14 STUDENTS' BIOTECHNOLOGY CONFERENCE

BOOK OF ABSTRACTS

FINAL VERSION

PREPARED BY: *AGATA STACHURA AND ALEKSANDRA TUŃSKA*

Spis treści

HONORARY SPEAKER

Prof. Ewa Łojkowska

Professor Ewa Łojkowska, chairwoman of the Committee of Biotechnology of the Polish Academy of Sciences, Dean of IFB 2005-2012. She graduated from University of Nicolaus Copernicus in Toruń in 1977 with MSc in biology. In 2001 she was awarded with the Professor title in biological sciences. A specialist in the field of molecular factors determining the pathogenicity of bacteria, bacterial plant pathogen biodiversity, and epidemiology, as well as the molecular basis of plant resistance to bacteria and molecular taxonomy. The second focus of her research includes issues related to biologically active plant secondary metabolites and their potential use for human health protection, as well as biotechnological methods for propagating rare and endangered plant species (such as Polish orchids and insectivorous plants). She is the founder of a scientific school that encompasses research on biodiversity, taxonomy, and the molecular factors determining the pathogenicity of bacterial plant pathogens.

"During the inaugural lecture, I will discuss the biography and academic, teaching, and organizational achievements of Professor Anna Jadwiga Podhajska. She is the initiator of the establishment of the IFB UG and MUG and has significantly contributed to shaping the functioning principles of our faculty."

TUESDAY 6TH JUNE

SESSION 1

LABORATORY OF MOLECULAR BIOLOGY

TITLE: Analysis of protein activity of variants DnaA V211A and R245A in process of initiation of plasmid replication

SPEAKER: ***Mateusz Modzelewski***

SUPERVISOR(S): ***Katarzyna Węgrzyn, PhD;***

DnaA is a bacterial protein that functions in the initiation of chromosomal DNA replication; hence, this enzyme controls one of the most crucial mechanisms in cell life. In the first step of initiation, Escherichia coli DnaA binds itself to specific sequences of DNA (DnaA-box) in the replication origin (oriC). The protein complex formation on DNA results in the double helix melting at oriC DNA unwinding element (DUE). Next, one of the single-stranded DNA (ssDNA) of DUE is recruited to the DnaA complex on DnaA-boxes, stabilizing the whole complex and initial DUE unwinding. Furthermore, DnaA participates in the initiation of some plasmid DNA replication, except the process is less known than chromosomal DNA replication. Plasmid RK2 has the replication origin (oriV) consisting of DUE, four DnaA-boxes bound by host DnaA and five direct repeats (iterons) bound by plasmid encoded replication initiator, TrfA protein. The presence of both DnaA and TrfA proteins is required to initiate RK2 replication. In this project, I focused on the role of DnaA in the initiation of plasmid replication. For investigation I purified variants of DnaA, the wild-type (WT) and with single amino acid substitution V211A and R245A, which were shown previously to have no binding activity to ssDNA of DUE oriC. To measure the affinity of DnaA variants to ssDUE top and ssDUE bottom strands of oriV, I used Biolayer interferometry (BLI). It showed weaker affinity to both DNA molecules for variant R245A compared to WT and V211A. Unlike oriC, where helicase assay indicated that mutants have no ability to recruit helicase, experiments using oriV showed that mutants still hold enzymatic activity, although with various efficiencies. Therefore, the DnaA role in plasmid DNA replication may differ from replication initiation of bacterial chromosomes; however, understanding this role needs further research.

LABORATORY OF PHYSICAL BIOCHEMISTRY

TITLE: Analysis of the effect of thymoquinone on the stability of cholecalciferol using the HPLC technique

SPEAKER: ***Paweł Chodorowicz***

SUPERVISOR(S): ***Leszek Kadziński****, PhD;*

Vitamin D3 has multiple beneficial effects in humans: it helps the body absorb calcium and phosphorus, plays a role in supporting immune function and regulation of cardiovascular functions. However, its preparations are prone to oxidation and degradation.

The stability of cholecalciferol (Vitamin D3) is critical for its efficacy as a dietary supplement and pharmaceutical agent. To achieve high stability preparations of Vitamin D3 often include stabilizers – antioxidants, which are often artificial or require high concentrations to function.

Thymoquinone, a bioactive compound derived from Nigella sativa, is known for its potent antioxidant properties. This makes it a potential substance which could be used in their place to stabilize vitamin D3 preparations.

Currently it's used in many preparations, however, its effect on the stability of Vitamin D3 itself remains unknown.

The aim of this research is to investigate the impact of thymoquinone on the stability of cholecalciferol under various storage conditions and compare its efficiency to BHT - Butylated hydroxytoluene.

