) is the second most common neurodegenerative disease defined by the gradual degeneration of dopaminergic (DA) neurons and the presence of intracellular α-synuclein (αSyn) aggregates known as Lewy bodies (LB). Multiple studies suggest that αSyn may also be involved in viral infections contributing to neurodegeneration. The pathological changes induced by the virus may trigger an inflammatory response and result from both increased αSyn expression and aggregation, as well as the direct activation of glial cells.

Still, there is a lack of information about the link between amyloidogenic proteins such αSyn, viral infection and their synergistic impact on inflammatory responses in the brain.

The aim of this study was to investigate the synergistic effect of viral RNA-mimetics – loxoribine (LOX) and polyinosinic–polycytidylic acid (Poly (I:C) with amyloidogenic protein – alpha-synuclein (αSyn) on primary rat neuronal-glial co-cultures.

In this study, we used primary neuronal-glial co-cultures prepared from the cerebellum of 5–7-day-old Wistar rats pups (both genders). For experiments culture cells were pre-incubated for 1 hour with RNA-mimetic LOX and Poly (I:C). Then αSyn, pre-aggregated for 6 h, was added and cultures were incubated for 72 h. The viability and number of neuronal and microglia cells were assessed by fluorescence microscopy.

We showed that LOX (1µg/ml) and Poly (I:C) (100 ng/ml) incubated with or without αSyn (10nM – 50nM) had no effect on CGC cell culture viability which remained above 95%. It was also found that various concentrations of αSyn did not affect neurons. In contrast, 6 h pre-aggregated αSyn (10nM – 50nM) incubation with viral Poly (I:C) decreased neuronal cell number and tend to increase the proliferation of microglia by both – LOX and Poly(I:C) compared to control group after 72 h incubation.

These results indicates a potential synergistic effect between αSyn and viral RNA-mimetics, in neuronal-glial co-cultures.

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P13. ANTIDIABETIC DRUG AS POTENT PROTECTION IN ISCHEMIC BRAIN INJURY

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Ischemic brain injury is among the leading causes of human deaths and disabilities. An acute reduction in cerebral blood flow results energy failure, glutamate release, ionic disbalance, oxidative stress, mitochondrial damage, neuroinflammation and cell death. Multiple attempts have been made to develop neuroprotective treatments by using glutamatergic activity inhibitors, ion channel modulators, free radical scavengers, anti-inflammatory agents, etc., but none of them has been approved as an efficient medicine for ischemic stroke treatment yet. Imeglimin is a novel oral antidiabetic drug approved for treatment of type 2 diabetes that among other potential effects also alters mitochondrial processes. To provide insight into the pharmacological properties of imeglimin we aimed to assess the effect of intraperitoneal injection (109 μg/kg) on ischemic brain injury in different age groups: young (2-3 months-old), middle-aged (10 months-old) and aged (24 months-old) rats, and to determine whether imeglimin directly affects brain mitochondrial functions.

Imeglimin treatment 24 h before in vitro global cerebral ischemia in acute brain slices reduced infarct size only in young and middle-aged rat groups. Elucidating the mechanism of neuroprotection, we found that direct addition of imeglimin to isolated mitochondria of 2-3 months-old and 10 months-old rat brains suppressed NADH-linked oxidative phosphorylation and enzymatic activity of respiratory chain complexes I. The opposite stimulating effect on complex II activity was observed within the same groups as previously mentioned. In the aged group (24 months) imeglimin had no effect in reducing cerebral infarct size or directly modulating mitochondrial respiratory and enzymatic functions.

In conclusion, we expanded knowledge about potential effects of imeglimin in the brain by demonstrating a direct stimulating effect on mitochondrial complex II activity and an age-dependent its beneficial effects on brain under in vitro ischemia.

P14. COMPARISON OF THE ENERGY METABOLISM OF LPS-STIMULATED MICROGLIA WITH THE EFFECTS OF ITACONIC ACID

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Background: Microglia are the resident immune cells of the central nervous system (CNS) and they play a key role in the adaptation of CNS during various pathological processes such as bacterial or viral infection, ischemic stroke, and neurodegenerative disease. One of the most upregulated genes in microglia upon pro-inflammatory stimulation is IRG1 which results in the synthesis of itaconate. Itaconate is regarded as an immunomodulatory metabolite that influences the production of pro-inflammatory cytokines as well as the energy metabolism in macrophages and microglia. Recent evidence suggests that the transition between activated macrophage phenotypes is dependent on energy metabolism as it was shown that the transition from the pro-inflammatory to the anti-inflammatory phenotype requires oxidative phosphorylation. Itaconate is known to inhibit oxidative phosphorylation via inhibition of succinate dehydrogenase (SDH), however its full role in the metabolic reprogramming of microglia or macrophages is not yet clear. The aim of this study was to investigate the changes in microglial energy metabolism upon pro-inflammatory stimulation and to compare them with the changes caused by itaconate.

Methods: Experiments were carried out in cultured BV-2 and primary cerebellar granule cells (CGC), or isolated rat brain mitochondria. BV-2 cells were stimulated with 500 ng/ml LPS for 24h. Rat brain mitochondria were isolated using the differential centrifugation method. Oxygen consumption rates were measured in permeabilized BV-2 or CGC, and isolated mitochondria using a polarographic oxygen sensor (Oroboros O2k Oxygraph). Glycolytic activity was evaluated by measuring the real-time proton flux in intact BV-2 cells using the O2k-pH ISE-Module. The activity of NADH:ubiquinone oxidoreductase (complex I) was evaluated in isolated mitochondria spectrophotometrically by measuring the rotenone-sensitive oxidation of NADH.

