THE PROGRAMME AND ABSTRACT BOOK

Jędrzej Sniadecki's Memorial Conference
"Frontiers in Molecular Life Sciences" (JSMC-2023)
Vilnius, Lithuania, May 23-25, 2023

Gyvybės mokslų centras

Foreword

The 2nd Jędrzej Sniadecki's Memorial Conference (JSMC-2023) "Frontiers in Molecular Life Sciences" is organized jointly by the Polish Biochemical Society and the Lithuanian Biochemical Society on 23–25 May 2023 at Vilnius University. The JSMC-2023 will continue to commemorate the 250th anniversary of Sniadecki's birth following the 1st Belarusian-Lithuanian-Polish Jędrzej Sniadecki's Memorial Conference organized in Grodno on 8–9 November 2018. Aiming to strengthen the historical focus and to broaden the audience, the 2nd JSMC is organized in conjunction with the 13th International Conference on the History of Chemistry 2023 (13th ICHC).

Jędrzej Śniadecki was born in Żnin (Poland), in the then Polish–Lithuanian Commonwealth. He studied Medicine and Chemistry in Kraków and continued his studies later in Italy and Scotland. The professional life of Prof. Jędrzej Sniadecki was related to Vilnius University, where he worked as the first professor of Medicine and Chemistry and wrote his prominent scientific books on chemistry, biology, and medicine. Jędrzej Sniadecki was the first head of the Chemistry Department at Vilnius University (1797–1822).

The JSMC-2023 provides a great chance to interact, present the latest research achievements and develop a partnership for the members of Polish and Lithuanian biochemical societies. In this electronic issue, the conference programme and the collection of abstracts are provided for the JSMC-2023 participants.

CONFERENCE PROGRAMME

May 23 (Tuesday)		
Time	***Session details***	
15:30-17:00	Registration	*Maironio str. 11*
16:30-16:45	Unveiling of the memorial plaque to Jędzej Sniadecki.	*A.Volano str. 2, Ministry of Educations, Science and Sport*
17:00–7:15	Photo of all participants.	
17:15–7:30	Opening of the conference, welcome	
17:30-18:30	Key lecture. The history of chemistry in Lithuania. *Rimantas Vaitkus.*	
18:30-20:00	Welcome party	*Maironio str. 11*
May 24 (Wednesday)		
09:00-11:00	Registration	*Saulėtekio av. 7*
09:30-10:30	Plenary lecture. Science History Institute lecture: 20 years of scientific heritage in Europe: Trends and perspectives. *Marta C. Lourenço*	*Saulėtekio av. 3*
Session: Molecular foundations of life *Moderators: Saulius Serva, Marek Figlerowicz*		*LSC, Saulėtekio av. 7 Room R106*
10:50-11:00	Welcome at Life Sciences Center (LSC). *Edita Sužiedėlienė, Daumantas Matulis*	
11:00-11:30	Plenary lecture. Chemical epigenetics: from disease mechanisms to diagnostic tools. *Saulius Klimašauskas, Vilnius University*	
11:30-11:50	Type III CRISPR antiviral defense in bacteria. *Gintautas Tamulaitis, Vilnius University*	
11:50-12:10	Tackling tumor heterogeneity with scRNA-Seq *Linas Mažutis,, Vilnius University*	
12:10-12:30	Viruses of bloom-forming cyanobacteria - infection strategies. *Dariusz Dziga, Jagiellonian University, Krakow*	
12:30-12:45	Understanding and engineering plant-endophyte interactions for improved crop growth and resilience. *Danas Baniulis. Lithuanian Research Centre for Agriculture and Forestry*	
12:45-13:00	Plant stress response induced by seed exposure to cold plasma and electromagnetic field. *Vida Mildažienė, Vytautas Magnus University*	
13:00-14:00	***Lunch***	
Session: Biomedicine *Moderators: Vida Mildažienė, Joanna Dulińska-Litewka*		
14:00-14:30	Plenary lecture. Mitochondrial potassium channels and cardioprotection. *Adam Szewczyk, Nencki Institute of Experimental Biology*	
14:30-14:50	Neuronal sialylation in neural circuitry remodelling: insights from a mouse model and human surgical tissue. *Urtė Neniškytė, Vilnius University*	
14:50-15:10	Insight into cell communication: from apoptotic bodies down to exomeres. *Aistė Jekabsone, Lithuanian University of Health Sciences*	
15:10:15:30	One step closer to 3D bioprinted meniscus. *Jakub Rybka, Adam Mickiewicz University*	
15:30-16:00	***Coffee break***	
Session: Genetic diversity and Systems biology *Moderators: Česlovas Venclovas, Jozef Dulak*		
16:00-16:30	Plenary lecture. Mechanisms and processes generating genetic variation - genomics and archaeogenomics. *Marek Figlerowicz, Institute of Bioorganic Chemistry, Polish Academy of Sciences*	
16:30-16:50	Structural variation of baruol biosynthesis gene cluster in Arabidopsis accessions is associated with the climatic gradient. *Agnieszka Żmieńko, Institute of Bioorganic Chemistry Polish Academy of Sciences*	
16:50-17:10	Genomic characterization of invasive *Neisseria meningitidis* strains isolated in Lithuania between 2009-2019, *Milda Plečkaitytė, Vilnius University*	
17:10-17:30	SARS-CoV-2 genomic surveillance: the Lithuanian story. *Emilija Vasilūnaitė, Vilnius University*	
17:30	***Transfer to hotels***	

18:30-20:00	***Conference dinner*** *(for ticket holders only)*
May 25 (Thursday)	*LSC, Room R102*
Session Bioinformatics *Moderators: Justas Dapkūnas, Marek Figlerowicz*	
09:30-10:00	Plenary lecture. Application of bioinformatics to study diversity and distribution of bacterial DNA polymerases *Česlovas Venclovas, Vilnius University*
10:00-10:20	Sharing scientific data: the crystallography experience. *Saulius Gražulis, Vilnius University*
10:20-11:00	***Coffee break***
Session Biomedicine and Biotechnology *Moderators: Milda Plečkaitytė, Dariusz Dziga*	
11:00-11:30	Plenary lecture. Induced pluripotent stem cells and gene editing for disease modelling and experimental therapies. *Jozef Dulak, Jagiellonian University*
11:30-11:50	miRNA and gut microbiota signatures of vulnerability to food addiction in mice and humans. *Aurelijus Burokas, Vilnius Universiity*
11:50-12:10	The inhibitors and nanoparticles in cancer therapy. *Joanna Dulińska-Litewka, Jagiellonian University*
12:10-12:25	Design of isozyme-selective inhibitors via engineering of chimeric carbonic anhydrases. *Joana Smirnovienė, Vilnius Universiity*
12:25-12:40	Development of monoclonal antibodies against human endoplasmic reticulum proteins as a tool for their immunodetection and quantification. *Martynas Simanavičius, Vilnius University*
12:40-14:00	***Lunch***
14:00-15:30	Poster session
15:30-16:00	***Coffee break***
Closing session *Moderators: Saulius Serva, Adam Szewzcyk, Vida Mildažienė*	
16:00-16:10	Life Sciences Center – the story of success. *Daumantas Matulis, Life Sciences Center*
16:10-16:25	Advancing genome editing technologies: An EMBL - Vilnius University partnership. *Stephen Knox Jones, EMBL Partnership Institute*
16:25-17:30	Panel discussion about the collaboration opportunities between Polish and Lithuanian biochemical societies and research groups
17:30	**End of the conference**

ABSTRACTS OF ORAL PRESENTATIONS

1. MOLECULAR FOUNDATIONS OF LIFE

1.1. CHEMICAL EPIGENETICS: FROM DISEASE MECHANISMS TO DIAGNOSTIC TOOLS

Saulius Klimašauskas

Institute of Biotechnology, Life Sciences Center, Vilnius University

Epigenetic regulation in vertebrates involves chemical variation of one-carbon groups on cytosine residues in CpG dinucleotides by enzymatic production of 5-methylcytosine and its oxidized forms 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine [1]. Genomic distribution of these modified cytosines varies in different cell types, environmental conditions and disease states and is associated with many biological processes such as embryogenesis, establishment of cell identity, and development of pathological conditions, including cancer. We seek to develop novel efficient chemo-enzymatic tools for genome-wide profiling of epigenetic DNA modifications. Our major strategy is based on selective covalent labeling of unmethylated or modified CpG sites in the genome in vitro [2-5] or inside metabolically engineered mammalian cells in vivo [6] followed by analysis of the tagged sites using our recently established high-resolution mapping techniques [3,4]. These novel tools enable advanced epigenome studies and open ways to developing next generation diagnostics.

References:

1) Kriukienė et al. Chem. Soc. Rev., 2012, 41: 6916–6930.
2) Kriukienė et al. Nat. Commun. 2013, 4: 2190.
3) Staševskij et al. Mol. Cell, 2017, 65: 554–564.
4) Gibas et al. PLOS Biol., 2020, 18: e3000684.
5) Ličytė et al. Open Biol., 2022, 12: 210302.
6) Stankevičius et al. Mol. Cell, 2022, 82: 1053–1065."

1.2. TYPE III CRISPR ANTIVIRAL DEFENCE IN BACTERIA

Gintautas Tamulaitis

Vilnius University, Life Sciences Center, Institute of Biotechnology

Recent studies of prokaryotic antiphage defence mechanisms revealed a multitude of new cyclic nucleotide signalling molecules that play a crucial role in switching infected cells into an antiviral state. Defence pathways including type III CRISPR, CBASS, PYCSAR and Thoeris use cyclic nucleotides as second messengers to activate a diverse range of effector proteins [1]. These effectors typically degrade or disrupt key cellular components such as nucleic acids, membranes or metabolites, slowing down viral replication. To combat infection type III CRISPR system combines transcription-dependent DNA degradation by crRNA guided ribonucleoprotein (RNP) complex [2] with the cyclic oligoadenylates (cAn)-dependent immunity pathway [3]. In response to the viral RNA binding, the RNP complex synthesizes cAn molecules of various ring size (n=3–6). We demonstrated that cA4 or cA6 acts as signalling molecule that binds CARF domain of stand-alone Csm6 protein to activate its effector HEPN domain for nonspecific ribonucleolytic activity [3]. Bioinformatic analysis revealed that the sensor CARF or SAVED domain are found fused with different enzymatic effector domains [4]. I will present some examples of the molecular mechanisms by which type III CRISPR accessory proteins confer protection against viruses in bacteria.

References:

1) Athukoralage, J., White, M.F. (2022) Cyclic Nucleotide Signaling in Phage Defense and Counter-Defense. Annual Reviews Virology, 9(1), p.451-468.
2) Tamulaitis, G., Venclovas, Č, Siksnys, V. (2017) Type III CRISPR-Cas immunity: major differences brushed aside. Trends in Microbiology, 25(1): 49–61.
3) Kazlauskiene, M., Kostiuk, G., Venclovas, Č., Tamulaitis, G. and Siksnys, V. (2017) A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. Science, 357(6351), p. 605-609.
4) Makarova, K.S., Timinskas, A., Wolf, Y.I., Gussow, A.B., Siksnys, V., Venclovas, Č. and Koonin, E.V. (2020). Evolutionary and functional classification of the CARF domain superfamily, key sensors in prokaryotic antivirus defense. Nucleic acids research, 48(16), p. 8828-8847.

1.3. TACKLING TUMOR HETEROGENEITY WITH scRNA-SEQ

Linas Mazutis

Institute of Biotechnology Vilnius University

Tumors are intrinsically heterogeneous and are composed of different cell types. Deciphering this heterogeneity might be challenging, yet it is critical for our fundamental understanding of tumorigenesis. In this talk I will present how single-cell RNA-Seq helps us to unravel the different cell states that exist within tumors and uncover new biological mechanisms that drive tumor survival and metastasis.

1.4. VIRUSES OF BLOOM-FORMING CYANOBACTERIA - INFECTION STRATEGIES

Dariusz Dziga[1], Adam Antosiak[1], Antonia Łobodzińska[1], Barbara Klimczak[1], Przemysław Malec[2], Nada Tokodi[1,3], Sarit Avrani[4], Sigitas Sulcius[5]

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[3] *Department of Biology and Ecology, University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia*
[4] *Department of Evolutionary and Environmental Biology, University of Haifa*
[5] *Laboratory of Algology and Microbial Ecology, Nature Research Centre, Vilnius, Lithuania*

There is growing interest in the investigation of how cyanophages impact the dynamics of cyanobacterial populations. Understanding the strategies employed by viruses to ensure their effective multiplication is of crucial importance. However, only few studies have provided information on the mechanisms of cyanophage infection in bloom-forming freshwater cyanobacteria. Therefore, experimental biochemical and physiological analyses are a major challenge in order to deepen current understanding of the strategies employed by different cyanophages. In collaboration with Nature Research Centre (Vilnius) and University of Haifa we have initiated a detailed characterisation of cyanobacterial physiology in the course of infection. Three viral-host models have been investigated: cyanophages infecting freshwaters Aphanizomenon flos-aquae, Microcystis aeruginosa and Raphidiopsis raciborskii. The current research is focused on: (i) the susceptibility of different strains and species to CL131, Ma-LMM01 and fR cyanophages and the course of infection, (2) the energy and carbon flow during viral infection with special emphasis on photosynthesis efficiency, (3) the proteome modification of the infected strains. Our observations indicate that the analysed freshwater cyanophages employ different mechanisms to control biochemical pathways of the host and to redirect the energy and carbon flow to viral demand.