To do this I've employed a modified normal phase High-Performance Liquid Chromatography (HPLC) technique to quantitatively analyze the concentration of cholecalciferol in the presence of thymoquinone over time.

Tested samples were incubated for four weeks in different temperatures ranging from 5 to 50 degrees Celsius in order to simulate both ambient and accelerated aging environments.

Results showcase that thymoquinone affects the stability of cholecalciferol, additionally, this effect is correlated with the concentration of thymoquinone.

LABORATORY OF PHYSICAL BIOCHEMISTRY

TITLE: Influence of polyethylene glycol on bioencapsulated β-galactosidase. Modeling and optimization of composite's synthesis process.

SPEAKER: ***Aron Gorbacz***

SUPERVISOR(S): ***Leszek Kadziński****, PhD;*

The objective of this study is to investigate the effect of the concentration and molecular mass of polyethylene glycol (PEG) on the activity and stability of beta-galactosidase bioencapsulated in xerogel. Xerogels serve as a perfect matrix for enzyme bioencapsulation due to their porous structures which improves the accessibility of enzyme's active sites. In order to do that a Box-Behnken design of experiment model was used to obtain the most information within the least amount of synthesized probes. Enzyme activity and stability assays were conducted where beta-galactosidase was bioencapsulated in xerogel containing various concentrations of PEG of various molecular masses. The results indicate that only the molecular weight of PEG significantly influences the activity and stability of beta-galactosidase. PEG of low molecular weight increases enzymatic activity whilst high molecular weight PEG decreases it. The study demonstrated that the appropriate selection of PEG concentration and molecular weight is crucial for optimizing the activity and stability of beta-galactosidase in xerogel. These findings have significant implications for the biotechnological applications of beta-galactosidase, particularly in the context of enzyme stabilization in polymer matrices.

LABORATORY OF BIOPHYSICS

TITLE: Interaction of doxorubicin with platinum nanoparticles with diameters of 5 and 70 nanometeres

SPEAKER: ***Marceli Bogusławski***

SUPERVISOR(S): ***Jacek Piosik,*** *PhD, DSc;*

Doxorubicin (DOX) is regarded as one of the most crucial chemotherapeutic agents due to its efficacy. Unfortunately, it is also associated with severe side effects. To minimize these adverse effects, researchers propose combined therapies with other drugs or advanced drug delivery platforms. One of the approaches that could be promising is the combination of doxorubicin with platinum nanoparticles (PtNPs), which possesses anticancer activity and could potentially enhance the drug delivery.

The interactions between PtNPs of two different sizes (5 nm and 70 nm) and DOX was investigated using spectrometric methods and isothermal titration calorimetry (ITC). Additionally, to analyze the aggregation pattern for PtNPs with DOX, dynamic light scattering (DLS) was exploited. Furthermore, the impact of PtNPs on the mutagenicity of DOX was evaluated using the Ames assay on Salmonella enterica serovar Typhimurium TA98.

The results indicated that the titration of PtNPs of both sizes with doxorubicin resulted in an increase in molar extinction and a decrease in DOX fluorescence. Moreover, the enthalpy changes values revealed that the interaction between 5 nm PtNPs and DOX is exothermic, while between 70 nm PtNPs and DOX is endothermic. The hydrodynamic diameter increased in both cases, which suggests aggregation. Finally, the Ames assay confirmed that both sizes of PtNPs influence the mutagenicity of doxorubicin.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: The impact of DMVs formed by SARS-CoV-2 proteins on antigen presentation pathways

SPEAKER: ***Magdalena Marchewa***

SUPERVISOR(S): ***Andrea Lipińska,*** *PhD;*

Double membrane vesicles (DMVs) are induced in cells infected with certain positive sense ssRNA viruses. They work as viral factories – providing a safe and closed environment for viral genome replication. One of viruses taking advantage of this phenomenon is Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus is responsible for the most recent pandemic causing millions of deaths worldwide. Its (+)ssRNA genome consists of approximately 30 000 nucleotides that encode 16 non-structural proteins, 4 structural proteins and 9 accessory proteins. Non-structural proteins (Nsps) that are embedded in the endoplasmic reticulum (ER) membrane cause it to curve creating DMVs. Disruption of ER may impact early antigen presentation events, as its crucial components: the transporter associated with antigen presentation processing (TAP) or MHC class I and II reside in the ER.