Results: LPS stimulation significantly decreased the ADP-stimulated mitochondrial respiration in BV-2 cells oxydizing pyruvate and malate or pyruvate, malate and succinate as substrates. Proton leak-associated respiration was not affected by LPS, and maximal uncoupled respiration was also decreased by LPS. LPS stimulation also significantly increased the glycolytic capacity of BV-2 cells. Since one of the hallmarks of activated macrophages is increased intracellular itaconate, we next measured its effect on glycolytic activity and mitochondrial respiration. Glycolytic activity was significantly decreased by itaconate treatment. We measured respiration in isolated mitochondria and permeabilized CGC cells and found that itaconate inhibits respiration both with pyruvate and malate, and with succinate as substrates. Since itaconate is only known to inhibit SDH, we measured the enzymatic activity of mitochondrial complex I in the presence/absence of itaconate and found that itaconate inhibits complex I in a non-competitive manner.

Conclusions: LPS causes a reduction in microglial mitochondrial respiration which is comparable to the effect of itaconate on isolated mitochondria and CGC cells. Itaconate inhibits the enzymatic activity of complex I which may contribute to the reduction in respiration. Glycolysis is enhanced in LPS stimulated microglia but is inhibited by itaconate.

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P15. AGE-RELATED VARIATIONS IN HYPOXIA INDUCED LESIONS TO WISTAR RAT BRAIN MITOCHONDRIA

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Risk of ischemic stroke increases by aging. Despite this, understanding of the molecular basis of age-associated differences in the pathogenesis of this disease is still limited. Therefore, in this study we compared 90 minutes hypoxia induced lesions to 7 days, 2-3, 7-10 and 24-26 months-old rats brain mitochondria respiration and mitochondrial permeability transition pore (mPTP) sensitivity to Ca2+ with particular focus on mitochondrial complex I.

Data indicate that hypoxia inhibited cortical mitochondrial respiration rate of animals from all age groups and reduced mitochondrial calcium retention capacity (CRC) in 2-3, 8-10- and 24-26-months animals' groups. Hypoxia inhibited cerebellar mitochondrial respiration in 7 days, 2-3 and 24 - 26-month-old groups, but had no effect on 8–10-month-old group. CRC after hypoxia were reduced in 8 - 10 and 24-26 months - old rats' cerebellum. Western blot analysis showed an age-related decrease of complex I protein NDUFS2 levels, while enzymatic activity of mitochondrial complex I was increasing with aging. Additionally, the size of the hypoxia-induced infarct zone was measured; all animals, regardless of age, had similar brain tissue damage after 90 minutes of hypoxia.

Altogether, these findings suggest that, despite age-related differences in mPTP sensitivity to Ca2+ or hypoxia-induced acute mitochondrial dysfunction; brain necrosis is not likely mediated by mPTP opening.

P16. EXOGENOUS MITOCHONDRIA INDUCE NEURONAL DEATH IN ISOLATED RAT BRAIN CELL CULTURES

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Mitochondria are endogenous cell organelles, however, recent studies have determined presence of exogenous mitochondria in cerebrospinal fluids of post-stroke patients and in animal stroke models. What is the role of exogenous mitochondria and whether they can cause activation of cell death in central nervous system under pathological conditions is not investigated yet. In our study, we aimed to investigate whether exogenous free mitochondria or synaptosomes with mitochondria affect viability of neuronal and glial cells.

We isolated mitochondria and synaptosome fractions from 2–3-months Wistar rats. Primary neuronal-glial cell cultures were prepared from 5–7-days Wistar rat cerebella. Cell cultures were treated with different mitochondrial and synaptosome concentrations for 48 hours. After incubation cell viability was evaluated with fluorescent microscope using Hoechst 33258 and Propidium Iodide. Isolectin GS IB4 AlexaFluor 488 was used to identify microglial cells. Synaptosomal mitochondrial respiration was measured by OROBOROS high-resolution respirometer using 0.5 mg protein/ml of fresh isolated synaptosomes.

We determined that isolated synaptosomes contained functional mitochondria which was indicated by the rate of ADP-stimulated oxygen consumption in the presence of pyruvate, malate and succinate as substrates. Incubation of neuronal-glial cell cultures with such synaptosomal preparations at 0.05 mg protein/ml concentration significantly decreased total neuronal number by 23 % comparing to control cultures, though there were no signs of necrotic neuron death at such concentration of synaptosomes. While exogenous free mitochondria significantly reduced neuronal number in cultures by 55% at 0.025 mg protein/ml concentration, such treatment also significantly increased numbers of necrotic neurons. Synaptosomes and free exogenous mitochondria did not affect total microglial numbers and viability.

In conclusion, our data show that synaptosomes containing functional mitochondria can induce neuronal loss without cell death, whereas exogenous mitochondria can cause neuronal necrosis and related neuronal loss in mixed neuronal-glial co-cultures.

P17. ANTIFUNGAL AND ANTIVIRAL EFFECTS OF ESSENTIAL OILS

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The spreading of drug-resistant microorganisms and viruses is a cause of great concern and a formidable threat to human health and well-being. To combat this threat, various alternative antimicrobial agents are being investigated. Lately, plant-based compounds and extracts are in focus due to the long history of plant usage in folk medicine for treating infectious diseases and their "generally recognized as safe" status. Of these, essential oils (EOs) show great potential for various applications as some have been shown to possess both antimicrobial and antioxidant, anticancer, anti-inflammatory, and other properties. During this work, the antifungal properties of EOs of 9 medicinal plants (Corymbia citriodora, Cymbopogon winterianus, Salvia sclarea, Citrus bergamia, Pinus sylvestris, Juniperus communis, Eucalyptus radiata, Rosmarinus officinalis, Laurus nobilis) were analyzed. Yeast cultures on solid growth media were either placed in direct contact with an EO or exposed to its vapor and the intensity of antifungal activities of EOs was evaluated by comparing the size of growth inhibition zones they produced. Corymbia citriodora and Cymbopogon winterianus EOs showed the strongest antifungal activities against potential pathogens Candida albicans and Rhodotorula mucilaginosa as well as yeast Saccharomyces cerevisiae. R. mucilaginosa seemed to be the most sensitive, while C. albicans – the most resistant to the effect of EOs. The effect of EO exposure on the morphology of yeast cells was also investigated using light microscopy. Some extent of increase of cytoplasmic granules and vacuole or entire cell shrinkage was observed in the case of most EOs tested. The antiviral properties of EOs were investigated using the S. cerevisiae LA-lus virus as a model system. The strength of the antiviral effect was evaluated by comparing the residual viral polymerase activity of control and EO-affected viral particles (VPs). This was accomplished by carrying out the synthesis of viral transcripts with radio-labeled nucleotides. Two antiviral modes of action of EOs were investigated – mixing VPs with EOs directly or exposing VPs to EO vapor. It was observed that some EOs seemed to inactivate VPs more effectively when applied in the vapor phase, rather than being in direct contact with the VP mixture. Pinus sylvestris and Juniperus communis EOs, both rich in α-pinene, showed the strongest antiviral properties of the 9 EOs tested. The obtained data on the antifungal and antiviral efficacy of EOs will further extend their application in human healthcare.