1.5. UNDERSTANDING AND ENGINEERING PLANT-ENDOPHYTE INTERACTIONS FOR IMPROVED CROP GROWTH AND RESILIENCE

Danas Baniulis, Inga Tamošiūnė, Jurgita Vinskienė, Elena Andriūnaitė, Rytis Rugienius

Lithuanian Research Centre for Agriculture and Forestry

Detrimental effects of the agricultural practices on agro-ecosystems as well as the effect on human health, emphasize a need for environmentally benign approaches to maintain sustainable agricultural production. Plant microbiome engineering-based innovations have a potential application for environmentally friendly agriculture practices. During the last decade, application of high-throughput DNA sequencing technologies largely contributed to the understanding of plant-associated microbial diversity, and it opened up possibility for breeding or cultivation-based engineering of the plant microbiome. However, deeper understanding of plant and endophytic bacteria interactions is required to establish plant microbial communities capable to improve plant stress and disease resilience. Monitoring of gene expression and signaling using biochemical techniques enable phenotyping of plant-microbial interactions at a molecular level and are capable to provide insights into the functional basis of microbial community formation. Plant-microbial and microbial-microbial interaction knowledge-based design of microbial consortia could stimulate recovery of a balance within microbial communities that is linked to a healthy plant holobiont. The microbiome engineering approach could benefit the development of robust planting stock for variety of clonally propagated woody and herbaceous crops that are affected by degradation of microbial diversity due to application of in vitro techniques for planting stock propagation or germplasm preservation.

1.6. PLANT STRESS RESPONSE INDUCED BY SEED EXPOSURE TO COLD PLASMA AND ELECTROMAGNETIC FIELD

Vida Mildaziene

Vytautas Magnus University

The potential of seed irradiation with low temperature plasmas or cold plasmas (CP) and electromagnetic field (EMF) for increasing agricultural production is under intensive investigation. Short term treatments induce significant, complex and persistent changes in plant metabolism. Upregulated photosynthesis results in better plant growth, stimulated secondary metabolism leads to better plant establishment, fitness and stress resistance.

The accumulated body of evidence indicates the high complexity of seed response to CP and EMF treatment on the molecular level. The most important novel findings on such response induced in dry seeds are changes in EPR signal, DNA methylation, balance of phytohormones, expression of genes and proteins, enzyme activities, modified seed microbiome. CP-induced changes in ROS production were reported in the germinating seeds. The events of seed response to the CP stress signal are further developed in the growing plant as multiple interrelated changes in gene expression, due to DNA methylation, changes in protein expression, enzyme activities (including enzymes of photosynthetic system and enzymes of secondary metabolism). Modulation of secondary metabolism that is followed by an increased plant fitness and resistance to abiotic stress, as well as modified plant communication with microorganisms - both pathogens and plant growth promoting microorganisms, e.g. N-fixating rhizobacteria.

2. BIOMEDICINE

2.1. MITOCHONDRIAL POTASSIUM CHANNELS AND CARDIOPROTECTION

Adam Szewczyk

Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 02-093 Warsaw, Poland

Various potassium channels have been identified in mitochondria. There are eight potassium channels known to contribute to the potassium flux via inner mitochondrial membrane: ATP-regulated channel, calcium-regulated channels of large (large, intermediate and small conductance), voltage-regulated Kv1.3 and Kv7.4 channels, two-pore-domain TASK-3 channel and SLO2 channel. The primary function of these channels is regulation of the mitochondrial membrane potential. Additionally, mitochondrial potassium channels alter cellular respiration and reactive oxygen species synthesis. The focus of the presentation will be on cardioprotective role of mitochondrial potassium channels induced by drugs, kinases and gases such as carbon monoxide or hydrogen sulfide. Additionally, an interaction with respiratory chain of mitochondrial channels will be reviewed.

This study was supported by a grant 2019/34/A/NZ1/00352 from the National Science Centre, Poland

2.2. NEURONAL SIALYLATION IN NEURAL CIRCUITRY REMODELLING: INSIGHTS FROM A MOUSE MODEL AND HUMAN SURGICAL TISSUE

U. Neniskyte, U. Kuliesiute, R. Propokovicius, U. Kisieliute, G. Luksys, S. Rocka

Vilnius University

Neuronal glycocalyx contributes to neuronal synapse formation, neuron excitability as well as neuron-autonomous and microglia-dependent brain network remodelling. Specifically, the sialic acid, which terminates the glycans on neuronal surface, is required for appropriate brain development and function, while aberrant neuronal sialylation and/or desialylation leads to neuropathological conditions, such as epilepsy.

To investigate the sialylation in circuit remodelling, we used mouse brain cultures for biorthogonal metabolic labelling of de novo synthesized sialic acid. We modulated neuronal sialylation with chemical inhibitors of sialic acid synthesis and cleavage to test how it shapes neuronal network. Furthermore, we analysed surgically resected human brain tissue to characterise the composition of glycans in healthy and epileptic brain. Finally, we investigated the expression of genes and enzymatic activities that regulate sialylation during mouse brain development as well as healthy and epileptic human brain.

We demonstrated that sialylation of neurons is tightly regulated during the periods of high plasticity such as hippocampal development. Furthermore, we found aberrant sialidase activity and glycocalyx composition in human epilepsy samples, indicating the role of sialic acid in circuitry diseases. Our findings indicated that sialic acid synthesis, distribution and cleavage from neurons and their synapses is an important factor for neuronal network remodelling.

2.3. INSIGHT INTO CELL COMMUNICATION: FROM APOPTOTIC BODIES DOWN TO EXOMERES

Aiste Jekabsone, Deimantė Kulakauskienė, Deimantė Narauskaitė, Zbigniev Balion

Lithuanian University of Health Sciences

The extracellular vesicle and particle research field exploded with new data over the recent decade, erasing the barriers between the different extracellular vesicle classes and extending the size range of these intercellular communicators up and down from nearly cell-sized apoptotic blebs to several nanometer particles. Exosomes, microvesicles, migrasomes, oncosomes, mitovesicles, exomeres, chromatimeres are smurfing from cell to cell, travelling large distances, ignoring biological barriers and exchanging intracellular material all over the body, between the gut, the airway, the brain and the heart. In this talk, we will overview the role of different extracellular vesicles and particles in physiology and pathology, with a specific focus on vesicular communication between the airway and the brain during viral infections, vesicular crosstalk in the tumour microenvironment and beyond, involvement of extracellular vesicles in neurodegeneration and current trends in cell-derived vesicle application.

2.4. ONE STEP CLOSER TO 3D BIOPRINTED MENISCUS

Jakub D. Rybka

Center for Advanced Technology, Adam Mickiewicz University, Poznan

The field of regenerative medicine is growing rapidly and holds promise for restoring physiological functions through advanced tissue engineering techniques such as 3D bioprinting. Using an exogenous protein scaffold known as ECM, cells can be anchored and the structural, mechanical, and growth-stimulatory conditions of bioprinted constructs can be defined to mimic fibro-chondrocytic meniscal tissue. The ECM is highly biocompatible, exhibits low immunogenicity, and promotes chondrogenesis, making it a suitable candidate for bioprinting meniscus implants for therapeutic purposes.

We propose a novel method for formulating bioink using ECM extracted from porcine menisci, specifically designed for 3D bioprinting of meniscal implants. Our method is cost-effective and allows for scalable extraction of ECM from easily accessible slaughter material. The bioink produced exhibited good properties and supported the survival of hMSC-AT, suggesting its usefulness in tissue engineering. Further experimentation is needed to determine the long-term effect of the bioink on cell differentiation and to establish the superiority of the pre-culture method.

This work was supported by the National Center for Research and Development TECHMATSTRATEGIII/0027/2019-00 grant.

3. GENETIC DIVERSITY AND SYSTEMS BIOLOGY

3.1. MECHANISMS AND PROCESSES GENERATING GENETIC VARIATION - GENOMICS AND ARCHAEOGENOMICS

Marek Figlerowicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Biodiversity, at the level of individual species as well as at the inter-species level, is a phenomenon that has always fascinated people. Since the discovery of DNA and RNA, it has been becoming increasingly clear that diversity of living organisms is underlain by a wide spectrum of mechanisms and processes generating genetic variation. The latter causes that individuals representing the same species are different, and that each species can evolve, and thus, adapt to changing environmental conditions.

Unfortunately, for many years, studying the mechanisms that generate genetic variation was extremely difficult due to the lack of methods for determining the sequence of whole genomes or transcriptomes. Thus, the development of next generation sequencing technologies became a real breakthrough. Consequently, the genetic basis of both many biological phenomena and pathological processes can now be studied. It has also been shown that by studying genetic variation, one can learn about the past of individuals and whole populations. As a result, a new research field, called archeogenomics was established and in a short time, became one of the fastest growing branches of genomics. In contrast to traditional genomics, the major object of archeogenomic studies is not contemporary but ancient DNA.

3.2. STRUCTURAL VARIATION OF BARUOL BIOSYNTHESIS GENE CLUSTER IN ARABIDOPSIS ACCESSIONS IS ASSOCIATED WITH THE CLIMATIC GRADIENT

Małgorzata Marszałek-Zeńczak[1], Anastasiia Satyr[1], Paweł Wojciechowski[1,2], Krzysztof Brzeziński[1], Marek Figlerowicz[1], Agnieszka Żmieńko[1]

[1] *Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan, Poland;*
[2] *Poznan University of Technology, Faculty of Computing and Telecommunications, Institute of Computing Science, Poznan, Poland*

Metabolic gene clusters (MGCs) are formed by groups of neighbouring genes, which are together involved in a common biosynthetic pathway. In the model plant Arabidopsis thaliana, four MGCs have been described so far, all involved in the specialized triterpene biosynthesis. Among them, the arabidiol/baruol gene cluster, located on chromosome 4, is the largest and least characterized one. We studied gene copy number variation in this MGC in a population of over one thousand Arabidopsis accessions, with a combination of bioinformatic and experimental approaches. In nearly one-third of the population we discovered a chromosomal insertion, about 25 kb in size, containing divergent duplicates of two cluster genes: BARS1 (which encodes baruol synthase) and CYP705A2 (which encodes a cytochrome P450 oxidase). We named them BARS2 and CYP705A2a, respectively. We next showed that BARS2 encodes a novel oxidosqualene synthase. Accessions with the duplication more frequently originated from geographical locations with lower latitude and were associated with warmer climates, compared to the reference-like accessions. Moreover, they also presented different root growth dynamics (slower growth) and altered gene expression profiles within the cluster. Our findings indicate the potential role of arabidiol/baruol gene cluster structural variation in Arabidopsis adaptation to different climates and phenotypic diversity.

3.3. GENOMIC CHARACTERIZATION OF INVASIVE NEISSERIA MENINGITIDIS STRAINS ISOLATED IN LITHUANIA BETWEEN 2009-2019

Emilija Sereikaitė, Rūta Plepytė, Aurelija Petrutienė, Dovilė Stravinskienė, Indrė Kučinskaitė-Kodzė, Vilmantas Gėgžna, Aurelija Žvirblienė, Milda Plečkaitytė

Life Sciences Center, Vilnius University

Neisseria meningitidis causes invasive meningococcal disease (IMD), resulting in considerable mortality and long-term consequences. Lithuania had one of the highest incidence of IMD in Europe in the past twenty years; nevertheless, molecular typing of meningococcal isolates has not been carried out. This study characterized IMD isolates (n=294) from Lithuania from 2009 to 2019 by means of multilocus sequence typing (MLST) and identification of antigens FetA and PorA. The more recent (2017-2019) genogroup B isolates (n=60) were genotyped by studying vaccine-related antigens to evaluate their protection by the four-component (4CMenB) and two-component (MenB-Fhbp) vaccines using the genetic methods. Most (90.5%) of the isolates belonged to genogroup B. MLST determined that clonal complex 32 (74.02%) was predominant. Genogroup B strain P1.19,15: F4-28: ST-34 (cc32) accounted for 64.1% of IMD isolates. The general level of strain coverage by the 4MenB vaccine was 94.8% (CI 85.9-98.2%). The majority of genogroup B isolates (87.9%) were covered by a single vaccine antigen, most often Fhbp peptide variant 1 (84.5% of isolates). Altogether, 88.1% (CI 77.5-94.1) of isolates were predicted to be protected by the MenB-Fhbp vaccine. Ultimately, both serogroup B vaccines appear to offer protection against IMD in Lithuania.

3.4. SARS-COV-2 GENOMIC SURVEILLANCE: LITHUANIAN STORY

Emilija Vasiliūnaitė

Institute of Biotechnology, Life Sciences Center, Vilnius University

The first cases of COVID-19 in Lithuania were identified in February 2020. For the first year of the pandemic, several sequencing efforts were made in different institutions, resulting in over 1000 SARS-CoV-2 sequences published in GISAID. A year later – in February 2021 – a national sequencing programme was established. Under the supervision of the National Public Health Surveillance Laboratory (NPHSL), four Lithuanian institutions sequenced and published over 40,000 SARS-CoV-2 genomes throughout the heat of the pandemic. A sequencing capacity of around 10 % of total cases was obtained and resulted in the identification of a novel VOI (variant of interest) lineage B.1.620 of questionable origin as well as human-mink-human transmission cases. The Lithuanian SARS-CoV-2 genomic surveillance story depicts the importance of worldwide sequencing efforts to avoid the sudden, unexpected emergence of highly divergent and possibly more pathogenic lineages. Our sequencing efforts started as an unorganised, time-consuming and expensive approach; however, lessons were learned and today we have a professional sequencing facility established for current and emerging pathogen surveillance.