The main hypothesis of my research is that DMVs formation affects antigen presentation processes. To investigate that problem I generated cell line models expressing transmembrane Nsp proteins that are the minimal DMV inducers (Nsp3 and Nsp4). For this purpose I used MelJuSo human melanoma cell line that expresses both MHC class I and class II. The expression of Nsp3 and Nsp4 genes has been confirmed by western blotting and flow cytometry. The generated cell lines will be next used for MHCI and MHCII level measurements, DMVs visualization using microscopy and tests of TAP activity.

I believe a more detailed understanding of the DMV formation process and its impact on the cell is crucial in developing antiviral therapies targeting DMVs in the future.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: Development of a model for structural studies of viral proteins and the antigen presentation pathway

SPEAKER: ***Pola Szydarowska***

SUPERVISOR(S): ***Andrea Lipińska****, PhD;*

Cryo-electron microscopy (Cryo-EM) has revolutionized structural biology by providing atomic-level details of macromolecular structures. This technique has gained widespread attention in recent years due to a series of technical advancements, often referred to as the 'resolution revolution'. Cryo-EM tomography 3D reconstructions offer invaluable insights into the functional mechanisms of macromolecules and their involvement in various biological processes. Cryo-EM is particularly valuable for studying large, dynamic protein complexes and membrane proteins that are unable to form crystal structures essential for X-ray crystallography imaging. Additionally, Cryo-EM complements other structural biology techniques, such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy.

The antigen presentation pathway is a crucial component of the immune system, playing a central role in identifying and eliminating pathogens and abnormal cells. During viral infection, cells express viral proteins that are cleaved by the proteasome and transported by the Transporter Associated with Antigen Processing (TAP) into the endoplasmic reticulum (ER). Inside the ER, with the assistance of chaperone proteins, viral antigens are loaded onto Major Histocompatibility Complex class I (MHC I) molecules. Finally, the MHC I molecules are transported to the cell surface, where cytotoxic T cells can bind to them and eliminate infected cells. Some viruses have evolved to evade this pathway by inhibiting and/or targeting crucial elements of this pathway for proteasomal degradation. This strategy is often utilized by members of the Herpesviridae family to establish persistent infection within the host. Viral TAP inhibition may prevent conformational changes and lead to transporter stabilization, which can be utilized to resolve its structure with high resolution. This strategy was previously implemented, resulting in the first image of the TAP transporter trapped by the Herpes simplex ICP47 protein.

The aim of my research is the development of a model system based on the HEK 293 GnTi- cell line (cells lacking N-acetyl-glucosaminyltransferase I (GnTI) activity and therefore devoid of N-glycans) for structural studies of viral proteins and the antigen presentation pathway, specifically focusing on the complex formed by the herpesvirus UL49.5 protein with TAP. This model will address instances where viral proteins lead to the proteasomal degradation of certain components of the antigen presentation pathway, hindering our ability to study these complexes in large amounts. To overcome this challenge, I introduced the Cas9 nuclease into the cell line to selectively delete (after introducing complementary single guide RNA) specific ubiquitin ligases responsible for the degradation, thereby preventing the breakdown of key components and enabling the robust production of these complexes for structural analysis. In this model, KLHDC3 was chosen for knock-out due to recent findings indicating its involvement in TAP degradation in the presence of UL49.5. Additionally, I compared the HEK GnTi- cell line to the more commonly used

lenti HEK cells, by analysis of MHC I and TAP transport levels, TAP transport activity using fluorescent peptides, and TAP susceptibility to degradation by UL49.5 in those modified HEK cells.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: Analysis of neutralizing properties of mouse sera after immunization with chimeric HBV/SARS-CoV2 virus-like particles.

SPEAKER: ***Weronika Kłopotek Główczewska***

SUPERVISOR(S): ***Katarzyna Grzyb,*** *PhD;*

The ongoing COVID-19 pandemic has enforced unprecedentedly quick development of vaccines targeting SARS-CoV-2. Most vaccines have demonstrated high efficacy in clinical trials and are now available for widespread use. However, the effectiveness of these vaccines is being questioned due to the emergence and spread of SARS-CoV-2 variants of concern, which contain multiple mutations in the spike (S) protein—the primary target of COVID-19 vaccines. Therefore, analyzing the neutralizing properties of existing and candidate vaccines against previously circulating and newer variants is crucial in current research. This importance highlights the role of neutralization assays, which are valued for their speed and accuracy.