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P18. ENGINEERING OF CHIMERIC CARBONIC ANHYDRASES VA, VI, AND XII AS MODELS FOR TARGET ISOZYMES

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A family of human carbonic anhydrases (CA) consists of 12 catalytically active members that play role in cancer, glaucoma, altitude sickness, and other diseases development. Because of the high percentage of amino acid sequence homology, it is challenging to design isozyme-selective inhibitors. In addition, the production of CA isoforms VA, VI, and XII is complicated for large-scale drug candidate screening. Also, there is no crystal structure of CA VA available.

To solve the problems of low purification yields and difficult crystallization of CAs, as well as understand the mechanism of sulphonamide inhibitor recognition, we have engineered chimeric carbonic anhydrases that resemble the active sites of CA VA, CA VI, or CA XII. We used the off-target isozyme CA II as a core protein and mutated five to seven amino acids in the active site to that characteristic for the CA VA, CA VI, or CA XII. The detailed kinetic, thermodynamic and X-ray crystallographic analysis revealed that engineered chimeric CAs recognized and bound inhibitors with similar affinities and binding modes as target isoforms (CA VA, CA VI, and CA XII), but not as the off-target isoform (CA II). The chemical compounds that bound CA VA, CA VI, or CA XII more strongly than CA II, switched their preference and bound more strongly to the engineered chimeric carbonic anhydrases.

As a result, chimeric carbonic anhydrases VA, VI, and XII are good models of target CA isoforms – CA VA, CA VI, and CA XII. They have high purification yield, could be easily crystallized and recognizies most of the inhibitors as target isozymes. For those reasons, chimeric carbonic anhydrases could be used for large-scale inhibitor screening and X-ray crystallographic studies.

P19. ANTIBACTERIAL EFFICACY OF *HERMETIA ILLUCENS* LARVAE FAT

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The rising spread of antibiotic-resistant bacteria has encouraged researchers to explore alternative sources of antibacterial agents. In recent years, the use of insect-derived compounds has gained significant attention due to their diverse bioactive properties. The larvae of the black soldier fly (*Hermetia illucens*) are among the most lipid-rich insects compared to others. One of the advantages of cultivating these larvae is that they have gained recognition as a sustainable and efficient source of high-quality compounds (fat, protein, and chitin) due to their ability to convert organic waste into valuable biomass. Black soldier fly larvae (BSFL) have emerged as a novel and potentially beneficial resource in the field of antibacterial research. The BSFL fats are dominated by saturated (lauric, palmitic, etc.) and unsaturated (oleic) fatty acids. Fats of BSFL and their extracts exhibit antimicrobial activity against Bacteroides and Clostridium species, as well as inhibit antibiotic-resistant pathogenic bacteria, e.g., Klebsiella pneumoniae. The potential applications of black soldier fly fat as an antibacterial agent are vast. Its broad-spectrum activity combined with the use of eco-friendly materials, makes it a promising alternative to traditional antibiotics. The aim of this work was to produce BSFL fat extracts and evaluate their antibacterial properties. Several BSFL fat extracts were produced by applying acidic and alkaline extraction techniques. Microbiological methods were used to study the inhibitory effects of fat extracts on bacterial cultures. Minimum inhibitory and minimum biocidal concentrations of the tested materials were determined. It was demonstrated that extracts obtained by alkaline extraction often have stronger activity, but act against a narrower spectrum of strains than acidic extracts. Microorganisms with probiotic properties, such as Lactobacillus brevis and Lactobacillus plantarum, are resistant to BSFL fat extracts. On the contrary, opportunistic pathogens, such as Listeria monocytogenes and Salmonella typhimurium, could be effectively neutralized by BSFL fat extracts. The present study demonstrates the potential of Hermetia illucens larvae extracts as natural antibiotics.

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P20. PROTECTIVE EFFECT OF *PELARGONIUM SIDOIDES* ROOT EXTRACT ON PERIODONTAL TISSUE

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Periodontitis is a chronic inflammatory disease that affects the tissues supporting the teeth, including the gums, periodontal ligament, and alveolar bone. It is a prevalent condition, affecting more than 10% of the global population and is a leading cause of tooth loss worldwide. *Pelargonium sidoides* root extract (PSRE) contains biologically active compounds that have demonstrated the potential to modulate bacterial virulence, stimulate the host's immune responses, and reduce inflammation. These properties make PSRE an intriguing candidate for the development of new non-aggressive therapeutic agents for managing periodontal infections. Overall, the exploration of PSRE and its biologically active compounds represents an exciting avenue in the development of new therapeutic approaches for managing periodontitis. The aim of the study is to evaluate the changes in molecular markers MMP-3 and TIMP-1 in relation to clinical signs of periodontitis and assess the protective effect of Pelargonium sidoides root extract (PSRE).

Methods: Saliva samples from three groups were collected: a control group of 20 healthy individuals, a group of 20 patients with periodontitis, and a group of 20 periodontitis patients treated with PSRE.