4. BIOINFORMATICS

4.1. APPLICATION OF BIOINFORMATICS TO STUDY DIVERSITY AND DISTRIBUTION OF BACTERIAL DNA POLYMERASES

Česlovas Venclovas, Kęstutis Timinskas, Albertas Timinskas, Darius Kazlauskas

Institute of Biotechnology, Life Sciences Center, Vilnius University

Understanding molecular function and mechanism of proteins requires "wet" lab experiments that are often laborious, expensive, and not always successful. On the other hand, currently available huge amounts of genome and protein sequence data enable researchers to make new discoveries using computational methods. Availability of protein sequence data and highly effective computational methods motivated us to explore DNA synthesis machineries in the bacterial world. The actual DNA synthesis in both DNA replication and repair is performed by DNA polymerases. Bacterial DNA polymerases belong to five evolutionary distinct families: A, B, C, X and Y. Our aim was to explore the abundance of each polymerase family, the presence of distinct groups in each family and their representation in individual bacterial species. We also aimed to understand the organizational principles of multisubunit polymerases. To this end we identified and analyzed DNA polymerases of all five families in more than 3000 representative bacterial genomes. Analysis of individual polymerase families revealed several new groups. Strikingly, we discovered that most bacteria have error-prone DNA polymerases expected to form multisubunit complexes. This finding suggests that protein-protein interactions represent a common evolutionary solution to control the activity of mutagenic DNA polymerases involved in DNA repair.

4.2. SHARING SCIENTIFIC DATA: THE CRYSTALLOGRAPHY EXPERIENCE

Saulius Gražulis, Andrius Merkys, Antanas Vaitkus

Vilnius University, Life Sciences Centre, Institute of Biotechnology

Science of the 21st century is crucially dependent on data availability. With most data "born digital" at ever increasing rates, sharing data among scientists becomes of paramount importance. The awareness of this is expressed concisely in the FAIR data publication principles and in the declarations of Open Data movement.

Historically, crystallography was and still is a field of science where data sharing traditions are very strong. From the early days when digital computers started to be used in scientific research, crystallographers processed their data in machine-readable form, used computers to explore crystal symmetries, and established early databases of scientific results. The CSD of CCDC, the PDB, the NDB, the PDF, the ICSD and the Pauling File are examples of early crystallographic databases distributed among crystallographers, some as Open Data (the PDB and NDB), and some under commercial licenses. As the need for open data became more pressing, crystallographers established the COD (Crystallography Open Database, https://www.crystallography.net) to collaborate and share data on small organic, metal-organic and inorganic crystals. Standards established and supported by the IUCr greatly facilitate data sharing between research groups and making different applications compatible. The reuse of data and the means of ensuring data quality will discussed.

5. BIOMEDICINE AND BIOTECHNOLOGY

5.1. INDUCED PLURIPOTENT STEM CELLS AND GENE EDITING FOR DISEASE MODELLING AND EXPERIMENTAL THERAPIES.

Józef Dulak

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow

Induced pluripotent stem cells (iPSC) thanks to the ability to differentiate into almost all cell types offer enormous possibilities for investigating disease mechanisms, drug effectiveness and safety and for experimental regenerative approaches.
In this lecture I will review our research on iPSC and CRISPR/Cas9 gene editing for investigating the iPSC-differentiation to cardiomyocytes, endothelial cells and other cell types affected in neuromuscular and cardiac diseases. We have generated patient-derived iPSC cells for modelling of Duchenne and Becker muscular dystrophy, LAMA2-type dystrophy, spinal muscular atrophy and amyotrophic lateral sclerosis. Potential application of iPSC-derived cardiomyocytes for cell therapy of heart failure will be addressed in regard to our recently published and ongoing studies.

References:

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5.2. miRNA AND GUT MICROBIOTA SIGNATURES OF VULNERABILITY TO FOOD ADDICTION IN MICE AND HUMANS

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Food addiction is a multifactorial disorder mainly characterized by a loss of control over food intake that may promote obesity and alter gut microbiota diversity and composition. We have investigated the changes in miRNAs expression and the microbiota promoted by food addiction in animals and humans and their involvement in the mechanisms underlying the behavioural hallmarks of this disorder. Sharp similitudes were found between the miRNAs signatures in the medial prefrontal cortex of our animal cohort and the miRNAs circulating levels in our human cohort allowing to identify several miRNAs of potential interest for the development of this disorder. Interestingly, our animal and human cohorts showed close similitudes in the gut microbiota signatures as well. These signatures suggest non-beneficial effects in the Proteobacteria phylum and protective effects in the development of food addiction in Actinobacteria and Firmicutes phyla in both cohorts of humans and mice. Notably, e Blautia from the Firmicutes phylum was downregulated in addicted individuals and mice. We demonstrated the beneficial effects of Blautia by administering lactulose and rhamnose prebiotics that increased the relative abundance of Blautia in mice feces and prevented the development of food addiction. By understanding the crosstalk between this behavioral alteration, these novel epigenetic mechanisms and gut microbiota, we provide a new advance toward future treatments for food addiction and related disorders.

5.3. THE INHIBITORS AND NANOPARTICLES IN CANCER THERAPY

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Despite the continuous development of medicine, cancer still remains a group of diseases with one of the highest mortality rates as the high diversity of cancer or metastasis – a process in which circulating tumor cells (CTC) through the bloodstream travel to the other organs where they form a secondary tumor. Metastatic dissemination occurs very early in the malignant progression of cancer but the clinical manifestation of metastases often takes years. Ras/Raf/MEK/ERK, Ras/PI3K/PTEN/Akt/mTOR or AR/Akt/WNT-cadherin pathways play key roles in the regulation of cell growth. Inhibitors targeting these pathways have many potential uses from suppression of cancer, and proliferative diseases as well as aging. We have shown e.g. that ILK silencing has anti-tumor implications for melanoma and bladder cancer, AR/AKT silencing for prostate cancer. But not only inhibitors but also superparamagnetic iron oxide nanoparticles (SPIONs) are broadly applicable in medicine. We used SPIONs in the context of CTC capture and neutralization, as well as magnetic drug delivery. N-cadherin is one of the mesenchymal markers on the surface of CTC and actively promotes cancer cell migration, VCAM-1 is a cell adhesion molecule and was shown to be closely associated with the invasiveness of various cancers therefore, we used both antibodies to decorate SPION in order to target a range of CTC.

This study was supported by National Science Centre - the grant no. NCN 2020/39/B/NZ5/03142.

5.4. DESIGN OF ISOZYME-SELECTIVE INHIBITORS VIA ENGINEERING OF CHIMERIC CARBONIC ANHYDRASES

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A family of 12 catalytically active human carbonic anhydrases (CA) play a role in cancer, glaucoma, epilepsy, altitude sickness, and other diseases. Due to a high percentage of amino acid sequence homology between isozymes, it is challenging to design the isozyme-selective inhibitor.
To understand the structural differences between several CA isoforms, we have engineered chimeric carbonic anhydrases CA Va, CA VI, or CA XII using CA II as a core. By applying biochemical and biophysical methods we investigated thermal stability, catalytic activity, and inhibitor binding affinities of target and engineered CA isozymes. We performed data analysis mainly using Thermott – a novel application for fitting thermal shift assay data, and PLBD – Protein-Ligand Binding Database.
The detailed analysis revealed that 5-7 mutations in the active site of CA II "switched" the enzyme to CA VA, CA VI, or CA XII. The chimeric isoforms started to recognize inhibitors as the target isozyme but not the off-target. In conclusion, the strategy of engineering chimeric carbonic anhydrases has been proven to be successful in designing isozyme-selective inhibitors.

5.5. DEVELOPMENT AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO HUMAN ENDOPLASMIC RETICULUM PROTEINS AS TOOLS FOR DETECTION AND QUANTIFICATION

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The research of human endoplasmic reticulum (ER) chaperones as potential blood biomarkers of neurodegenerative diseases and other illnesses is gaining increasing interest. Monoclonal antibodies are great biotechnological tools for the investigation of various proteins and their roles. Antibodies could be used in the development of immunoassays for the quantification of their targets. In this study, we describe the development and characterization of hybridomas secreting monoclonal antibodies to human ER proteins calreticulin (CRT), binding immunoglobulin protein (BiP), and ERp57 (protein disulfide-isomerase A3). Three monoclonal antibody collections were generated and described by ELISA, Western blot and immunofluorescence microscopy. Monoclonal antibody pairs against CRT and ERp57 were selected and quantitative ELISA tests were developed. The results show that monoclonal antibodies generated against recombinant proteins are specific to natural proteins and are suitable reagents for the investigation of human chaperones as potential blood biomarkers.

ABSTRACTS OF POSTER PRESENTATIONS

6. BIOINFORMATICS

6.1. CRYSTALLOGRAPHY OPEN DATABASE: CURATION, VALIDATION AND (RE)USE

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Crystallography Open Database (COD) is the largest open-access FAIR collection of small-molecule crystal structures that currently contains nearly 500 000 entries describing organic, inorganic, organometallic compounds and minerals. The COD is a curated database which employs the Crystallographic Information Framework (CIF) as well as open-source software tools to ensure that the data are presented in a uniform machine- and human-readable way. The database can be searched in multiple ways such as calling a RESTful API endpoint to find entries with specific crystallographic parameters or executing a chemical (sub)structure search on a set of manually curated SMILES strings which currently covers more than 44% of the entire dataset. To supplement the manually curated chemical descriptions, the COD team has recently developed an automated chemical perception pipeline capable of deriving information about bond types and atom charges from crystallographic data with minimal human intervention. The generated descriptions were cross-checked with the SMILES descriptions as well as chemical information extracted from additional sources using a novel graph isomorphism-based algorithm. The application of the algorithm allowed to detected and correct issues both in the pipeline and in the original peer-reviewed chemical descriptions thus showcasing its applicability in a wider scientific context.

6.2. PROTEIN THERMOSTABILITY PREDICTION USING SEQUENCE REPRESENTATIONS FROM PROTEIN LANGUAGE MODELS

Ieva Pudžiuvelytė, Kliment Olechnovič, Darius Kazlauskas

Vilnius University, Life Sciences Center, Biotechnology Institute, Bioinformatics Department

Earlier studies show that protein's sequence and structural properties influence the thermostability of the macromolecule. Furthermore, one of the most recent achievements in the field of deep learning are protein language models that have not yet been used to classify proteins based on their thermostability. Therefore, it was decided to apply protein representations from the protein language model to make inferences about thermostability of the biological macromolecules.

Language models are transformer architecture-based language models trained in an unsupervised fashion to predict probabilities of elements in sequences. Simultaneously, the process of training creates sequences' embeddings – the real numbered vectors. These representations can be transferred as input to specific application models trained using a supervised learning method. Such transition - 'transfer learning' - is practically useful because the computationally-heavy task to train the language model can be excluded from the development of the application model.

This work presents a method - an ensemble of feed-forward neural networks (FNN) - to predict thermal stability of proteins. To train each of the FNN, ProtTrans model is used to generate embeddings for proteins of organisms with annotated growth temperatures. The model takes the generated embedding to predict the thermostability class of the input protein.

6.3. DIVERSITY AND DISTRIBUTION OF BACTERIAL DNA POLYMERASES

Kęstutis Timinskas, Darius Kazlauskas, Albertas Timinskas and Česlovas Venclovas

Vilnius University Life Sciences Center

Bacterial DNA polymerases belong to five families: A, B, C, X and Y. Representatives from several species have been studied in some detail; however, a comprehensive analysis of DNA polymerases in bacteria is still missing. Therefore, we performed a large-scale computational analysis to explore the diversity and distribution of bacterial DNA polymerases. We found that polymerases of A, C, and Y families are widespread, whereas B and X families are much less common. All bacteria have replicative C-family polymerases and nearly all also have A-family polymerases. Particularly, the 5'-3' exonuclease domain of A-family is critically important. The distribution of Y-family is skewed towards bacteria with large genomes. We identified a group of A-family polymerases lacking any apparent exonuclease activity but containing an uncharacteristic β-clamp binding motif. We also identified a group of unusual B-family polymerases predicted to form a multimeric complex, analogous to E. coli Pol V. Furthermore, within Y-family we found a number of groups predicted to function as various multimeric complexes. Strikingly, the majority of bacteria have error-prone polymerases expected to form multisubunit complexes. This finding suggests that protein-protein interactions represent a common evolutionary solution to control the activity of highly mutagenic DNA polymerases involved in DNA repair.

6.4. STRUCTURAL MODELING AND COMPUTATIONAL ANALYSIS OF UNCHARACTERIZED ANTI-CRISPR CAS PROTEINS

Lukas Valančauskas, Darius Kazlauskas, Česlovas Venclovas

Institute of Biotechnology, Life Sciences Center, Vilnius University

Prokaryotic CRISPR-Cas systems can provide immunity against invading bacteriophages. However, some phages can inhibit CRISPR-Cas systems by using anti-CRISPR (Acr) proteins. Acrs have been shown to interact with Cas proteins in multitude of ways thereby reducing the ability of Cas proteins to bind or hydrolyze nucleic acids.

There is a large and diverse set of proteins, experimentally identified as Arcs, but with unknown mechanism of action. To explore structures of these Arcs, we applied a large-scale structure modeling using AlphaFold. Highly divergent nature of Acrs was supported by the lack of prevailing or unifying structural motifs common to analyzed Acr models. Nonetheless, domain organization of Acr clusters was investigated using various sequence and structure search methods. This in some cases revealed divergent domain organization even between related Acrs. Screening for possible Acr-Cas protein interactions was also performed by modelling complexes, uncovering possible modes of action for a set of Acrs.

As a result, this study provides not only structural and domain-level characterization of many Acrs, but also various computational approaches applicable for analysis of these highly divergent proteins.

6.5. MODELING OF PROTEIN-NUCLEIC ACID INTERACTIONS USING TEMPLATE-BASED AND FREE DOCKING METHODS

Rita Banciul, Kliment Olechnovič, Justas Dapkūnas

Institute of Biotechnology, Life Science Center, Vilnius University

Protein-nucleic acid interactions are essential in many cellular processes, such as transcription, DNA modification and others. It is important to know the structures of the resulting complexes, as they form the basis of our understanding of these processes. Experimental methods such as X-ray crystallography can provide high-resolution structures, but they are expensive and time consuming. Thus, there is a need for computational methods that can model protein-nucleic acid complexes providing insights into interaction mechanisms.