SARS-CoV-2 is classified as a BSL-3 pathogen, complicating its use in neutralization assays by restricting the protocol and reducing the number of facilities that can perform such research. Promising alternatives include lentiviral pseudoparticles—a replication-defective lentivirus that displays the S protein on its surface. This system can be used in BSL-2 conditions allowing researchers to switch the S protein variant on the particles' surface. In previous studies, chimeric HBV/SARS-CoV2 VLPs that expose selected conserved epitopes of the SARS-CoV-2 spike protein were designed and utilized in mouse immunization studies. These VLPs induced a strong and specific antibody response; however, their neutralizing potency against different SARS-CoV-2 variants was not evaluated. In my thesis, I adjusted the neutralization assay protocol and characterized lentiviral pseudoparticles displaying three S protein variants: Wuhan, Delta, and Omicron, to assess the neutralizing properties of previously collected mouse sera. The study demonstrated that pseudoparticles were produced at a similar titer irrespective of the transfection reagent and successfully incorporated the S protein. Variations in the protocol, such as infection time, did not affect the outcomes. Moreover, the neutralization assay with reference spike-neutralizing antibodies matched the anticipated values for each variant. Finally, a neutralization test with mouse sera showed no neutralization against all tested variants. Nevertheless, this study confirms that lentiviral pseudoparticles are a reliable alternative to wild-type viruses for use in the BSL-2 laboratory.

SESSION 2

LABORATORY OF BIOMOLECULAR SYSTEMS SIMULATIONS

TITLE: Designing new substances against antibiotic-resistant strains of anthrax

SPEAKER: ***Hubert Mielziuk***

SUPERVISOR(S): ***Rajmund Kaźmierkiewicz,*** *PhD, DSc;*

Bacterial resistance, particularly multidrug resistance, is a global public health threat. Bacteria develop survival mechanisms in the face of antibiotics, leading to difficulties in treating infections. The consequences are severe: increased mortality, higher healthcare costs and economic losses. It is necessary to understand these mechanisms and develop prevention and treatment strategies, including new therapies and antibiotics.

To date, two different cellular receptors for anthrax toxin have been identified, TEM8 and CMG2. It is also known that their extracellular domains bind to one of the components of the toxin with low and high affinity, respectively. Given the low expression of TEM8 in tissues, inhibition of this receptor with the ligands I designed in this thesis may cause fewer potential side effects compared to the CMG2 that is commonly present in the body, making it a promising candidate as a key therapeutic target in the course of anthrax infection.

The aim of this work is to design new substances against antibiotic-resistant strains of Bacillus anthracis. The constructed molecules will be tested for their potential use as drugs, as well as estimating their potential toxicity.

To prepare the TEM8 receptor structure for the calculations, I used the SwissModel web server, while the Gromacs software was used for energy minimisation. I identified potential ligand-receptor interaction sites using the LigBuilder V3 software. The same program was used to design one million potential ligands, matching one active site I selected from among all those detected. I used the online tool SwissADME to determine the pharmacokinetic properties of the constructed ligands, and the servers MouseTox, admetSAR; and the VEGA software to estimate their potential toxicity. The ligands I constructed and selected with the highest probability to be an effective drug could be good candidates for synthesis and further in vitro / in vivo studies.

LABORATORY OF BIOMOLECULAR SYSTEMS SIMULATIONS

TITLE: Designing new HIV-1 reverse transcriptase inhibitors

SPEAKER: ***Wiktor Wójcicki***

SUPERVISOR(S): ***Rajmund Kaźmierkiewicz,*** *PhD, DSc;*

The Human Immunodeficiency Virus (HIV) is a lentivirus of retrovirus family that when untreated causes lethal AIDS (acquired immunodeficiency syndrome). Two species of HIV have recently been discovered: HIV-1 and HIV-2. In 2022 globally 630 000 people died from HIV related causes. In Poland, the same year 22 people died and 2384 were infected with HIV virus. There is available treatment for HIV infection is HAART or highly active retroviral therapy. Main goal of HAART is to stop replication of virus by using various classes of inhibitors. One of the classes used in this therapy are inhibitors of reverse transcriptase (RTIs). Main goal of this research is to design new compounds which would inhibit action of HIV-1 reverse transcriptase.

Software used for generation of candidate compounds is Autogrow4 used in tandem with QuickVina2Docking for molecular docking of new molecules to enzyme model. The obtained molecules of new compounds were sorted by its affinity to enzyme. The best obtained inhibitors underwent the ADME analysis performed by the web service SwissADME. From around 100 000 candidate molecules I have chosen 10 molecules which shows great affinity to reverse transcriptase and are orally bioavailable according to their physiochemical properties. They also have potentially low toxicity. The aim of this work is to design new substances against antibiotic-resistant strains of Bacillus anthracis. The constructed molecules will be tested for their potential use as drugs, as well as estimating their potential toxicity.

LABORATORY OF BIOLOGICALY ACTIVE COMPOUNDS

TITLE: Green synthesis of silver nanoparticles using Sea Buckthorn (Hippophae rhamnoides) - a plant with antioxidant properties.