Results: The concentration of MMP-3, a molecular marker associated with inflammation, was significantly higher in the saliva of the periodontitis patient group compared to the healthy control group. However, after treatment with PSRE, the MMP-3 level in the saliva of the patient group decreased and returned to a level similar to that of the control group. This indicates that PSRE may have a positive effect in reducing inflammation associated with periodontitis. The level of TIMP-1, which is a tissue inhibitor of matrix metalloproteinases and plays a role in regulating the extracellular matrix turnover was also measured. The TIMP-1 concentration in the saliva of periodontitis patients was significantly lower compared to healthy individuals. However, after treatment with PSRE, the TIMP-1 concentration in the patient group's saliva increased significantly, indicating that PSRE may help restore the levels of TIMP-1 and potentially promote tissue healing.

In conclusion, the study suggests that the biologically active compounds present in Pelargonium sidoides root extract (PSRE) have the ability to decrease inflammation and provide protection to periodontal tissue. These findings support the potential use of PSRE as a non-aggressive therapeutic agent for managing periodontal infections. However, it is important to note that further research is needed to validate these results and establish the effectiveness and safety of PSRE in larger patient populations.

P21. FUNCTIONALIZATION OF YEAST VIRUS AS NON-INFECTIOUS MODEL SYSTEM IN THE SEARCH FOR ANTIVIRAL AGENTS

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The COVID-19 pandemic, ushered on by the coronavirus 2 causing severe acute respiratory syndrome, struck humanity in 2020. The primary tool in the fight against the pandemic is now vaccination, which helps the body's immune system become able to recognize the cause and develop resistance against the coronavirus. Discovering different small-molecule drugs that inhibit certain non-structural proteins of SARS-CoV-2 and so diminish its negative consequences of the infection was the second strategy for combating the threat. Third, and also important, is searching for various antiviral agents with the capability to inactivate viruses on various surfaces and thus prevent further transmission among the populace. To test various potential antiviral compounds, a non-infectious model system was developed basing on the S. cerevisiae LA-lus virus. Purified S. cerevisiae LA-lus virus is composed of a capsid and own genomic dsRNA. Such a virus particle still possesses RNR synthesis due to Gag-Pol protein RNA dependent RNA polymerase activity. This allows to test not just the denaturing nature of antiviral agents, but also the possible inhibition of important functions of viral particles. By comparing the residual viral polymerase activity of control and agent-affected viral particles, the potency of the antiviral impact was assessed. The synthesis of viral transcripts using radiolabeled nucleotides was the method employed in order to accomplish the testing. Different disinfection formulations have been addresssed first. Unexpectedly, several essential oils seemed to exhibit profound antiviral capabilities.

P22. STUDIES ON INHIBITION OF EFFLUX OF ANTIFUNGALS IN CANDIDA SPP. YEASTS

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The number of dangerous and fatal infections caused by pathogenic Candida yeasts is increasing rapidly every year. One of the biggest problems encountered in treating these infections is the ability of cells to acquire resistance to known antifungal drugs. The most common mechanism of resistance is multidrug resistance pumps. Usually, in resistant strains activity of such pumps is upregulated, leading to increased antifungal drug efflux out of cells. Because of that, the concentrations of drugs within the cells are too low to inhibit the growth of pathogenic Candida yeasts. One possible way to increase the concentrations of antifungals inside the cells is an inhibition of the activity of efflux pumps, using alternative substrates, i.e. drugs used for the treatment of chronic diseases.

The aim of this study was to evaluate the ability of statins - drugs to lower the level of cholesterol in the blood – to inhibit the efflux of antifungals. The effects of statins alone or in combinations with antifungals were tested against different strains of C. glabrata and C. albicans, including strains mutant in ABC family efflux pumps – ΔCDR1 and ΔCDR2. Statins inhibited the growth of all strains of C. albicans and growth of ΔCDR1 and ΔCDR1/ΔCDR2 cells was inhibited stronger than the wild type and ΔCDR2. The effect of statins on the growth of C. glabrata yeasts was similar for all tested strains, and MIC90 was observed at the highest tested concentration of statins – 256 μg/ml. Statins and fluconazole showed synergistic effects only against the growth of wild type cells of C. albicans, while the combination of nystatin and statins did not show synergism. The effects of polyene class drug nystatin on Candida yeasts can be observed using real-time methods. This drug increases the amount of lipophilic anion PCB^- bound by C. albicans cell membranes, however this effect could not be registered on C. glabrata cell membranes. Nystatin inhibited cell respiration of the subcultures of both tested species.

These findings show that statins strengthen the effects of antifungal drugs and might be helpful in the treatment of Candida infections.

P23. EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM STEM CELLS ON BISPHOSPHONATE INDUCED DAMAGE IN OSTEOBLASTS

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Osteonecrosis of the jaw is a complication of long-term use of bisphosphonates which are used for the treatment of excessive bone resorption, hypercalcemia and combination of other risk factors, such as dental or periodontal diseases, glucocorticoid therapy, chemotherapy and smoking. Pathogenesis of the disease is still not completely clear and there is no single effective treatment. Therefore, it is important to perform scientific research in order to better understand bisphosphonate-related mechanisms for osteonecrosis of the jaw development. This research addresses the role of osteoblasts in the disease model, which has been significantly less studied compared to osteoclasts, as bisphosphonate-related osteonecrosis of the jaw is known to be associated with induction of apoptosis in osteoblasts. This may be an important marker of early detection of the processes of osteonecrosis. We also examine non-cellular regeneration therapy – extracellular vesicles (EVs) derived from adipose-derived mesenchymal stem cells. The viability of osteoblasts treatment with different concentrations of bisphosphonates and isolated EVs was investigated using PrestoBlueTM cell viability dye and fluorescence microscopy. It was found that bisphosphonates decrease viability of osteoblasts when compared to control in concentration and time time dependant manner. Meanwhile, co-treatment of cells with both bisphosphonates and EV samples showed an increase in osteoblast viability when compared to cells treated with bisphosphonates alone. In addition, studies of the energy profile of osteoblasts found that bisphosphonates markedly reduced mitochondrial and glycolytic activity. Meanwhile, EVs partially reduced mitochondrial damage and did not affect glycolysis. The obtained results prove that adipose-derived mesenchymal stem cells EVS have a positive effect on the process of bone regeneration and osteonecrosis prevention.