When structures of homologous protein-nucleic acid complexes are known, the interactions can be predicted using template-based modeling. To this end, we have developed the PPI3D web server. It allows querying the non-redundant set of interactions from the Protein Data Bank, identifying the templates and modeling the structures of protein-nucleic acid complexes.

When closely related templates are not available, free docking can be employed. We present a newly developed FTDMP software system for docking, scoring, and ranking diverse protein interactions, including protein-nucleic acid complexes. Rigid body docking of protein and diverse simulated conformations of DNA is performed to account for DNA flexibility. The protein–DNA and protein-RNA docking benchmarks are used to evaluate the docking results.

7. BIOMEDICINE

7.1. INVESTIGATION OF THE PERSISTENCE OF SARS-COV-2 SPIKE PROTEIN-SPECIFIC ANTIBODIES INDUCED BY SARS-COV-2 INFECTION

Aurelija Žvirblienė[1], Martynas Simanavičius[1], Aistis Šimaitis[2], Indrė Kučinskaitė-Kodzė[1]

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Data on the persistence of the SARS-CoV-2-specific immune response are of great importance for managing the outbreaks and planning measures for increasing heard immunity to the virus. In the current study, the dynamics of antibody responses was investigated after the well-documented outbreak of SARS-CoV-2 infection in a private Lithuanian company during the first wave of the pandemic. Virus-specific IgG and IgM were monitored 2, 6 and 13 months after the outbreak via rapid IgG/IgM serological test and SARS-CoV-2 S protein-specific IgG ELISA. Six months after the outbreak, 95% (CI 86–99%) of 59 previously infected individuals had virus-specific antibodies irrespective of the severity of infection. One-third of seropositive individuals had virus-specific IgM along with IgG indicating that IgM may persist for 6 months. Serological testing 13 months after the outbreak included 47 recovered individuals that remained non-vaccinated despite a wide accessibility of COVID-19 vaccines. The seropositivity rate was 83% (CI 69–91%) excluding one case of confirmed asymptomatic reinfection in this group. Between months 6 and 13, IgG levels either declined or remained stable in 31 individuals and increased in 7 individuals possibly indicating an exposure to SARS-CoV-2 during the second wave of the pandemic. Summarizing, this study revealed that detectable levels of SARS-CoV-2-specific antibodies persist up to 13 months after infection for the majority of the cases.

7.2. 4NQO-INDUCED MIRNA EXPRESSION CHANGES IN LEUKOCYTES OF TYPE 2 DIABETES MELLITUS PATIENTS ARE ASSOCIATED WITH CARDIOVASCULAR COMPLICATIONS

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The development of type 2 diabetes mellitus (T2DM) is closely associated with oxidative stress (OS), which in turn increases the risk of complications in various organs and tissues. However, epigenetic regulatory mechanisms underlying T2DM pathogenesis in response to OS remain unclear. Various microRNAs (miRNAs) have been associated with T2DM, however, their clinical application has not yet been fully elucidated.

In the present study, four selected miRNAs (miR-16-5p, miR-17-5p, miR-106a-5p, and miR-223-3p) were quantified in leukocyte samples treated with 4-nitroquinoline 1-oxide (4NQO), which induces OS in cells. The study cohort consisted of 38 T2DM 21 non-diabetic patients (NDP).

MiR-106a-5p levels in T2DM cases were lower than in NDP, while expression of other miRNAs was similar between the groups. MiR-17-5p expression was higher in patients with primary arterial hypertension (PAH+) than in those without the condition (PAH-) both before and after 4NQO treatment. MiR-106a-5p levels were higher in PAH+ cases only after 4NQO treatment, whereas miR-16-5p expression differed only in untreated samples. Furthermore, several associations were observed between the miRNAs and other clinicopathological patients' parameters.

In conclusion, our preliminary results indicate that the analyzed miRNAs may play a role in the development of T2DM-associated cardiovascular complications.

7.3. SIGNATURE OF MICRORNA EXPRESSION IN TRIPLE-NEGATIVE BREAST CANCER PATIENTS UNDERGOING NEOADJUVANT THERAPY

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Breast cancer is the most common malignancy and the leading cause of cancer-related deaths among women. Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that accounts for 10-15% of all cases. Due to the loss of three certain receptors in TNBC cells, treatment options are limited, and the disease is associated with a poor prognosis. MicroRNAs (miRNAs) are small non-coding RNA molecules whose dysregulation can alter the expression of specific genes, affecting cancer pathogenesis.

This study aims to provide insights into the role of selected miRNAs in TNBC progression. 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 whole blood and formalin-fixed paraffin-embedded (FFPE) tissue specimens from patients with TNBC undergoing neoadjuvant chemotherapy. Finally, statistical analysis was performed to compare miRNA expression levels across the different specimens.

7.4. THE STUDY OF INTERACTIONS BETWEEN FLUORINATED BENZENESULFONAMIDE COMPOUNDS AND CARBONIC ANHYDRASES I – XIV

Dovydas Lužeckas, Mantas Žvirblis, Aurelija Mickevičiūtė , Daumantas Matulis, Virginija Dudutienė, Joana Smirnovienė

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Carbonic anhydrases (CA) are enzymes which catalyse a simple but essential reaction for all living organisms - carbon dioxide hydration/dehydration to bicarbonate ion. Most oftenly, isoforms are found in different organs or cell compartments. In human body, 15 CA isoforms are found, 3 of which are inactive. Cytosolic isoforms are I – III, membrane – bound – CAIV, CA IX, CA XII and CA XIV, secretory – CA VI and mitochondrial – CA Va and CA Vb. In the case of cancer, large amounts of carbon dioxide and lactic acid molecules are formed. Tumorigenic cells are adapted to low pH values by utilising two isoforms of carbonic anhydrases – CA IX and CA XII. Thus, a lot of research is done to find inhibitors which could target these isoforms.

In this study the main goal was set to study interactions between fluorinated benzene – sulphonamide inhibitors and isoforms of I-XIV carbonic anhydrases. Fluorescent thermal shift assay was used to measure the thermal stability of carbonic anhydrases and determine the binding affinities of inhibitors.

The analysis of binding data helped to identify several selective inhibitors of anticancer targets – CA IX and CA XII. The lead compounds could be further developed as anticancer drug-candidates.

7.5. DENDRITIC CELLS' ROLE IN OVARIAN CANCER IMMUNOTHERAPY VACCINE

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Background.
According to the current range of ovarian cancer immunotherapy approaches, it is appropriate to improve dendritic cells vaccines (DCV) for better cancer management. We aimed to investigate allogenic DCV preparation with different ovarian cancer (OC) cell lines (A2780, SKOV3, COV362, OV7), their molecular profile, and their impact on dendritic cell maturation.
Methods.
Flow cytometry analysis was used to determine the expression of stemness related markers in cancer cell lines and surface markers of matured DCs. Gene expression was assessed by RT-PCR in OC cell lines and later in matured DCs. DCs were matured with prepared lysates of OC cell lines and their mixture. Pearson correlation analysis was used to investigate the relationship of the ovarian cancer cell lines molecular profile and dendritic cells maturation level.
Results.
Studied OC cell lines have different molecular profiles. Individual OvCa cell lines and their mixture have different effects on the maturity of dendritic cells. The highest expression of genes associated with immunogenicity and the lowest with tolerogenicity – were achieved using a mixture of all OC cell lines lysates for the maturation of DC. The OV7 cell line with the mesenchymal and stem-like phenotype is able to induce the highest expression of maturation markers on DC. Genes encoding the major transcription factor for epithelial-mesechymal transformation SNAIL1 and the multidrug resistance active transport carrier ABCG2 are strongly correlated with essential DL maturation markers CD80 and MHCII and CD86 and STAT1 transcripts ($p < 0.05$).
Conclusion.
In early phase clinical trials where it is not always possible to use the patient's autologous tumor material could be used the mixture of OC cell lines lysates for allogenic DCV preparation. Single-cell transcriptional profiling could be further investigated to improve allogenic DCV immunotherapy of ovarian cancer.

7.6. THE EFFECTS OF MATERNAL HIGH-FAT DIET CONSUMPTION ON OFFSPRING NEURODEVELOPMENT

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There is growing evidence that maternal high fat diet (mHFD) increases the risk of neurodevelopmental disorders in the offspring. One of the pathways that mediate the effect of mHFD on offspring neurodevelopment are changes of maternal and offspring gut microbiota. Our aim was to evaluate how mHFD alters maternal microbiota, leading to the changes of offspring microbiota, brain morphological aberrations and abnormal behavior in the offspring. Female C57Bl/6J mice were fed a control diet (CD, 10% fat) or high-fat diet (HFD, 60%) from weaning to lactation. We investigated the behavioral phenotype of the offspring. Gut microbiota were determined by 16S rRNA amplicon sequencing. PFA-perfused offspring brains were imaged using T2-weighted MRI (9.4T). The changes of brain structure were determined by high-resolution voxel-based morphometry. We determined that the consumption of HFD caused metabolic dysfunction in the dams and changed the relative abundance of different gut bacteria genera. mHFD changed the composition of offspring gut microbiota as well with microbiota alterations more prominent in mHFD female offspring. In contrast, the exposure to mHFD caused more structural alterations in mHFD male offspring. Offspring behavior depended more on brain structural features rather than gut microbiota composition, as mHFD disturbed the behavior of male offspring more than that of females. Our findings showed that mHFD altered the composition of the offspring gut microbiota, brain structure and behavior in a sex-dependent manner.

7.7. EFFECTS OF MICROGRAVITY ON THE PHYSIOLOGY OF YEAST CELLS WITH DIFFERENT TELOMERE LENGTH AND RESISTANCE TO CHEMICAL AND PHYSICAL AGENTS

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Eukaryotic cells chromosome ends are protected from degradation by telomeres, which are affected when cells undergo various environmental stresses. Recent studies showed that astronauts have shorter telomeres than ground controls before and after spaceflight [1] and shorter telomeres are associated to cellular aging and longevity [2]. In this work, the effect of microgravity on the resistance of three Saccharomyces cerevisiae strains with different telomeres lengths to chemical and physical agents was measured by comparing cells grown under normal gravity and simulated microgravity.
The study attempted to evaluate chemical and physical factors such as resistance to formic and acetic acids, antifungal compounds, namely amphotericin B, nystatin dihydrate, clotrimazole, and electric shock. Microgravity was simulated using a rotary cell culture system, chemical resistance was assessed using the microplate dilution method, and the cells were exposed to an electric shock by a pulsed electric field generator.
The early results show a slight difference between S. cerevisiae strains grown under normal gravity and microgravity treated with organic acids, antifungal compounds, and electric shock.
This study concludes that telomeres length has an impact on cell physiology and moreover are affected by microgravity, due to this a follow up research is necessary for evaluation of telomere length variation.

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7.8. THE ROLE OF ARHGAP29 IN REGULATING BREAST CANCER CELL MIGRATION

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One of the major fundamental challenges in tumor biology is to understand how cells migrate in the three-dimensional extracellular matrix (ECM) during metastasis, where disturbances in the mechanisms controlling cell migration significantly increase the lethality of all tumors, promoting metastasis from the primary tumor to multiple secondary tumors. ARHGAP29 is one of the Rap2-binding proteins that activated promotes forward movement of the cell, as well as inhibits RhoA. The aim of this study was to activate RhoA using Rap2-dependent ARHGAP29.
In this study we used triple-negative breast cancer (TNBC) MDA-MB-231 cells, which is one of the most aggressive tumors, accounting for 10-15% of breast tumor diagnoses worldwide. MDA-MB-231 Cas9 cell were used as control line, two knockdown lines of ARHGAP29 were generated, MDA-MB-231 ARHGAP29 knockout lines was kindly provided by R. Prekeris from Colorado University. We tested these cell lines for focal adhesion density using immunostaining with paxillin. Also, using time-lapse microscopy, differences in directionality, distance, length, and speed were evaluated. Characterization of mechanisms regulating TNBC migration will provide new insights into the mechanisms regulating TNBC metastasis and will lead to new drug targets and therapies.

7.9. TNF-α AS A NOVEL BIOMARKER FOR POSTOPERATIVE COMPLICATIONS IN LEFT-SIDE COLORECTAL CANCER SURGERY

Kornelija Rauduvytė, Agata Mlynska, Žilvinas Gricius, Agnė Šeštokaitė, Simona Rūta Letautienė, Kristina Žukauskaitė, Audrius Dulskas, Rasa Sabaliauskaitė, Augustinas Baušys

National Cancer Institute

Colorectal cancer (CRC) is one the most common type of cancer worldwide. Surgery remains the only potentially curative treatment option for it. Despite recent medical progress, postoperative complications still occur in about 40-50% of CRC patients. There is a lack of tools that could reliably predict postoperative complications before the surgery. Therefore, this study aims to investigate a variety of biomarkers for postoperative outcomes after left-side CRC surgery.

In this prospective longitudinal study, 40 patients undergoing left-side CRC surgery were included. Blood samples were collected on the baseline, postoperative day 6 and 30. ELISA and RT-qPCR were performed to evaluate 13 serum cytokines and 2 plasma miRNAs expression as biomarkers for postoperative outcomes. Univariate and multivariate logistic regression analyses were performed to adjust for common risk factors (age, gender, CCI, TNF-α , IL-8, tumor size, lymph node status, white blood cells and c-reactive protein concentration).