SPEAKER: ***Nowak Antonina***

SUPERVISOR(S): ***Aleksandra Królicka,*** *PhD, DSc;*

This research explored the green synthesis of silver nanoparticles (AgNPs) using Sea Buckthorn (Hippophae rhamnoides) fruit extract with high antioxidant properties. Among 13 investigated plant species, Sea Buckthorn fruits exhibited the strongest antioxidant activity, making them ideal for AgNPs synthesis. After green synthesis optimization with three different methods (water bath, microwave and sonication), water bath with extract adjusted to pH 10 was chosen as the one yielding the most effective nanoparticles. Synthetized AgNPs demonstrated significantly higher antibacterial activity against Gram-negative bacteria (Escherichia coli) compared to Gram-positive bacteria (Staphylococcus aureus). Combining synthesized AgNPs with a Sea Buckthorn leaf extract demonstrated a synergistic effect, reducing the required AgNPs dose 4-8 fold for E. coli and 8 fold for S. aureus. This research highlights the potential of Sea Buckthorn-based AgNPs as a promising, eco-friendly approach for combating human pathogenic bacteria.

LABORATORY OF BIOLOGICALY ACTIVE COMPOUNDS

TITLE: Diverse effects of Drosera zigzagia extracts on multidrug-resistant environmental strains of Escherichia coli

SPEAKER: ***Anna Sawik***

SUPERVISOR(S): ***Marta Potrykus****; PhD;*

Antibiotic resistance has emerged as one of the major global health challenges of the 21st century, causing more than 700,000 deaths per year, with this number predicted to rise dramatically if radical actions are not taken. As emerging contaminants, antibiotic resistance genes (ARGs) can spread and persist in the environment via vertical gene transfer by bacterial proliferation and horizontal gene transfer mediated by mobile genetic elements, which generally transfer ARGs within the host genome or between the genomes of different bacterial hosts under external stress, facilitating the development of multidrug-resistant bacteria and thus posing a serious threat to public health.

Over the past few decades, the extensive long-term use of antibiotics has accelerated the dissemination of diverse ARGs and the proliferation of antibiotic-resistant bacteria (ARB) in the environment. Water is considered an ideal channel for the dissemination of ARGs and ARB into the environment. In the context of the rapid urbanization of cities, such emerging pollutants (ARGs and antibiotics) can drift towards receiving water bodies and end up in the marine ecological environment. The accumulation of antibiotics in coastal areas, transported there through rivers and streams, can provide major selective pressure for the proliferation of ARB and the horizontal transfer of ARGs. According to the WHO, by 2050, infectious diseases caused by drug-resistant microorganisms could result in the deaths of 10 million people annually.

One way to address this problem is by examining biologically active compounds present in both single- and multicellular organisms. Many of these compounds are found in herbs widely used in traditional medicine. Such medicines serve as a good alternative to synthetic drugs, with lower production costs and fewer allergic reactions. An example of such plant is Drosera zigzagia, a carnivorous plant that produces secondary metabolites with antimicrobial properties. Due to the limited nutrient availability in the habitats of Drosera spp., these plants have evolved certain adaptive mechanisms. These conditions drive sundews to generate a varied array of secondary metabolites, including naphthoquinones and flavonoids. Additionally, by cultivating Drosera zigzagia under in vitro conditions and employing various extraction techniques, the quantity of biologically active compounds could be enhanced.

In conclusion, the focus of this study was to investigate the biodiversity of environmental Escherichia coli strains isolated from different locations of Gdańsk Bay in terms of their genetic profiles and resistance to antibiotics and by treating them with D. zigzagia extracts explore alternative strategies to combat and prevent multidrug resistance dissemination in the environment.

LABORATORY OF PLANT BIOCHEMISTRY

TITLE: Effect of knock out of gene encoding for phospholipid:sterol acyltransferase on physiology and development of Arabidopsis thaliana grown under abiotic stress conditions

SPEAKER: ***Grużewska Patrycja***

SUPERVISOR(S): ***Katarzyna Jasieniecka-Gazarkiewicz,*** *PhD;*

Plants, like all living organisms, are exposed to various stress factors. With increasing climate change, abiotic stress has become the main cause of losses in agricultural crops. The rising human population puts pressure on increasing the efficiency of biomass production, which accounts for an estimated 80-90% of total food produced. Creating plants that are resistant to abiotic stress may be the solution to this global problem. Therefore, genes involved in plant responses to abiotic stress are being sought.