P24. ENZYMATIC ACTIVITY OF THE MICE ORGANS CATALASE UNDER THE INFLUENCE OF ST. JOHN'S WORT EXTRACT

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St. John's Wort, botanically known as *Hypericum perforatum L.,* is a widespread medical herb widely used in Lithuania and all over the world. The aerial parts of the plant are rich in antioxidants such as flavonoids, carotene, and vitamin C. Although the plant is well known for its anti-inflammatory, antidepressant, antimicrobial, antiviral and antioxidant effects, the studies for its capability to reduce oxidative stress in the brain are sparse. Though antioxidant properties of some phenolic compounds of St. John's Wort have been proved to be effective in vitro, absorption of these compounds from the gastrointestinal tract, the further metabolism, tissue uptake and possibility to pass blood-brain barrier remains unclear. There is also insufficient knowledge about the further fate of these compounds, depending on the dose and the mode of entry into the body.

The present study aimed to elucidate possible protective effects of *Hypericum perforatum L*. extract in alleviating the toxicity of aluminum on catalase (CAT) activity in mice brain and liver.

The experiments were done on BALB/c laboratory mice. The CAT activity in mice organs homogenates determined spectrophotometrically. Results expressed as the mean ± SEM.

Results showed that aluminum decreases CAT activity in the liver and brain of mice by 13.9% and 88.4%, respectively, compared to control group. In the liver *Hypericum perforatum L*. extract reduced CAT activity by 19.4% in comparison with control mice. The effect of St. John's wort extract on CAT activity in the liver of aluminum-treated mice was practically minimal. However, the effects of St. John's wort extract could be clearly seen in the brain. The extract decreases CAT activity by 74.5%, compared to control. But administration of St. John's wort extract to the aluminum group showed a large increase in CAT activity.

P25. RELATIONSHIP BETWEEN OXIDATIVE STRESS AND LEFT VENTRICLE MARKERS IN PATIENTS WITH CHRONIC HEART FAILURE

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With respect to structural and functional cardiac disorders, heart failure (HF) is divided into HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF). HFrEF is known to be the outcome of myocardial ischemia and infarction. Notably, HFpEF is associated with dysregulated metabolism and chronic hypertension, contributing to oxidative stress and myocardial dysfunction. Oxidative stress (an imbalance between the increased formation of reactive oxygen species (ROS) and the elimination or neutralization of ROS by an antioxidant system) plays an important role in the development of chronic HF and correlates with left ventricle dysfunction and hypertrophy in the failing heart. So, oxidative and antioxidative stress biomarker levels should be higher in HFpEF.

The aim was to compare oxidative stress (nitrotyrosine, dityrosine, protein carbonyl, malondialdehyde, oxidized HDL) and antioxidative biomarker (total plasma antioxidant capacity, catalase (CAT)) levels in the blood between HFrEF and HFpEF patient groups.

60 CHF patients were enrolled in the study and divided into two groups: HFpEF (<40%, n=27) and HFrEF (≥40%, n=33). Nitrotyrosine, dityrosine, protein carbonyl, malondialdehyde, oxidized high density lipoprotein cholesterol (HDL) and antioxidative readings' (total plasma antioxidant capacity, catalase) concentration was measured in the blood.

No statistically significant differences between the groups in the aforementioned variables were observed in the current study. Further, we divided the entire study sample into two groups based on the median values of malondialdehyde, protein carbonyl and oxidized HDL levels. No statistically significant differences of serum oxidative/antioxidative stress markers and left ventricular ejection fraction values were found between the groups of different malondialdehyde concentration (≤ 114.29 μg/L vs. > 114.29 μg/L), different protein carbonyl (≤ 259.95 U/mL vs. > 259.95 U/mL) and oxidized HDL levels (≤ 3.06 pg/L vs. > 3.06 pg/L). There was no correlation between the readings too.

Oxidative stress and antioxidative biomarker levels in the blood between HFrEF and HFpEF patient groups do not differ.

P26. NANOPORE SEQUENCING-BASED ANALYSIS OF MODIFIED DNA

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Every cell of a multicellular organism carries the same genome. Amazingly though, epigenetic regulation of gene expression drives cell differentiation towards various cell types, with dedicated functions and features. One of the fundamental epigenetic mechanisms is DNA modification. In the mammalian genome, cytosines in CpG dinucleotides are often methylated to give 5-methylcytosine (5mC) which is usually associated with silenced chromatin. This epigenetic mark is brought about by specific enzymes DNA methyltransferases. Further, through the action of oxygenase TET proteins, 5mC can be converted to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) which are further recognized by regulatory proteins [1]. Some lower eukaryotes use 6-methyladenine (6mA) as an epigenetic mark too. Moreover, the versatility of modified DNA bases is even wider in prokaryotes and viruses.

DNA modification repertoire is not limited to the natural entities though. Biotechnological approach has developed an assay called mTAG (methyltransferase-directed Transfer of Activated Groups) where engineered DNA methyltransferases can site-specifically introduce extended alkyl groups. The approach has been used for site-specific DNA labelling with a multitude of applications, ranging from optical genotyping to whole genome methylation profiling [2].

The rapidly advancing Nanopore Sequencing technology has a unique perspective of ability to detect modified bases directly in the native DNA sequence at a single-base resolution. We are taking advantage of using a simple MinIon device from Oxford Nanopore Technologies to detect modified bases, both natural and biotechnologically engineered, in model DNA sequences. Further, we are open for collaboration in analysis of any modified DNA relevant to your scientific interests.

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P27. ENZYMATIC N-DEMETHYLATION OF CAFFEINE MEDIATED BY NDM PROTEINS

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Caffeine and related methylxanthines are high-value chemicals used in pharmaceuticals as stimulants, diuretics, bronchodilators, and vasodilators, and for the treatment and/or prevention of axial myopia, glaucoma, and macular degeneration [1]. Most methylxanthines are difficult to synthesize chemically because of the difficulty in achieving selective alkylation of each nitrogen atom. Genes encoding caffeine-degrading bacterial enzymes can be very useful for diagnostic tests, and the production of valuable chemicals, medicines, animal feed, and biofuels. NdmABCDE enzymes catalyze the specific N-demethylation of alkylxanthines, which leaves a specific methyl group open to the chemical derivative. These enzymes could be useful to produce pharmaceutically useful modified xanthine analogs, which are currently synthesized by complex multi-step processes [2]. Thus, the study of caffeine degradation products is important from both a health and an environmental point of view.