Patients who suffered postoperative complications (n = 16; 40%) were found to have an increased baseline serum median of TNF-α (50.39; IQR = 2.37–590.50 pg/mL vs 2.28; IQR = 2.28–14.16 pg/mL, p = 0.009) and IL-8 (15.88; IQR = 5.16–49.96 pg/mL vs 7.06; IQR = 3.84–13.26 pg/mL, p = 0.031). At univariate analysis, TNF-α, gender and CCI were identified as potential risk factors associated with postoperative complications ($p < 0.1$). Multivariate analysis confirmed a higher than 2.47 TNF-α level (OR = 15.97; 95% CI 2.30–337.13, p = 0.018) and male gender (OR = 9.48; CI 1.44–189.46, p = 0.047) as risk factors for postoperative complications after CRC resections. Results presented here suggest, that serum TNF-α could be used to identify high-risk surgical CRC patients.

In conclusion, our study demonstrated that patients with an increased TNF-α level are at a higher risk for postoperative complications after left-side CRC surgery.

7.10. NON-DIGESTIBLE CARBOHYDRATES AS A PREVENTIVE MEASURE AGAINST FOOD ADDICTION

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Food addiction is a multifactorial brain disorder, that results from multiple interactions between genetic and environmental factors. Therefore, gut microbiome is believed to play an important role in the development of this addictive behavior. Thus, we aimed to increase Blautia spp. abundance in the gut of mice with non-digestible carbohydrates supplementation to evaluate the possible protective role against the development of food addiction.

Mice received non-digestible carbohydrates - lactulose and rhamnose with drinking water during the whole experimental protocol. An operant protocol of food addiction has been performed. A qPCR was conducted to confirm the effectiveness of targeted prebiotics in increasing Blautia genus abundance in the gut. 42 % of control mice became addicted, whereas none of the mice receiving lactulose or rhamnose achieved the addiction criteria (0%). Rhamnose was more effective in decreasing compulsivity-like behavior, impulsivity, and motivation. qPCR results confirmed that rhamnose significantly increased Blautia genus concentration in the gut.

To conclude, both lactulose and rhamnose have a protective effect in the development of food-addictive-like behavior. However, rhamnose displays stronger effect on distinct addiction-like criteria. These findings confirm that Blautia spp. exerts a protective role in the development of food addiction.

7.11. IMPACT OF THE PDGFRA GENE POLYMORPHISMS ON THE EFFECTIVENESS OF LATERAL EPICONDYLITIS TREATMENT USING PLATELET-RICH PLASMA

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Background: The effectiveness of PRP (platelet-rich plasma) therapy varies between patients. This could be due to genetic variability.
Aim: The aim of the present study was to analyse the impact of SNPs (single nucleotide polymorphisms) of the PDGFRA (platelet derived growth factor receptor alpha) gene on the effectiveness of lateral epicondylitis treatment with PRP therapy.
Materials and Methods: The treatment efficacy was analysed over time (2, 4, 8, 12, 24, 52 and 104 weeks after PRP injection) on 107 patients using patient-reported outcome measures: VAS (visual analog scale), QDASH (quick version of disabilities of the arm, shoulder and hand) and PRTEE (patient-rated tennis elbow evaluation). Three SNPs of the PDGFRA gene (rs1316926, rs6554164, rs766819) were analysed.
Results: The AA homozygotes of the rs1316926 polymorphism had lower values of VAS, QDASH and PRTEE comparing to G allele carriers. T allele carriers of the rs6554164 had higher values of ΔVAS, ΔQDASH and ΔPRTEE compared to CC homozygotes. Higher values of ΔVAS were observed also in the rs766819 AA homozygotes and higher PLT (platelets) and PCT (plateletcrit) in PRP preparation were found in the A allele carriers (rs766819).
Conclusion: PDGFRA gene polymorphisms affect the effectiveness of lateral epicondylitis treatment using PRP.

7.12. INFLUENCE OF SELECTED CAROTENOIDS ON EQUILIBRIA IN MONOLAYERS AT THE WATER/AIR INTERFACE

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Biological membranes determine the existence of every living cell. Despite the development of many fields of science, working with natural cell membranes still poses problems, which is why research is carried out with the participation of model systems such as monolayers.
The tested systems contained phosphatidylcholine and selected carotenoids (retinol and β-carotene) necessary for the proper functioning of the body. Vitamin A is the general name of chemical compounds in the carotenoid group, including beta-carotene and retinol. Vitamin A affects the proper functioning of the immune system, eyesight, and reproductive system, as well as the skin's condition.
In order to study the effect of the compounds mentioned above on the physicochemical parameters of membranes, tests were carried out using model systems - monolayers at the water/air interface using the Langmuir method. Based on the obtained results, the formation of a 1:1 complex between the components of the mixed monolayers was assumed. Then, the specific surfaces of pure substances and their mixtures with phosphatidylcholine in various volume relationships, stability constants, and formation energies of the resulting complexes were determined.
On this basis, it was found that retinol and β-carotene affect the properties of model cell membranes made of phosphatidylcholine.

7.13. IMPAIRMENT OF MITOCHONDRIAL BKCA CHANNEL ACTIVITY CAUSED BY INDUCTION OF SENESCENCE IN VASCULAR SMOOTH MUSCLE CELLS

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Reactions of cells to the some endogenous or exogenous factors is the induction of their senescence. The presence of senescent smooth muscle cells and endothelial cells are also localized in the vascular plaque. The changes in the functioning of mitochondria are the important features of senescent cells that affect the development of the entire aging process. Still little is known about involvement of mitochondrial potassium channels in these processes. The aim of the study was to estimate potential changes in mitochondrial large conductance calcium-activated (mitoBKCa) channels in vascular smooth muscle cells undergoing cellular senescence. Human smooth muscle cells were treated with a single dose of H_2O_2 induces visible senescence within seven days after addition. Our results confirmed the appearance of basic markers of senescence processes in the studied smooth muscle cells. Changes in the mitochondrial network under the influence of H_2O_2 were determined and transcription differences in genes encoding proteins involved in mitochondrial biogenesis and function were described. The decrease in the level of BKCa channels protein was observed regardless of the unchanged specific mRNA expression. The typical electrophysiological activity of mitoBKCa was measured in the control cells, but was absent in senescent smooth muscle cells.
This research was funded by the National Science Center, grants nos. 2019/34/A/NZ1/00352 and OPUS UMO-2018/31/B/NZ3/02931.

7.14. EFFECT OF 4-PHENYLBUTYRIC ACID (4-PBA) ON PHYSICOCHEMICAL PROPERTIES OF PHOSPHATIDYLCHOLINE LIPOSOMES

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Chaperones, such as 4-phenylbutyric acid (4-PBA), have been gaining increasing attention for their cytoprotective, neuroprotective, and anti-apoptotic properties. Evaluation of physicochemical parameters such as zeta potential or particle size is essential for determining the nature of membrane-chaperone interactions. However, these interactions are difficult to analyze due to the complexity of the biological membrane structure. Therefore, the use of liposomal nanostructures is particularly advantageous since they are relatively simple physical models characterizing cell membranes.
We investigated the effect of 4-PBA on selected physicochemical properties of phosphatidylcholine liposomes. Zeta potential was monitored by electrophoretic light scattering (ELS), while hydrodynamic diameter and polydispersity index were assessed by dynamic light scattering (DLS). Measurements were carried out as a function of the pH of the electrolyte solution (0.9% NaCl), at 298 K and 310 K.
4-phenylbutyric acid caused changes in the values of the above-analyzed parameters, with the changes depending significantly on the pH, as well as the temperature. However, since there are few reports in the literature on the 4-PBA's interactions with lipid membranes, and the molecular mechanisms of its action have not yet been fully understood and elucidated, we hope our results will contribute to expanding knowledge on the chaperone.

7.15. THE CYTOTOXIC EFFECT OF SALINOSPORAMIDE A - A NOVEL PROTEASOME INHIBITOR, ON GLIOBLASTOMA CELL LINE LN-229

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Glioblastoma (GBM) is the most aggressive cancer of the central nervous system. The primary treatments including surgical resection, radiotherapy and temozolomide-based chemotherapy, are yet inefficient, thus the novel and effective treatment options are required. Since proteasomes are known to play a vital role in the physiology of GBM, proteasome inhibition might be exploited as a strategy for treating GBM. Salinosporamide A (SalA, marizomib) is a second-generation, irreversible proteasome inhibitor (PI) with a more lipophilic structure enabling penetration across the blood-brain barrier. The in vitro antitumor activity of this PI was studied in glioblastoma LN-229 cell line treated with 5 nM -500 nM SalA for 24h. SalA significantly decreased the viability of GBM cells as shown by the MTT and the ATP-based test. Moreover, the subsequently tested concentrations of 50 and 100 nM of SalA caused activation of apoptotic cell death as demonstrated by increased activity and expression of Casp-3 and overexpression of cleaved-PARP1. Additionally, the SalA-dependent proapoptotic effect was accompanied by an up-regulation of endoplasic reticulum (ER) stress markers such Ire1α and Chop. These preclinical studies show that SalA can cause ER stress and apoptosis in glioblastoma cells suggesting a potential antitumor effect of this PI in brain malignancies.

7.16. APITOLISIB INHIBITS GROWTH OF HUMAN GLIOBLASTOMA CELL LINES BY INDUCING APOPTOSIS AND AUTOPHAGY

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A dysregulated the phosphatidylinositol 3-kinase/mammalian target of rapamycin (PI3K/mTOR) signaling pathway is common in glioblastoma, making this axis an attractive target for therapeutic manipulation. Given that PI3K/mTOR activation promotes tumor growth, metastasis, and resistance to cancer therapies, PI3K/mTOR inhibitors are therapeutic agents developed for various types of human tumors and they show promise in the treatment of cancer. In this study, we evaluated the efficacy of dual inhibitor Apitolisib (GDC-0980) in GBM and assessed the mechanisms of A-172 and U-118 MG GBM tumour cell line suppression. We show that GDC-0980 inhibited PI3K/mTOR signaling in investigated GBM cell lines. It has been demonstrated that GDC-0980, blocking PI3K/mTOR, induced time- and dose-dependent cytotoxicity and enhanced induction of apoptosis in investigated glioma cell lines. The strongest activation of apoptosis was exhibited in A-172 line after 48 h of incubation with 20 μM GDC-0980. Furthermore, GDC-0980 inhibited mTORC1, which led to induction of autophagy in both cell lines. In conclusion, here in this study, we first discovered that dual PI3K/mTOR blockade by GDC-0980 markedly supressed survival of human GBM cells and induced apoptosis. These studies show that GDC-0980 may be a candidate for further evaluation as a chemotherapeutic agent for anti-GBM therapy.

7.17. CYTOTOXICITY AND ELECTROPHORETIC LIGHT SCATTERING STUDIES OF HUMAN MALIGNANT GLIOBLASTOMA CELL LINES UPON QUERCETIN AND KAEMPFEROL TREATMENT

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The therapeutic potential of natural compounds has attracted great interest. However, in many cases, it is still necessary to collect additional information about their physicochemical properties as they determine their bioavailability and therapeutic efficiency.

We aimed to determine the drug-like potential of quercetin (QCT) and kaempferol (KMF) as anti-glioblastoma agents. We combined an experimental approach with a theoretical analysis of the crucial properties of these polyphenols. Analysis of the physicochemical parameters of QCT and KMF demonstrated that both flavonoids show favorable properties as orally bioavailable and central nervous system (CNS) active compounds.

Moreover, we have shown that QCT and KMF change the cell's zeta potential only in high pH but not in acidic pH. This tendency may indicate their better membrane permeability in low pH values. Considering the lower pH of cancer cells' environment compared to their regular counterparts, undisrupted intracellular penetration of potential anticancer drugs in the low pH range might be an essential factor in determining their efficiency. These results and the cytotoxic effect of QCT and KMF in GBM cells suggest good anti-potential of these polyphenols. Therefore, it seems reasonable to consider QCT and KMF as plausible candidates for pharmacological agents against brain malignancies.

7.18. A PRELIMINARY STUDY OF MARIZOMIB-INDUCED SENESCENCE IN MALIGNANT MELANOMA CELL LINES

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Malignant melanoma is considered as one of the most dangerous skin cancers, and current treatment methods are still insufficient. There is a need to search for new ways of treating this cancer and senescence induction might be one of them. Cellular senescence is a process that causes cell cycle arrest and prevents cells proliferation. Furthermore, senescence is an element of two-steps anticancer strategy: induction of senescence in tumor cells and subsequent elimination of senescent tumor cells with senolytic drugs. In our research we wanted to check, if proteasome inhibitor – marizomib – induces senescence in A375 and G361 melanoma cell lines. We performed a proliferation assay with crystal violet, evaluated SA-β-galactosidase expression and examined cell cycle with flow cytometry. We showed decreased proliferation of both investigated cell lines, treated with marizomib, in comparison to untreated cells. Furthermore, we have observed a rise of G2 phase in cell cycle and increased expression of SA-β-galactosidase in both exposed cell lines. All gathered findings suggest that marizomib has an antiproliferative activity on examined melanoma cancer cells. Further research is required to ensure that the senescence is responsible for this effect.

8. BIOTECHNOLOGY

8.1. INVESTIGATION OF NEUTRALIZING ACTIVITY OF MONOCLONAL ANTIBODIES AGAINST RECEPTOR BINDING DOMAIN OF SARS-COV-2 SPIKE PROTEIN

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SARS-CoV-2 caused the COVID-19 pandemic and became a major problem threatening public health and the global economy. Monoclonal antibodies (MAbs) with broad neutralizing activity have high therapeutic and diagnostic potential and are still relevant. Receptor-binding domain (RBD) located on the surface of the S protein mediates viral entry to the cell by interacting with the cellular ACE2 receptor. Therefore, anti-RBD MAbs tend to have high potency for virus neutralization. Moreover, while new virus variants are emerging, inhibitory MAbs against conservative regions of RBD would be critical. These MAbs would be beneficial for diagnostics measuring the level of neutralizing antibodies against SARS-CoV-2. This study aimed to evaluate the capability of the newly generated MAbs to block S protein interaction with ACE2 receptor. For this, a surrogate neutralization assay based on ELISA, determining MAb-dependent inhibition of the interaction between recombinant ACE2 and S proteins of different SARS-CoV-2 variants, was investigated. The obtained data demonstrated the feasibility of tested MAbs to block S protein interaction with ACE2. The results suggest that epitopes within the RBD, which are recognized by the newly generated MAbs, are responsible for the attachment to ACE2 receptor, hence these MAbs could be used alone or in a mix to develop diagnostic systems measuring SARS-CoV-2 neutralizing antibodies.