Under the influence of abiotic stress, changes in the composition of membranes occur. Hence, the activity of enzymes responsible for membrane remodeling may affect the ability of plants to adapt to changing environmental conditions.

One of the enzymes involved in membrane remodeling is phospholipid:sterol acyltransferase (PSAT). This enzyme catalyzes the conversion of sterols to sterol esters. While sterols are components of biological membranes, sterol esters are stored in lipid droplets in the cytoplasm. Therefore, PSAT regulates the amount of sterols in the membrane through its activity.

In this study, the effect of knockout of the gene encoding PSAT was tested in Arabidopsis thaliana grown under salt stress and low temperature stress conditions. Plants were cultivated in vitro and in vivo. Morphological observations were performed. The impact of this mutation on lipid content was examined using gas chromatography. Concentration of protein was also determined. Tests of the enzymatic activity of PSAT and the second enzyme that catalyzes the synthesis of sterol esters - acyl-CoA:sterol acyltransferase (ASAT) were performed to determine whether low temperature or high salinity affect the enzymatic activity. It was also checked whether their enzymatic activity is stimulated by magnesium or calcium ions.

Morphological differences were observed between the wild type and the mutants grown at low temperature. Mutation does not affect the content of lipids and proteins. It has been shown that abiotic stress affects the enzymatic activity of ASAT and that its activity is stimulated by magnesium ions

Research on this enzyme can add valuable knowledge that may result in a strategy for creating plants resistant to abiotic stresses.

LABORATORY OF PLANT MICROBIOLOGY

TITLE: Effect of colonization of Arabidopsis thaliana tissues by the beneficial strain Pseudomonas donghuensis P482 on plant growth and condition

SPEAKER: Natalia Półgęsek

SUPERVISOR(S): *Magdalena Rajewska,* *PhD;*

Pseudomonas is a genus of Gram-negative, motile bacteria that can colonize various environments such as soil, water, plants and animals. Many Pseudomonas species are among plant growth-promoting rhizobacteria. These bacteria promote plant growth by efficient colonization of roots, production of phytohormones, siderophores, which increase iron availability, and solubilizing phosphorus, which is crucial for plant metabolism. Volatile organic compounds (VOCs) produced by Pseudomonas inhibit pathogens and promote plant growth. Beneficial strains of Pseudomonas are widely used in agriculture as biological fertilizers or crop protection products. The isolation of new strains of this type of bacteria and the investigation of their mechanisms of interaction with plants are crucial for developing new strategies to support crop productivity and health.

Pseudomonas donghuensis P482 is an aerobic bacterium isolated from the rhizosphere of tomato, known for its antimicrobial properties and ability to colonize various plants. The present study investigated the effects of Pseudomonas donghuensis P482 and its mutants associated with c-di-GMP and the GacS/GacA system on Arabidopsis thaliana plants. The GacS/GacA system is a two-component regulatory system that controls the expression of genes responsible for secondary metabolites production and colonization mechanisms, while c-di-GMP is a secondary signal transmitter that regulates biofilm formation and other cellular processes.

The study was conducted to initially evaluate the effects of P482 wild type and mutants in the gacA and c-di-GMP metabolism-associated genes on plant weight growth, root system architecture, and to investigate the possible colonization of Arabidopsis thaliana roots. The study also aimed to optimize the methodology to facilitate the study of the above parameters.

The obtained results have shown that P. donghuensis P482 is capable of colonizing Arabidopsis thaliana roots. Mutations in genes associated with c-di-GMP metabolism and the GacS/GacA two-component system do not affect the colonization ability. It has been demonstrated that neither P482 or its mutants significantly impact root system architecture. However, the mutant defective in the gacA gene causes a significant increase in plant biomass in liquid in vitro cultures.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: Analysis of Extracellular Vesicles Secreted by Bacteria of the Dickeya Genus

SPEAKER: ***Katarzyna Kaczerska***

SUPERVISOR(S): ***Małgorzata Waleron,*** *PhD, DSc;*

Bacteria of the genus Dickeya are members of the Pectobacteriaceae family and attack economically important crops and ornamental plants. They can also be found in soil, water, and insects, which facilitates their transmission. Currently, the genus Dickeya can be found in every climate.

They secrete cell wall degrading enzymes, including pectinases and proteases, causing tissue maceration and diseases such as soft rot or black leg. Due to the presence of two membranes in the cell envelope of Gram-negative bacteria, secretion is conducted through protein complexes called secretion systems. There are 10 types of secretion systems (TxSS), from T1SS to T11SS, excluding T7SS. Additionally, some researchers describe the secretion of extracellular vesicles (EVs) as Type 0 Secretion System (T0SS).