In this work, the conditions for the expression of the ndmA and ndmD genes in the E. coli host were investigated and optimized. Respective recombinant NdmA and NdmD proteins involved in N-demethylation reaction of caffeine were purified using metal affinity chromatography, dialyzed, and concentrated. The activity of the NdmA and NdmD enzyme complex was determined by color reactions with L-tryptophan or Purpald reagents and by HPLC analysis.

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P28. INVESTIGATION OF THE RELAXANT EFFECTS OF 2-ACETYLFURAN AND 5-METHYLFURFURAL ON RAT PROSTATE AND AORTA

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A diverse range of furan ring containing compounds can be found in food, including herbs, and are utilized in the production of both synthetic and herbal medicines. While many furan-containing compounds are known to be toxic, certain compounds, such as antihypertensive drug prazosin, are employed in medical practice.

A recent in vivo study in pigs showed that the essential oil derived from the blossoming plant Elsholtzia ciliata possesses hypotensive properties [V. Zigmantaitė et.al., Pharmaceuticals (Basel), 2022]. It has been demonstrated that the dominant organic compounds in E.ciliata are dehydroelsholtzia ketone and elsholtzia ketone, both of which contain a furan ring in their structure. To investigate whether these compounds may be related with the relaxation effect on smooth muscles, the effects of well-known and commonly found furan ring-containing compounds, namely 2-Acetylfuran (2AF) and 5-Methylfurfural (5MFF), were examined on contraction of rat prostate and aorta.

Male Wistar rats weighing 600-800 g were used. Tension measurements were performed on isolated prostate strips of ventral lobe and intact aortic rings. To obtain a maximal contractile response, the preparations were exposed to Tyrode solution containing 100 mM KCl. The effects of 2AF and 5MFF were evaluated on preparations precontracted by phenylephrine. Data were fitted to the Hill equation.

The results showed that both 2AF and 5MFF caused a concentration-dependent reduction in phenylephrine-induced contraction of the prostate and aorta. The effect was more pronounced in the prostate. Both compounds almost completely inhibited the phenylephrine-induced contractions in both tissues. The half inhibition value (KB) of 2AF was found to be 0.65±0.12 µl/ml in the prostate (n=5) and 0.78±0.22 µl/ml in the aorta (n=5). The KB of 5MFF was 0.42±0.09 µl/ml in the prostate (n=4) and 0.96±0.19 µl/ml in the aorta (n=3, $p<0.05$ vs prostate). The observed relaxation effect of the tested compounds on phenylephrine-induced contraction suggests that they may affect α1-adrenergic receptors. To investigate this, the dose-response of phenylephrine in the presence of 2AF or 5MFF in the rat prostate was assessed. The results showed that the maximal contractions caused by phenylephrine in the presence of 2FMK or 5MFF (0.03 µl/ml) were similar to those in control conditions. However, both 2AF and 5MFF caused a rightward shift in the dose-response curves for phenylephrine. The concentrations of phenylephrine needed to induce half of the maximal effect (EC50) were significantly increased in the presence of 2AF or 5MFF, i.e., EC50 were 0.86±0.12 µM (n=5, $p<0.05$) and 0.69±0.23 µM (n=5, $p<0.05$), respectively, compared to control (0.24±0.07 µM, n=6).

In conclusion, the data show that 2AF and 5MFF had a relaxant effect on smooth muscles of the rat prostate and aorta. The effect was more pronounced in the prostate. The observed effects of 2AF and 5MFF may be related with their inhibitory action on α1-adrenergic receptors.

P29. CLONING AND ANALYSIS OF THE ANTIGEN-BINDING REGION OF HYBRIDOMA-DERIVED MONOCLONAL ANTIBODY SPECIFIC TO BETA-LACTAMASE

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Beta-lactamases (β-lactamases) are enzymes produced by bacteria that provide multidrug resistance (MDR) to beta-lactam antibiotics such as penicillins, cephalosporins, cephamycins, and others by breaking the structure of the antibiotic. Beta-lactam antibiotics are typically used to target a broad spectrum of gram-positive and gram-negative bacteria. Rapid and reliable detection of MDR bacteria infections is key when choosing a treatment. Antibody-based assays, such as immunochromatographic tests using monoclonal antibodies (MAbs) specific to β-lactamases, are promising diagnostic tools because they are fast, easy to use, and relatively cheap. To enhance the detection signal and improve the sensitivity of the system multimeric recombinant antibody can be applied. Displaying Fc-fused single-chain variable fragments (scFv) on virus-like particles (VLPs) is one way to increase the avidity of antibodies thus possibly improving the detection sensitivity. To generate recombinant antibodies first step is to determine the variable fragments to heavy (VH) and light (VL) chains of antigen-specific antibody. Here we describe the determination and analysis of the variable region sequences of hybridoma-derived MAb 7B6/F6 directed against β-lactamase ACT-14. The VH and VL fragments were cloned from total RNA, which was isolated from a stable hybridoma clone 7B6/F6, by reverse transcription and PCR amplification using a set of degenerative primers specific for the framework and constant regions of mouse IgG heavy and light chains. DNA sequencing was applied to identify the nucleotide sequence of antibody variable regions, followed by the analysis with various online data-bases and tools (IMGT/V-QUEST, IgBLAST, Geneious Biologics). We have eliminated the sequences with accidental frameshifts, stop codons, deletions or atypical amino acids and obtained plausible VL and VH sequences. Sequences of the complementarity determining regions (CDRs) of VL and VH were also defined, thus providing valuable information for the subsequent generation of recombinant antibodies. The identified VL and VH sequences were fused into two scFv variants (VL-VH and VH-VL) by overlaping PCR and will be further displayed on the surface of pseudotype (VLPs). In conclusion, our study provides new data on the anti-ACT-14 antibody by identifying its antigen-binding region, which is a first step in developing recombinant antibodies.