8.2. THE USE OF A BROADLY REACTIVE MONOCLONAL ANTIBODY FOR ANTIGENIC CHARACTERIZATION OF RECOMBINANT FISH PARVALBUMINS

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Fish is one of the main food allergens causing allergy to less than 1 % of the world's population. Parvalbumins are small (about 10-12 kDa) and highly stable calcium-binding proteins found in fish muscle, that share high sequence identity among different fish species. Monoclonal antibodies (MAbs) against a particular fish parvalbumin may represent a useful tool for fish allergy studies.
The MAb (clone 3F6, IgG1 subtype) against recombinant common carp parvalbumin (MBP-Cyp c 1) was generated by hybridoma technology and characterized using enzyme-linked immunosorbent assay (ELISA), Western blot and dot blot. The pattern of the cross-reactivity of the MAb 3F6 with different fish parvalbumins was analyzed. In total, 11 recombinant parvalbumins of different fish species, fused with maltose-binding protein (MBP), were expressed in E.coli and purified.
The MAb 3F6 demonstrated high affinity for the antigens. It cross-reacted with all studied recombinant fish parvalbumins, except for recombinant Northern pike parvalbumin. It was shown to recognize linear epitopes of target parvalbumins by Western blot.
The newly developed cross-reactive MAb against MBP-Cyp c 1 represents a promising tool for antigenic characterization of fish parvalbumins.

8.3. THE APPLICATION OF MILK PERMEATE AS AN ALTERNATIVE TO ISOPROPYL B-D-1-THIOGALACTOPYRANOSIDE (IPTG) AND REDUCED AMPICILLIN CONCENTRATIONS FOR THE PRODUCTION OF RECOMBINANT ENZYMES

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As the biotechnology industry is growing, it is constantly seeking cheaper and more sustainable alternatives to current practices. Currently, one of the most popular hosts for recombinant protein synthesis is *Escherichia coli* and most expression systems depend on Isopropyl β-D-1-thiogalactopyranoside (IPTG) for the induction of recombinant protein synthesis. In addition to its enormous price, IPTG can cause additional stress to host cells. In this study, milk permeate (MP), a waste product of the dairy industry, has been suggested as a potential alternative to IPTG due to its low cost and adherence to circular economy principles.

Moreover, reducing the concentration of ampicillin in the cultivation medium for recombinant enzyme production was investigated due to the increasing concern over the emergence of antibiotic-resistant bacteria. The study showed that recombinant enzymes could be synthesized in E. coli BL21 (DE3) cells using lower antibiotic concentrations (25, 5 μg/mL) and MP instead of IPTG. The cost-effectiveness of MP as an inducer for recombinant protein synthesis makes it an attractive alternative to IPTG, while the reduced use of antibiotics in the production process is a positive step towards addressing the issue of antibiotic resistance.

8.4. DEVELOPMENT AND CHARACTERISATION OF MONOCLONAL ANTIBODIES AGAINST THE SELECTED EPITOPE OF SARS-COV-2 SPIKE PROTEIN

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SARS-CoV-2 virus enters hosts cells after spike (S) protein interacts with ACE2 receptor. Therefore, antibodies with virus-neutralising activity are important for research and diagnostics.
This study aimed to develop new monoclonal antibodies (MAbs) against the selected epitope as a target for neutralising antibodies located within amino acid residues 491-500 of S protein. To generate MAbs, recombinant chimeric virus-like particles harbouring the target epitope were used as an immunogen. The reactivity of the MAbs with recombinant S proteins of virus variants was analysed by indirect ELISA. A surrogate neutralisation test was used to investigate the ability of MAbs to block the interaction of ACE2 receptor and S protein.
Two stable hybridoma clones (11H3 and 20D1) producing MAbs against the target epitope were generated. They recognised recombinant S proteins of different SARS-CoV-2 variants. However, 11H3 had a low affinity to the S protein of Omicron, and 20D1 failed to recognise this antigen. Furthermore, these MAbs did not block the interaction between the recombinant ACE2 and S protein. This study revealed a low importance of the investigated epitope for ACE2 and S protein interaction. Moreover, the mutations in the selected epitope affect the ability of antibodies to recognise the S protein of Omicron.

8.5. STRUCTURAL VARIABILITY OF PRION PROTEIN AMYLOID FIBRILS UNDER DIFFERENT AGITATION CONDITION

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Amyloidogenic peptides and proteins property to convert from their native functional states into fibrillar amyloid aggregates is associated with neurodegenerative disorders, such as prionopathies. It has been observed that the environment conditions in which amyloid aggregation takes place have an important effect on fibril polymorphism. One example of such environmental conditions, whose effect on amyloid structural variability is not fully understood, is agitation.

In this work, we examined the effect of three different agitation conditions on the aggregation kinetics of mouse prion protein fragment (MoPrP 89-230) and analyzed the secondary structure of the resulting fibrils.

Protein MoPrP 89-230 samples were incubated under three agitation conditions (200, 400 and 600 RPM) at 37 °C, using 50 mM sodium phosphate buffer containing 2 M guanidinium hydrochloride. The kinetics of aggregation were determined by recording the fluorescence intensity of the amyloidophilic dye thioflavin-T (ThT). The secondary structure of fibrils was determined by analyzing each sample's FTIR spectra.

The results suggest that the intensity of agitation has a minimal influence on primary nuclei formation. The apparent rate of elongation, however, was significantly different between all three conditions. In all three cases, a diverse collection of secondary structures were observed, with the highest variability detected under 200 RPM agitation conditions.

8.6. DEVELOPMENT OF MONOCLONAL ANTIBODIES AGAINST ANTIBIOTIC RESISTANCE PROTEINS OXA-48 AND OXA-134

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Antimicrobial resistance (AMR) is the largest global health threat in the 21st century and it requires urgent measures. Major reasons behind the emergence of AMR - misusing and overusing different antibacterial agents in the healthcare settings [1]. One of the steps in limiting the spread of AMR is accurate diagnostics of the disease and prescription of effective antibiotics. Currently applied diagnostic methods include phenotyping, biochemical and genotyping methods which tend to be expensive and protractive. Immunodetection, on the other hand, is rapid, specific, and sensitive method for identifying resistance factors. However, development of immunodiagnostic tests is limited by lack of highly specific monoclonal antibodies (MAbs).

The aim of this study was to generate and characterize MAbs against two antibiotic resistance factors – OXA β-lactamases OXA-48 and OXA-134, which are widespread in pathogenic Enterobacteriaceae and confer resistance to carbapenems [2]. Using hybridoma technology one specific anti-OXA-48 MAb and three specific anti-OXA-134 MAbs secreting cell lines were created. All four generated MAbs were shown to have high affinity towards respective antigens and recognize their linear epitopes. One anti-OXA-134 monoclonal antibody pair was shown to have potential to be applied in antibiotic resistance factors detection tool.

8.7. IS200/IS605 FAMILY INSERTION SEQUENCES – A SOURCE OF NOVEL GENOME EDITING TOOLS

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One of the most widely distributed ancient groups of mobile genetic elements among prokaryotes are IS200/IS605 family insertion sequences (IS). These sequences are flanked by LE and RE terminal imperfect palindromic motifs and may encode TnpA, TnpA and TnpB, or a single TnpB. While the TnpA transposase is responsible for IS transposition, the function of TnpB has been unknown. Recent studies have shown that TnpB proteins could be an evolutional ancestor of class 2 CRISPR-Cas systems effector proteins, which are widely adopted in genome editing.

In this study we aimed to characterize transposon-encoded TnpB from Deinococcus radiodurans. TnpB has been found to be a reRNA-guided nuclease, capable of cleaving double-stranded DNA substrates in the TAM-dependent manner. In vitro cleavage assays revealed the TAM and reRNA complementary target requirements for an efficient target DNA cleavage.

Further characterization of TnpB nucleases could expand the genome-editing toolbox by providing a new class of extremely compact non-Cas nucleases with different biochemical requirements for genome-editing applications. Their miniature size, compared to the Cas nucleases, is suitable for adeno-associated virus-based delivery and opens new horizons for therapeutic applications.

8.8. PmlABCDEF MONOOXYGENASE CATALYSES SYNTHESIS OF EPOXIDES AND N-OXIDES

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Oxygenation reactions are widely used in industry, and nowadays more attention is shifting to the enzymes that catalyse such reactions - various oxygenases. Non-heme diiron monooxygenase PmlABCDEF possesses a broad substrate specificity and can oxidize different chemical groups, including ring heteroatoms and C=C double bonds [1]. This enzymatic activity has potential for synthetic applications since it is a challenging task to selectively oxidize chemical groups of different reactivity in a single molecule by conventional chemical methods [2].
In this work, we investigated the selectivity of PmlABCDEF monooxygenase with substrates bearing two possible reaction sites – terminal C=C double bond and nitrogen atom in the pyridine ring. Hence, alkenyl-substituted pyridine compounds having different lengths of carbon chains were synthesized from 3-pyridinol and appropriate alkenyl bromides. Produced compounds were used in the bioconversion reactions with Pseudomonas putida KT2440 producing recombinant PmlABCDEF monooxygenase. Reaction products were identified as N-oxides and epoxides.

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8.9. FORMATION OF CALPROTECTIN (S100A8/S100A9) INHIBITS AGGREGATION OF S100A9 INTO AMYLOID COMPLEXES

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Pro-inflammatory S100A8 and S100A9 are members of the S100 calcium-binding protein family. Both of the proteins can be heavily upregulated in Alzheimer's disease (AD) patients' brain tissues. S100A9 forms neurotoxic amyloid fibrils, which may contribute to the pathology of AD. Differently from S100A9, S100A8 assembles into non-fibrillar aggregates in the brain tissues. Together, S100A8 and S100A9 form a heterodimeric complex (calprotectin (CP)), which plays an important role in inflammatory processes.

During the amyloid aggregation process, a transformation from the native state of the protein into cross β-sheet structures occurs. Existing evidence implies that S100A9 forms distinct aggregates, which retain a significant amount of α-helical motives alongside β-sheets. Alongside, there are no detailed studies of S100A8 and CP aggregation in vitro.

In this study, we monitored S100A8, S100A9 and CP aggregation using thioflavin T fluorescence assay, Fourier transform infrared spectroscopy (FTIR) and Atomic Force Microscopy (AFM). Results revealed that aggregated S100A8 contains atypical α-helical motives, similar to S100A9 fibrils. However, AFM imaging indicated S100A8 aggregates as spherical oligomers, differently from S100A9 short worm-like fibrils. Lastly, we observed that CP formation inhibits both S100A8 and S100A9 aggregation in vitro, revealing an important protective function of CP.

8.10. MONOCLONAL ANTIBODIES AGAINST SARS COV-2 NUCLEOCAPSID PROTEIN: DEVELOPMENT AND APPLICATION

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Detection of new cases of COVID-19 is essential for controlling the spread of human coronavirus SARS CoV-2. Nucleocapsid (N) protein of SARS CoV 2 is characterized by early and abundant synthesis during infection, which is considered a potential serological marker for application in diagnostic systems. In this research the main aim is to develop monoclonal antibodies (MAbs) specific to SARS CoV 2 virus N protein and apply them in a sandwich enzyme-linked immunosorbent assay (ELISA) system. Because there are several homologies of the N-terminal part of nucleocapsid with other coronaviruses N proteins, to reduce the likelihood of non specific antibody interactions with them a truncated version of the N protein (N121-419) was used to immunize BLAB/c line mice. By evaluating humoral response in experimental animals, it was revealed that after the immunization plasma cells are secreting antibodies specific to SARS CoV-2 N121-419 and SARS-CoV-2 N antigens. Hybridoma technology was used to to generate cell lines secreting MAbs specific both to SARS-CoV-2 N and N121-419. Favourable combinations between MAbs from the generated collection were evaluated by the competitive ELISA method. After the analysis thirty pairs of antibodies were formed. The recognition effectiveness was tested by the sandwich ELISA method. Eleven new MAbs combinations capable of attaching and visualizing SARS-CoV 2 N antigen in solution were confirmed. Additionally, for previously generated coating 4G6 MAb, which was developed against full-length SARS-CoV-2 N, two alternatives of anti-SARS-CoV-2 N121-419 MAb HRP compatible conjugates were detected. Considering these results, the usage of truncated SARS-CoV-2 nucleocapsid protein is equally effective in the development of specific MAbs with the benefit of avoiding interaction with homologous proteins of other coronaviruses.

This work was part of the project "Development of diagnostic antibodies against SARS-CoV-2" has received funding from European Regional Development Fund (project No 13.1.1 LMT K 718 05 0031) under grant agreement with the Research Council of Lithuania (LMTLT). Funded as European Union's measure in response to Cov-19 pandemic.

8.11. DISPLAY OF ACINETOBACTER BAUMANNII BLP1 PROTEIN C-TERMINAL FRAGMENT ON THE SACCHAROMYCES CEREVISIAE SURFACE

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Acinetobacter baumannii is an opportunistic gram-negative pathogen responsible for numerous hospital-acquired infection cases. Its multidrug-resistant strains cause intractable secondary infections while the situation was aggravated by the global pandemic. In 2017, WHO has classified the culprit among highest priority pathogens for new treatment development [1].