Extracellular vesicles, commonly secreted by cells, participate in intercellular interactions and removal of unwanted substances. Recently, it has been shown that EVs are secreted by the genus Pectobacterium, another representative of the Pectobacteriaceae family. EVs from Pectobacterium participate in pathogenicity against plants and can induce disease symptoms (Jońca et al. Int. J. Mol. Sci., 22(22), 2021).

This study aimed to isolate and describe EVs secreted by Dickeya dadantii 3937 and Dickeya solani IPO 2222 strains. Additionally, the content and number of produced vesicles were examined in cells with membrane instability and defective type 2, 3 and 6 secretion systems. Finally, the effect of the interactions between tested strains and Arabidopsis thaliana in vitro cultures was determined.

We performed the first successful isolation and characterization of EVs secreted by Dickeya dadantii 3937 and Dickeya solani IPO 2222. While the size distribution remained consistent across species, we have observed significant changes in the number of secreted vesicles when mutations were introduced to disrupt secretion systems and reduce membrane stability.

These findings provide a unique perspective on the secretion process of Dickeya EVs. Proteomic analysis revealed that precursors of proteases and pectate lyases are secreted via Dickeya EVs. The results of the in silico analysis of potential interactions between bacterial proteins carried by bacterial vesicles and proteins of Arabidopsis suggest that proteins of EVs produced by Dickeya can potentially influence numerous physiological pathways in plants. Finally, the direct interaction between all tested strains of Dickeya and Arabidopsis, which EVs can also mediate, altered the root morphology of Arabidopsis thaliana during the in vitro study, indicating the significant impact of Dickeya EVs on plant physiology.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: Pro-apoptotic effects of pieperlongumine toward tamoxifen in estrogen receptor-positive breast cancer cells

SPEAKER: ***Agata Szczepańska***

SUPERVISOR(S): ***Anna Kawiak,*** *PhD;*

Breast cancer remains a leading cause of cancer-related morbidity and mortality among women worldwide. Estrogen receptor-positive (ER+) breast cancer, which constitutes the majority of breast cancer cases, is typically treated with endocrine therapies such as tamoxifen. However, resistance to tamoxifen is a significant clinical challenge, necessitating the exploration of novel adjuvant therapies to enhance its efficacy.

This study investigates the pro-apoptotic effects of piperlongumine, in combination with tamoxifen in ER+ breast cancer cells. Piperlongumine, as a natural alkaloid isolated from long pepper (Piper longum) has garnered attention for its ability to selectively induce apoptosis in cancer cells. Furthermore, to explore the molecular mechanisms of piperlongumine actions, the JNK signalling pathway was investigated.

In this research, two ER+ breast cancer cell lines were used: the MCF-7 and the T-47D. Cells were treated with various concentrations of tamoxifen, piperlongumine, and their combination. To determine the effects of piperlongumine in ER+ breast cancer cells two cell viability assays were performed: MTT and colony forming assay. Apoptosis was evaluated through flow cytometry using annexin V and 7-AAD, and the mechanism of piperlongumine actions was confirmed by the AlpaScreen method.

The results demonstrated that piperlongumine significantly enhances the apoptosis in ER+ breast cancer cells. The analysis of piperlongumine molecular actions, application of JNK pathway inhibitor and AlpaScreen method, confirmed that this bioactive compound induced apoptosis in ER+ breast cancer cells via the JNK cascade. The flow cytometry results confirmed that in combination with tamoxifen, the population of apoptotic cells significantly increases in comparison to the treatment only with one of the examined compounds.

In conclusion, this study indicates that piperlongumine effectively potentiates the pro-apoptotic effects of tamoxifen in ER+ breast cancer cells through the activation of the JNK signalling pathway. This study highlights the potential of piperlongumine as a promising adjuvant therapy to impact the activity of tamoxifen in patients with ER+ breast cancer. Further, in vivo studies and clinical trials are warranted to validate these findings and explore the therapeutic potential of this combination in estrogen-receptor positive breast cancer treatment.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: Eradication of bacteria associated with Acne Vulgaris from ex vivo porcine skin by direct cold atmospheric plasma jet type DBD

SPEAKER: ***Weronika Szkoda***

SUPERVISOR(S): ***Wojciech Śledź,*** *PhD, DSc;*

Acne vulgaris is a chronic inflammatory skin disorder that commonly affects adolescents, with about 80% of individuals between ages 11 and 30 experiencing it. Globally, it ranks as the eighth most prevalent disease. One of the primary factors in its development is the abnormal colonization of hair follicles by Cutibacterium acnes. This bacterium is a commensal, lipophilic, Gram-positive organism that can survive on the skin's surface despite being considered as anaerobe. It owes it to possession of enzymatic systems which are able to detoxify oxygen. In addition to C. acnes, tissue samples from acne vulgaris patients often reveal other Gram-positive, opportunistic pathogens such as Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, and Streptococcus agalactiae.