P30. YEAST VIRUS-LIKE PARTICLES AS A POTENTIAL NANODELIVERY SYSTEM

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The self-assembly of different viral proteins into virus-like particles (VLPs) has been widely studied during the past decade. These nanosized entities closely resemble the structure of the native virus but lack the genetic material and are considered as non-infectious. Using different methods, such as genetic manipulations, non-covalent interactions, chemical coupling of different compounds, or physical encapsulation, these particles can be encapsulated with various compounds. Different VLPs are used for specific delivery of anticancer agents, oligonucleotides and plasmids, various proteins, and nanoparticles.

The aim of this study was to produce Saccharomyces cerevisiae L-BC virus Gag VLPs in yeast cells and investigate their capability for encapsulation. L-BC Gag VLPs were synthesized in S. cerevisiae cells and purified in homogeneous form by sucrose cushion and CsCl gradient ultracentrifugation methods. Purified particles were analyzed using transmission electron microscopy (TEM) and dynamic light scattering (DLS) methods. TEM and DLS results confirmed that synthesized Gag protein successfully self-assembles into VLPs with a diameter of ~40 nm, which closely resembles the size of the native L-BC virus. After confirmation of self-assembly, these particles were encapsulated with antimicrobial peptide nisin and red fluorescent protein mCherry by different methods. For encapsulation of nisin, the passive diffusion method was used, and bacteria sensitivity to nisin-loaded VLPs confirmed the successful encapsulation. Fluorescent mCherry protein was encapsulated using the genetic engineering method by fusing it to Gag protein, and the incorporation of 120 mCherry molecules into Gag VLPs was confirmed by TEM and DLS methods. These results illustrate the potential of these particles to be developed into a nanodelivery system.

P31. RENAL ISCHEMIA AND REPERFUSION: CHANGES IN CARDIOLIPIN COMPOSITION

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Cardiolipin is a signature mitochondrial phospholipid that has a unique structure with two negatively charged phosphates and four polyunsaturated fatty acids. Cardiolipin interacts with many mitochondrial proteins including electron transport chain complexes, ATP synthase, adenine nucleotide translocase, and ensures their optimal activity. Cardiolipin also has a role in mitophagy and apoptosis, therefore, loss of cardiolipin induces mitochondrial dysfunction that is followed by cell and tissue death. Since cardiolipin interacts with electron transport chain complexes, a major source for reactive oxygen species (ROS), it becomes an easy target for peroxidation. Oxidative burst is a hallmark of reperfusion after ischemia (I/R), therefore, in these conditions cardiolipin is oxidized - its structure and amounts are modified, leading to mitochondrial and cellular damage. Heart and brain cardiolipins have been investigated quite thoroughly, however there is little to no information about what happens with cardiolipins during renal I/R.

Methods. We used adult male Wistar rats for an in vivo kidney I/R model. The rats were anesthetized, abdomen was opened, and ischemia was induced by clamping renal arteries for 30, 40 and 60 minutes. After that the clamps were removed and 30-minute reperfusion was induced. Kidney mitochondria were isolated by differential centrifugation and mitochondrial lipids were extracted using modified Folch's method. Human renal cell line (RPTEC/TERT1) was used for an in vitro hypoxia/reoxygenation model. To induce hypoxia the cells were kept inside a hypoxic chamber (2 % oxygen atmosphere) for 24 hours. For reoxygenation the cells were placed in regular incubation conditions with fresh medium change for 24 hours. Cell lipids were extracted using modified Folch's method. Lipid fractions from RPTEC cells and from rat kidney mitochondria were used for cardiolipin analysis which was performed using Acquity UPLC system coupled with Xevo TQD mass spectrometer.

Results. The dominant cardiolipin in rat kidneys was tetralinoleoyl cardiolipin (CL (18:2)4). The amount of it decreased by 30% after 30 min ischemia and 30 min reperfusion, however, it was restored to control levels after 60 min ischemia and 30 min reperfusion. The amounts of minor cardiolipin species CL (18:2)3(18:1) and (18:1)2(18:2)(16:1) decreased after 60 min ischemia and 30 min reperfusion by 15 % and 52 % respectively compared to control. We also investigated oxidation products of CL (18:2)4 – eight species containing up to 8 additional oxygen atoms were identified and their amounts were not significantly affected by ischemia/reperfusion. Similar results were observed in human renal cells: 24-hours hypoxia increased the dominant cardiolipin species CL (18:1)2(18:2)(16:1) by 45 % and reduced it after 24-hour reoxygenation by 76 % compared to control. The amount of (18:2)3(18:1) decreased after 24-hour hypoxia and 24-hour reoxygenation by 6 %. However, the amount of CL (18:2)4 decreased by 48 % after 24-hour hypoxia and increased over 3 folds after 24-hour reoxygenation compared to control.

We conclude that the amount of tetralinoleoyl cardiolipin was increased during reperfusion/reoxygenation, while the amounts of other minor cardiolipin species decreased. These results indicate that reperfusion/reoxygenation could initiate cardiolipin remodeling favored towards CL (18:2)4 formation.

P32. LIPOPOLYSACCHARIDES AND PROPERTIES OF THEIR AQUEOUS SOLUTIONS

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Lipopolysaccharide (LPS) is the major surface membrane component present in almost all Gram-negative bacteria and it is essential to both the form and function of the outer membrane. Lipopolysaccharides are known to be responsible for the production of various inflammatory cytokines produced by monocytes and macrophages. The role of LPS in Gram-negative bacterial diseases and its widespread application in various cell stimulation experiments provide a conceptual framework for studies targeting the isolation, purification, and detailed characterization of chemical interactions and structure of LPS. The main problem with LPS purification protocols is the contamination of the final product with proteins and nucleic acid, which hinders further quality assurance of the studies. Although, the structure and pathophysiological functions of LPS have been extensively studied, the studies of physical properties such as aggregation of LPS in aqueous solution are limited. According to other researches there is evidence that only endotoxin in aggregated structure is biologically active.