As an additional barrier to control spread, vaccines may provide a solution. However, there have been no candidates in clinical trials. The pathogen's ability to form biofilms and conceal most surface proteins under its capsule pose major challenges in finding feasible antigen targets. Fortunately, Blp1 is an exposed protein that has a conservative C-terminal domain universal to most prevalent *A. baumannii* clinical strains [2].

Yeast display of antigens enables oral vaccine development. First, yeast *Saccharomyces cerevisiae* has GRAS status. Moreover, glycans in the cell wall function as natural immunostimulants. The antigen of interest is anchored on the yeast surface using cell wall proteins, such as a-agglutinin in our implementation [3].

The aim of this study was to display the C-terminal 163 amino acid fragment of Blp1 protein on the surface of *S. cerevisiae*. The antigen was fused with Aga2 subunit for display. To confirm its expression and detect location, western blot and immunofluorescence assays were performed. The surface display efficiency was determined by flow cytometry analysis, which indicated that 48.5% of singlets displayed antigen. The results suggest that this platform is an amenable strategy to develop oral *A. baumannii* vaccine prototype.

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8.12. LYSOZYME AMYLOID FIBRIL STRUCTURAL VARIABILITY DEPENDENCE ON INITIAL PROTEIN FOLDING STATE

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Aggregation of amyloid proteins is associated with various amyloidoses, including neurodegenerative Alzheimer's or Parkinson's diseases. Although for many years scientists have been trying to find out the mechanisms of amyloid aggregation, this field is still not fully explored. Studies show that various environmental conditions, such as protein concentration, solution ionic strength, pH or temperature can change the course of aggregation and affect the polymorphism of the resulting amyloid fibrils. In this work, we investigated whether this type of variability is also present in the case of hen egg-white lysozyme (related to lysozyme amyloidosis).

Four temperatures (50 – 65°C) were chosen based on the results obtained from a thermal shift assay under the selected experimental conditions (PBS containing 2M guanidine hydrochloride, pH 7.4). Aggregation was monitored under constant 600 rpm agitation, using the fluorescent dye thioflavin-T. The structures of the resulting amyloid fibrils were analyzed using Fourier-transform infrared spectroscopy and atomic force microscopy.

The results showed that the initial protein folding state affected not only aggregation parameters (lag time and apparent rate constant) and the resulting amyloid fibril secondary structure variability, but also their self-replication propensity. In addition, these changes occurred over a small temperature range where the protein's folding state changed.

8.13. THE USE OF MONOCLONAL ANTIBODIES FOR THE IMMUNODETECTION OF BACTERIAL B-LACTAMASES CONFERRING TO ANTIMICROBIAL RESISTANCE

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According to the World Health Organization (WHO) antimicrobial resistance (AMR) is one of the greatest threats to global health in this century. Misuse of antimicrobials is the main driver in the development of new AMR mechanisms and difficulties in treating common infectious diseases. Therefore, development of reliable and rapid diagnostic tools is a priority in the context of AMR. Bacterial β-lactamases that degrade β-lactam antibiotics can be used as potential targets for diagnostics.

The aim of this study was to develop β-lactamase specific monoclonal antibodies (MAbs) to use them as molecular tools for diagnostic purposes in multiplex lateral flow immunoassay (LFIA). This method is rapid, simple to perform and highly promising for point-of care diagnostics. Considering the WHO list of antibiotic resistant priority pathogens, we have selected four β-lactamases – ACT-14, NDM-1, PDC-195 and CMY-34 – as targets for MAb development. Recombinant antigens have been produced in *Escherichia coli* and large collections of mouse MAbs against each target have been generated by hybridoma technology (60 MAbs in total). The most promising pairs of MAbs have been selected to develop multiplex LFIA for detection of each β-lactamase. The assay has been optimized and currently is being tested with samples of resistant bacteria producing natural β-lactamases. We believe that LFIA for detection of β-lactamases has high diagnostic potential.

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8.14. NON-THERMAL PLASMA-INDUCED EFFECTS IN STEVIA REBAUDIANA: MISSING PIECES OF THE PUZZLE

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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. Stevioside (Stev) and rebaudioside A (RebA) are the most abundant out 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 RebA concentration and higher RebA/Stev ratio.

In the recent decade, seed treatment with non-thermal plasma (NTP) 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 plant, revealed some NTP-induced changes that can be reproduced in various cultivars from different seed sources, seed storage times or plasma generator types. NTP was shown to increase SGs amount up to 11 times, to increase RebA/Stev in less RebA than Stev-rich cultivars. The question of the tendency of NTP to decrease biomass remains to be addressed concerning how these changes influence economic benefit. The more detailed mechanism of effects interplay can be elucidated by transcriptome analysis in the future.

8.15. THE EFFECT OF DGAE FRAGMENT CONCENTRATION ON ITS SELF-AGGREGATION AND ASSOCIATION WITH TAU PROTEIN

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Protein aggregation into amyloid fibrils is associated with several widespread neurodegenerative disorders. Alzheimer 's disease is one of the most prominent cases, with an ever-increasing number of afflicted patients. The disorder is characterized by memory loss and is considered to be caused by amyloid-β extracellular plaques and neurofibrillary tangles made from hyperphosphorylated Tau. In vitro Tau protein filament formation is induced by the presence of polyanions such as heparin. A truncated form of Tau protein (dGAE), which can assemble into filaments without polyanions, forms a proteolytically stable core region in full-length Tau paired helical filament (PHF). There are a lot of disputed questions concerning the physiological and pathological consequences of PHF-core Tau self-assembly, therefore we aim to elucidate the influence of dGAE fragment on Tau protein aggregation. Looking at the results we can see that the aggregation halftime and lag time decrease when dGAE fragment concentration increases. FTIR spectra show that when dGAE aggregates, the resulting dGAE fibrils associate with monomeric Tau protein.

8.16. GENERATION AND CHARACTERIZATION OF RECOMBINANT DOG ALLERGENS AND ALLERGEN-SPECIFIC MONOCLONAL ANTIBODIES

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Dogs are one of the most commonly kept pets in the world. They are also a major source of allergens. High prevalence of dog allergens in every day environment makes them difficult to avoid. To cope with high number of allergic patients, large efforts are taken to offer informative and valuable allergy diagnostic tests. Currently, low-precision allergen extracts – naturally derived heterogeneous mixtures of allergens – are widely used in the allergy diagnostics and specific immunotherapy. The main problems of extracts are variations in their composition and the lack of standardization. As an alternative, recombinant allergen components have been introduced in allergy diagnostic systems.
In response to the increasing need for the diagnosis and treatment of allergic diseases caused by pets, recombinant dog allergen components Can f 2 and Can f 8 were synthesized in Escherichia coli. The antigenic and immunogenic properties of purified recombinant allergens were analysed. Monoclonal antibodies (MAbs) specific to recombinant Can f 2 and Can f 8 were generated, purified and characterized. These MAbs can be used for the identification and quantification of allergen components in dog allergen extracts, which is of great importance for their proper standardization.

8.17. ACTIVATION OF ABSCISIC ACID CATABOLISM IS INDUCED BY SEED TREATMENT WITH COLD PLASMA AND FOLLOWED BY A STRONG POSITIVE EFFECTS ON GROWTH AND PRODUCTIVITY OF BROAD BEAN (VICIA FABA)

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Aiming to explore the molecular reasons for the strong positive impact of seed treatment with cold plasma (CP) on agronomic plant performance, we followed CP-induced changes in seed abscisic acid (ABA) amount, the activity of ABA oxidizing enzyme ABA-8'-hydroxylase, effects of CYP450 inhibitor diniconazole on germination of broad bean (*Vicia faba*). Field experiment was performed to estimate the effects of seed treatment with CP on plant growth, photosynthetic activity, biomass gain and productivity. The obtained results indicate that CP-induced enhancement of germination rate (by 2.3 times) in vitro is related to a strong decrease in seed ABA content as well as to the increased activity of ABA-8'-hydroxylase (by 2.7 times compared to control). In addition, the ABA-8'-hydroxylase inhibitor diminished the stimulating CP effect on the seed germination rate. Seed treatment with CP resulted in slight inhibition (by 10%) of seedling emergence in the field and did not change the efficiency of the photosynthetic system. However, at the end of the vegetation season plant weight, stem number, root weight, number and weight of pods, and weight of nodules in the CP-treated group were 87, 59, 74, 88 and 90, 58% larger in comparison to the control group.

8.18. VARIATION IN THE CONTENT OF SECONDARY METABOLITES IN RADISH (RAPHANUS SATIVUS L.) MICROGREENS CAUSED BY SEEDS COLOR AND SEEDS TREATMENT WITH COLD PLASMA

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It has been shown that atmospheric cold plasma (CP) treatment affects seed germination kinetics, the content of phytohormones and antioxidants in seeds and in plants. These changes are also seed color dependent. Variation in the content of secondary metabolites is important for the nutritional values of microgreens. This study aimed to estimate how seeds treatment by CP and seed color affect the early growth as well as the content of photosynthetic pigments and secondary metabolites in sprouts of two cultivars of *Raphanus sativus*. Seeds of 'Fujitaseed' and 'Nichinou' cultivars were separated by the color to grey and brown and irradiated for 3 min using a scalable dielectric barrier discharge (DBD). Results showed that microgreens response to seed irradiation was strongly cultivar dependent. While DBD plasma had no effect on the content of metabolites in 'Nichinou' seeds sprouts, it was effective in 'Fujita' sprouts: antioxidant activity was increased (by 62% for sprouts from grey and 13% - from brown seeds, compared to control). The content of flavonoids and carotenoids was increased (by 25% and 49%, compared to control), and the amount of chlorophyll b was decreased (by 24%), but only in brown seed sprouts. Additionally, the DBD plasma reduced the total content of phenolic compounds (TPC) in grey seed sprouts of both cultivars by 12%, while it did not have an effect on brown seed sprouts.

9. GENETIC DIVERSITY AND SYSTEMS BIOLOGY

9.1. PROFILING THE TUMOR MICROENVIRONMENT OF CLEAR CELL RENAL CELL CARCINOMA USING SINGLE CELL RNA SEQUENCING

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Clear cell renal carcinoma (ccRCC) is the most prevalent renal cancer, accounting for over 75% of cases. The asymptotic nature of the disease often leads to diagnosis in III or IV stage, where survival probability drops to 59% and 20%, respectively. Moreover, approximately 30% of cases metastasize (1). ccRCC is characterized as a highly vascularized and immune infiltrated tumor, and tumor microenvironment heterogeneity has an impact on disease progression and response to therapy. Using droplet-based single-cell RNA sequencing we profiled 50,236 cells from paired tumor and healthy adjacent kidney tissues. Our analysis revealed high heterogeneity and inter-patient variability of the tumor microenvironment. We characterized the heterogeneous tumor vasculature and discovered a previously uncharacterized subpopulation associated with epithelial-mesenchymal transition. Additionally, cell-cell communication analysis revealed multiple modes of immunosuppressive interactions in the tumor microenvironment, including interactions between tumor vasculature and stromal cells with immune cells. Furthermore, expression of the genes involved in these interactions is associated with worse survival in the TCGA cohort. Overall, our analyses reveal the unappreciated involvement of tumor vasculature and stromal cell populations in shaping the tumor microenvironment and uncovers a novel tumor vasculature subpopulation associated with invasive phenotype.

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9.2. COMPARISON OF MICROORGANISMS AND GEOCHEMISTRY WITH WATER DEPTH FROM THE SHALLOW MARINE FE-MN NODULES

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Fe-Mn nodules are composed mainly of Mn and Fe with some valuable metals such as Cu, Co, Ni, REEs. They generally form atop or within the first few cm of seafloor, and usually distribute in deep-sea abyssal plains of world's oceans, but also in the shallow-water environment for instance Baltic Sea and several seas in the Arctic Ocean. Through the 2021 ARA12B Expedition, 451 Fe-Mn nodules were acquired from two sites at water depths of 73 m (St58i) and 150 m (St52) in the Arctic Ocean using dredge. Micro XRF analyses show that Mn was abundant in dendritic and porous structures, while Fe, Co and P were abundant in the Fe-oxide layer without water depth. However, major, minor and REEs analyses using ICP-OES and ICP-MS reveal that there are some differences between them. Nodules from St52 have higher SiO2, MnO, Ni, Zn, Cu, but lower Fe2O3, P2O5, Co and REEs than those from St58i. Therefore, nodules from St52 show higher Mn/Fe ratio than those from St58i. Microorganisms are observed under SEM observation from both sites and Mn- and Fe-bearing minerals are closely related with them. However, the contribution of microorganism for the formation of Fe-Mn nodules are not certain and is an important challenge in this research field.

10. MOLECULAR FOUNDATIONS OF LIFE

10.1. TIBA AND PACLOBUTRAZOL EFFECT ON ENDOGENOUS GIBBERELIN LEVELS AND GENE EXPRESSION WITHIN OVARIES OF HERACLEUM SOSNOWSKYI

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Nature Research Centre

Sosnowsky's hogweed (*Heracleum sosnowskyi* Manden.) is a plant known among Invasive Alien Species that highly concerns the EU [1]. It's ability to destroy native ecosystems and toxicity for human health is worrying. Hogweed spreads only by seeds and dies after reproduction. Crosstalk between plant hormones – gibberelin and auxin, is crucial for seed development and inducing seedlessness by their modulation [2,3]. We analysed the endogenous levels of gibberelins and gene expression within ovaries of terminal and satellite inflorescences, during their early development stages. To affect gibberellin homeostasis, auxin transport inhibitor TIBA and early gibberellin biosynthesis inhibitor Paclobutrazol, were used. Results revealed dissimilarity in quantity of gibberelins among two orders of umbels, suggesting a unique mechanism of gibberelin homeostasis. TIBA increased the hormone level only in terminal umbels supporting the idea of apical dominance. Interestingly, high levels or deprivation of hormones in particular inflorescences, could disturb seed development. qPCR analysis revealed a significant up-regulation of GA biosynthesis genes 20-ox, 3-ox in satellite umbels after Paclobutrazol treatment, which is in consequence of increased endogenous GAs content. Obtained results may contribute to the development of environmentally friendly strategies to control the invasion of monocarpic hogweed species.