During the study, E.coli bacteria were grown in a bioreactor. The experiments have been performed in two different conditions: in the first group bacterial cell lysis was achieved by ultrasound applications, and in the second group methanol-chloroform extraction was executed. Further steps were identical for both groups, which included enzymatic purification, removal of phospholipids, dialysis and lyophilization. The composition of the samples was analyzed by SDS-PAGE electrophoresis and staining with Coomassie blue and silver dyes.

Quantity and degree of purification was determined in both groups. Concentration of LPS was identified by spectrophotometric analysis based on thiobarbituric acid reaction with keto-deoxy-d-manno-8-octanoic acid (KDO) and Limulus amebocyte lysate (LAL) test. Aggregation of LPS molecules, like any other micellization of an amphiphilic molecule, can only be characterized under specific conditions. The aim of this study was to determine critical micelle concentration (CMC) in water and phosphate buffer at different temperatures using conductometry.

Conductometrically determined critical micelle formation concentration of LPS in water at room temperature (21°C), 37°C and 50°C. Comparative analysis of CMC between two separate methods of LPS purification at different temperatures was performed.

P33. EFFECT OF CULTIVATION CONDITIONS AND ADDITIVES ON CELLULASE INDUCTION IN TRICHODERMA REESEI FUNGUS CULTURE

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Currently, most of the world's energy needs are met by fossil fuel resources. Several different technologies can be used to transition to more sustainable energy sources. Cellulose is the most abundant organic material in the biosphere. Therefore, the isolation and industrial use of cellulases could be used in the production of biofuels from organic cellulose sources.

Trichoderma reesei is a mesophilic filamentous fungus, known for its efficient production of cellulase enzymes, which are necessary for breaking down crystalline cellulose into glucose. T . reesei produces three main types of cellulolytic enzymes: endogluconases, cellobiohydrolases, and □-glucosidases. These enzymes work synergistically depolymerizing cellulose and converting it into glucose [1]. However, the close relationship between lignin and cellulosic biomass creates barriers to the enzymatic hydrolysis of cellulose into fermentable sugars. Hemicellulose and lignin prevent cellulosic enzymes from functioning, inhibiting the activity of the enzymes and reducing matrix pore size. Different pretreatment methods have specific effects on different lignocellulosic components. However, a significant number of them can create some kind of obstacles to the action of cellulases [2].

In our research, we utilized the RTS-1C bioreactor to cultivate T. reesei ATCC 26921 cells. The optimization of growth conditions was crucial during the cultivation of T. reesei preculture in the bioreactor. Our study demonstrated that different preculture conditions could influence cellulolytic enzyme activity. In some cases, an increase in spore amount in the inoculate resulted in improved outcomes, while in other instances, the opposite effect was observed. Initially, a ridge formed during the growth of T. reesei preculture in the bioreactor, however, after a few hours, the cell culture started to grow spontaneously without any external intervention. This ridge formation seemed to be a consequence of unfavorable growth conditions. Nonetheless, this culture exhibited the highest enzymatic activity when cellulases were induced using chemically untreated wheat straw.

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P34. INSIGHTS INTO ABSORPTION AND EMISSION SPECTRA OF INDOCYANINE GREEN IN THE HEART

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Introduction: Voltage-sensitive dyes (VSD) for many years have been successfully used for optical electrophysiological recordings of electrical activity in excitable cells and tissues in many labs. However, none of the widely used VSDs have been approved by the FDA for use in clinical applications. Indocyanine green (ICG) fluorescent dye has been approved by the FDA for use in medical diagnostics. We demonstrated that ICG dye has voltage-sensitive properties with a dual-component (fast and slow) response in the Langendorff-perfused rabbit heart (Biophys J, 2016;110:723-732). Here, we explore the different spectral properties of both components for analysis of the fractional change in ICG fluorescence in response to voltage changes and compare these results together with absorption spectra in homogenates of the heart tissue.

Methods: A standard glass microelectrode and optical mapping, using a near-infrared ICG fluorescent dye, were used to simultaneously record electrical action potential (AP) and optical signal (OS) in a Langendorff-perfused rabbit heart that was fully stopped. We used light from LEDs to obtain excitation; emission was measured using an EMCCD camera with band pass filters and a spectrometer. We applied a graphical model with Gaussian functions to construct and evaluate the individual emission curves and calculated the voltage-sensitive portion of each component of the ICG fluorescence in the rabbit heart. We used the homogenates of the rabbit heart tissue to record the absorption of light by ICG with spectrophotometer.

Results: The ICG OS has a dual-component (fast and slow) response to membrane potential changes that accurately tracks the time of electrical signal propagation but clearly differ in their kinetics and voltage-sensitive spectral properties. The voltage-sensitive fraction of ICG fluorescence was not high relative to the fluorescence of standard VSDs. However, after averaging, the good signal-to-noise ratio (> 20 dB) of ICG rendered its signal suitable for observing cardiac electrical activity. The results revealed that each isolated component (fast and slow) emanates from a unique ICG pool in a different environment within the cell membrane and that each component is also composed of two constituents (ICG-monomeric and ICG-aggregated) (Scientific Reports 2017;7(1):7983). The light absorption by ICG was concentration dependent. The absorption spectra shoved 2 peaks. Increasing ICG concentration shifted absorption curve to the left and reduced the right peak, meanwhile the left peak was increased. These results propose that the light absorption by ICG depends on monomeric and aggregated state of the dye molecules in the membrane.

Conclusions: For fast and slow component of ICG fluorescence signal we propose the existence of different voltage-sensitive mechanisms: (I) electrochromism and field induced reorientation for the fast component; and (II) field induced dye squeezing that amplifies intermolecular interactions, resulting in self-quenching of the dye fluorescence, for the slow component. The ICG absorption spectrum has two components, and we propose that the absorption is dependent on the aggregation state of the dye.

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