10.2. SHORT-TERM HYPOXIA IN CELLS

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Decreasing oxygen supply to the cells and tissues of the body is defined as hypoxia and is associated with the pathophysiology of many disease states. A hypoxic environment imposes stresses that can damage or kill the organism in the absence of an appropriate response. However, hypoxic tissues develop necessary adaptation mechanisms both at the tissue and cellular level.

The cellular adaptations involve a dramatic alteration in gene expression, post-transcriptional and post-translational modification of gene products. Alternative splicing of pre-mRNA is one of the regulatory mechanisms through which cells are able to respond to hypoxic conditions. The preferable expression of certain splice variants over others is one of the major strategies chosen by cells while adapting to hypoxic stress.

In our study, we demonstrate that the short-term hypoxia in cells induces expression of genes which are enhanced in stressed cells. Obtained results revealed different mRNA isoform formation patterns in cells under short-term hypoxic conditions and prolonged hypoxia.

10.3. THE APPLICATION OF LACTADHERIN C2 DOMAIN FOR IN VITRO, EX VIVO AND IN VIVO LABELING OF PHOSPHATIDYLSERINE EXPOSURE

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Phosphatidylserine (PS) dynamics is an important feature of eukaryotic cells. Under normal conditions, PS is found in the inner leaflet of the plasma membrane. Disruption of this asymmetry, leading to the exposure of phosphatidylserine on the cell surface, is fundamental in apoptosis and many physiological processes. An efficient PS marker is needed to study these processes. Currently, different commercial PS labelling tools are available. However, these tools have significant limitations and are ineffective for in vivo labeling due to tissue damaging injection.
We have developed a genetically encoded PS marker based on C2 domain of lactadherin (MFG-E8) protein, which is applicable to label exposed PS in different research models. Biophysical analysis revealed C2 domain specificity to PS and that fused markers C2-SNAP, C2-mKate maintain this feature. These recombinant proteins are suitable for PS labelling on apoptotic cell surface in different cell lines. Because injections are not effective for marker delivery into tissues, we applied engineered AAVs as a delivery method. C2 domain constructs were delivered with AAVs and labeled apoptotic cells in tissue culture. Genetically encoded markers are also applicable in vivo due to observed effective brain expression of fused C2 markers after intravenous AAV injection in mouse.

10.4. INVESTIGATION OF PRIMARY MOUSE MICROGLIA ACTIVATION BY IMMUNE COMPLEXES OF VIRUS-LIKE PARTICLES

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Microglial cells are immune cells of the central nervous system and important part of innate immunity. Activation of microglia is associated with cell transformation to phagocytes, capable of releasing potentially cytotoxic substances including cytokines and chemokines with immunological and neuroregulatory effects. The aim of this study is to determine the influence of immune complexes on the release of cytokines in primary mouse microglia.

Primary microglia cell culture was prepared from newborn C57BL/6 mice and treated with virus-like particles (VLP) and immune complexes (IC) of VLP. IC were composed of VLP of WU polyomavirus (WUPyV) and mouse immunoglobulins specific to WUPyV. Different subtypes of immunoglobulins were used: IgG1, IgG2a and IgG2b. Microglia activation was studied by detection of released inflammatory cytokines, such as TNF-α and chemokines, such as CXCL1 using the enzyme-linked immunosorbent assay method and immunoblotting.

Our study shows that IC of WUPyV activate microglial cells. In addition, it was found that different amounts of various cytokines and chemokines were released after the treatment of prepared IC. In conclusion, our results show that IC of WUPyV provide cytokine and chemokine release which is a sign of microglia activation.

10.5. MICROGLIAL NLRP3 INFLAMMASOME ACTIVATION BY IMMUNE COMPLEXES OF VIRUS-LIKE PARTICLES

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The inflammasome is a vital component of innate immunity. The best-described inflammasome is NLRP3, which contains three major components – nucleotide-binding and oligomerization domain-like receptor, adapter protein apoptosis-associated speck-like protein (ASC) and caspase-1. NLRP3 inflammasome activation results in release of inflammatory cytokines, like IL-1β, and inflammatory cell death – pyroptosis. In our previous research we showed that viral proteins triggered NLRP3 inflammasome activation depending on their structure. The aim of this study was to extend the latter research and determine whether immune complexes of oligomeric proteins could change the NLRP3 inflammasome activation in macrophages.

Primary mouse microglia were selected as cell culture model. Cells were treated with spherical virus-like particles (VLPs) of these human polyomaviruses (PyV): KIPyV, WUPyV, and their immune complexes. NLRP3 activation was studied by evaluating cell viability, IL-1β and TNF-α cytokine release and the formation of ASC specks.

It was found that spherical PyV-derived VLPs and their immune complexes induced cell death, IL-1β secretion and ASC speck formation in microglia indicating NLRP3 inflammasome activation. In addition, immune complexes mediated a significantly higher cellular response compared to VLPs alone. To conclude, our results demonstrate that immune complexes can enhance inflammasome activation.

10.6. VIRULENCE-ASSOCIATED CHARACTERISTICS OF OPPORTUNISTIC PATHOGEN STENOTROPHOMONAS MALTOPHILIA FROM ENVIRONMENTAL AND CLINICAL ORIGIN

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Stenotrophomonas maltophilia is Gram-negative multidrug resistant biofilm forming bacterium widely distributed in the natural environment, while also known as an emerging opportunistic pathogen causing serious bloodstream, pulmonary, urinary tract infections. Although S. maltophilia infections mortality rate can reach nearly 70%, it is still not clear which traits are responsible for its ability to cause infections and how to distinguish between pathogenic and non-harmful S. maltophilia types. The aim of this work was to analyse clinical and environmental S. maltophilia isolates and compare their phenotypic and genotypic virulence related characteristics. Collection of 42 environmental and 33 clinical isolates was used in this study. For genotypic characterisation, antibiotic resistance and virulence genes detection was performed. For phenotypic characterisation, antibiotic resistance to most commonly used antibiotics was analysed. Biofilm formation, swarming and twitching motility were analysed at environmental (28°C) and human body (37°C) temperatures. Results indicated that only few environmental isolates showed similar phenotypic and genotypic traits to clinical isolates, majority of environmental isolates were not able to express virulence associated traits at host body temperature. Therefore, only small fraction of environmental isolates could be a source of *S. maltophilia* infections.

10.7. SEQUENCE-SPECIFIC RECOGNITION OF GUIDE AND TARGET STRANDS BY *Archaeoglobus fulgidus* ARGONAUTE PROTEIN

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Prokaryotic Argonautes (pAgos) provide innate immunity to exogenic nucleic acids, such as plasmid or bacteriophage DNA. All pAgos share a similar mechanism of action, they use DNA or RNA guides to recognize complementary DNA or RNA targets. Upon target recognition, pAgo either cleaves the target strand or employs effector proteins, which leads to degradation of invading nucleic acids or, in some cases, cell death. Knowledge of preferred guides and targets reveals valuable information about how pAgos obtain their guides and recognize invading nucleic acids.

The object of this study is an archaeal Argonaute protein AfAgo from *Archaeoglobus fulgidus*. In this study we use electrophoretic mobility shift assay (EMSA) to provide experimental evidence that AfAgo specifically recognizes RNA guides (gRNA) with 5′ AUU sequence. Studies show that pAgos may exhibit specificity to the guide strand 5‘ terminal nucleotide and respective complementary target strand nucleotide. However, this is the first example of more extensive specificity towards guide and target sequence. We also demonstrate that AfAgo:gRNA binary complex preferentially binds complementary DNA targets. These results challenge previous knowledge about AfAgo, since it was thought that AfAgo uses ssDNA guides and recognizes DNA targets, possibly due to the use of nonoptimal 5′-sequence guides in experiments.

10.8. ANTIFUNGAL ACTIVITY EVALUATION OF ESSENTIAL OILS AGAINST MALASSEZIA PACHYDERMATIS

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Lithuanian University of Health Sciences

With the increasing resistance of the microorganisms to antifungal and antimicrobial agents, it is very important to discover alternatives for effective treatments in inhibiting pathogenic microorganisms. It is known that due to the increasing resistance of bacteria and fungi, there will be a severe shortage of drugs in the near future for the effective antimicrobial treatment.

For the determination whether the essential oil has antibacterial or antifungal activity different techniques could be used. The antibacterial activity of essential oils is effectively tested by the agar disc diffusion method, where the essential oils are added to agar in which the bacterial strain is uniformly infected. The result obtained is evaluated according to the size of the disc area and it is determined whether the essential oil has an antimicrobial effect. After incubation, the inhibition zone reflects the antimicrobial effect.

In this study 11 samples of the dogs were collected from both ears. Nine dogs were not identified with clinical symptoms consistent with otitis symptoms characteristic of otitis media in two dogs shaking of the ears, digging, discharge, unpleasant odor. The samples taken were collected using TransSwab bacteriological tubes. All samples were inoculated on petri dishes using dextrose agar. Samples are grown for 5 days at 30 degrees. In all the samples the *Malassezia* was observed, following a cytological test *M.pachydermatis* was identified.

Efficiency evaluation results of the different essential oils in various concentrations on the *M.pachydermatis* will be presented. This study allows to conclude that essential oils is very promising alternative for the traditional treatment in veterinary medicine.

10.9. STUDY OF NEW ANTI-PHAGE DEFENSE SYSTEMS

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Bacteriophages are incredibly diverse and widespread viruses that constantly threaten bacteria. The continuing coevolutionary fight between bacteria and their viral predators has resulted in the development of sophisticated and diverse array of bacterial defense mechanisms such as well known restriction-modification and CRISPR-Cas systems. Discovery of these systems led to the development of precise molecular tools used in genetic engineering and genome manipulation, thus the identification of novel defense systems may result in the development of new tools for manipulating cells and genomes. On the basis of the discovery that anti-phage defense systems frequently form so-called "defence islands" in bacterial genomes, several groups have employed computational methods to detect new uncharacterized defense systems [1, 2]. Here, we present research of nine of these newly discovered systems which have well known nuclease, NTPase and DNA/RNA helicase domains. We showed that these systems are active and possess resistence against bacteriophages. Also, we successfully cloned these systems into high-copy plasmid DNAs and showed protein expression.

10.10. CYCLIC HEAT-STRESS IN THE DARK CHANGING THE RELATIVE LEVELS OF VIOLAXANTHIN AND ANTHERAXANTHIN IN ARABIDOPSIS THALIANA (L.) HEYNH SUGGESTS A POSSIBLE LINK BETWEEN VIOLAXATNHIN CYCLE PIGMENTS AND PLANT MEMORY MECHANISMS

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Due to the progressing climate change, the impact of heat stress on the functioning of photosynthetic organisms must be studied intensively. An important mechanism involved in the stress response is plant memory. One of its concepts states that after the first exposure to stress, the stress-response parameters change, after regeneration they return to values closer to the physiological ones, and after the next exposure the change is intensified. According to the established photoperiod, after 10 hours of darkness, three-week-old Arabidopsis thaliana (L.) Heynh were shielded from light and heat-primed. After 2 days at standard conditions, they were placed at 45 °C in the dark for 90 minutes. The same stressing procedure was repeated after two days. Using the HPLC method, the relative levels of photosynthetic pigments were studied. The level of antheraxanthin in once stressed A. thaliana was subtly increased, and the level of violaxanthin decreased, against the control. After the second stress, these changes intensified. These trends would indicate the existence of links between the studied pigments level and the mechanisms of plant memory under heat stress. The described pattern of changes in pigment levels obtained in the dark is identical to that of the de-epoxidation reaction requiring light.

10.11. LOSS OF THE BKCA CHANNEL CAUSES AN INCREASE IN MITOCHONDRIAL REACTIVE OXYGEN SPECIES IN GLIOBLASTOMA CELLS

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Mitochondrial potassium (mitoK) channels play an important role in cellular physiology. In cancer cells, inhibition of mitoK channels leads to an increase in mitochondrial reactive oxygen species, which leads to cell death. In glioma cells activity of the mitochondrial, large conductance calcium-activated potassium (mitoBKCa) channel is regulated by mitochondrial respiratory chain.
In our project, we used CRISPR/Cas9 technology in glioblastoma U-87 MG cells to generate knockout cell lines lacking the α-subunit of the BKCa channel encoded by the KCNMA1 gene. Mitochondrial patch-clamp experiments showed the absence of an active mitoBKCa channel in knockout cells. Additionally, the absence of this channel resulted in increased levels of mitochondrial reactive oxygen species. However, analysis of the mitochondrial respiration rate did not show significant changes in oxygen consumption in the cell lines lacking BKCa channels. The expression levels of selected mitochondrial genes, organization of the respiratory chain, and mitochondrial morphology did not show significant differences between the analyzed cell lines. In conclusion, we show that the pore-forming subunit of the mitoBKCa channel is encoded by the KCNMA1 gene in U-87 MG cells. Additionally, the presence of this channel is important for the regulation of reactive oxygen species levels in mitochondria.
This project was supported by Polish National Science Center grants No. 2015/18/E/NZ1/00737 and 2019/34/A/NZ1/00352.

Jędrzej Sniadecki's Memorial Conference "Frontiers in Molecular Life Sciences" (JSMC2023)

Išleido Lietuvos Biochemikų Draugija

Vilnius

ISBN 978-609-96030-2-5