There are numerous treatment options for Acne Vulgaris, including benzoyl peroxide, antibiotics, and retinoids, which can be used alone or in combination. Systemic treatments might involve oral antibiotics, hormonal therapies, and isotretinoin, tailored to the patient's needs. The global issue of antibiotic resistance highlights the necessity for new therapeutic strategies.

Cold atmospheric plasma (CAP) is a free-flowing mixture of electrons, ions, and neutral particles that operates on a macroscopic time and spatial scale. It is produced at normal atmospheric pressure through processes such as dielectric barrier discharge (DBD), glow discharge, corona discharge, or arc discharge. DBD cold plasma has a significant biological impact, leading to the destruction of bacterial cells, and thus, it holds potential as an adjunctive therapy that could reduce the reliance on antibiotics for acne treatment.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: THE INFLUENCE OF A PHOTODYNAMIC METHOD AND A NOVEL VENETIN-1 NANOPARTICLE ON A LUNG CANCER CELL LINE (A549)

SPEAKER: ***Julia Sadowska***

SUPERVISOR(S): ***Magda Rybicka-Misiejko,*** *PhD;*

Lung cancer remains the leading cause of cancer-related deaths worldwide, with non-small cell lung carcinoma (NSCLC) accounting for approximately 85% of cases. This study investigates the effects of red light and Venetin-1, a protein-polysaccharide complex derived from the coelomic fluid of the earthworm Dendrobaena veneta, on A549 lung cancer cells. Previous studies have shown that Venetin-1 has anti-tumor activity and potential as a component of photodynamic therapy (PDT).

The aim of my research was to investigate the effects of Venetin-1 in combination with red light irradiation on the viability, cell cycle, apoptosis, gene expression and proteome profile of A549 cells. In the study, I examined lung cancer cell line - A549 and control normal bronchial epithelial cell line - BEAS-2B.

First, cells were seeded and treated with either regular medium or medium supplemented with Venetin-1. Cells were then exposed to different doses of red light (25% red light (65.92 J/cm^2), 50% red light (131.89 J/cm^2), 75% red light (192.91 J/cm^2), or 100% red light (248.96 J/cm^2)) for 30 minutes, while control cells were kept in the dark. After 72 hours of incubation, cells were analyzed for cell cycle distribution and apoptosis by flow cytometry, proteome changes by mass spectrometry, and gene expression by qPCR.

The results showed that red light and Venetin-1 treatment induced the arrest of cells in G1 phase and increased their population in subG1 phase, indicating their apoptosis. Notably, a significant effect was observed at 75% red light power, which was selected for further analysis. In addition, compared to Venetin-1 treatment alone, Venetin-1 and red light treatment significantly decreased the number of live cells. No significant differences were observed in the control BEAS-2B cell line.

This study suggests that Venetin-1, especially when combined with photodynamic therapy, is a promising therapeutic strategy for treating lung cancer.

SESSION 3

LABORATORY OF PROTEIN BIOCHEMISTRY

TITLE: The binding mechanism of chaperone proteins Hsp70 and JDP class B to the surface of protein aggregates

SPEAKER: ***Agata Konieczka***

SUPERVISOR(S): ***Agnieszka Kłosowska****; PhD;*

Under conditions of stress, proteins can lose their native conformation and form dangerous amorphous aggregates or highly structured amyloid fibrils. To maintain proteostasis, organisms developed protein quality control system that includes chaperone proteins. Yeast chaperones called Ssa1 (Hsp70) and Sis1 (Hsp40, J-domain protein) are involved in protein refolding and disaggregation. In a canonical Hsp70 activity cycle, Sis1 recognises a protein substrate and transfers it to Ssa1, which undergoes conformational changes. However, those interactions are still not quite understood. In this project, I wanted to investigate the requirements for Sis1-Ssa1 complex to bind with the aggregate. Using Bio-layer Interferometry (BLI) method, I have shown that Sis1 binding to the luciferase aggregate is very weak. To study whether Sis1-agreagte interaction change after formation of Sis1-Ssa1 complex, I constructed, overproduced and purified Ssa1 V435F variant (unable to bind with the aggregate). Results showed that Sis1-Ssa1V435F complex also dose not bind with the aggregate. This implies that interaction Ssa1-agregate is essential for Sis1-Ssa1 complex. Then, I tested, whether Ssa1-agregate interaction will be abolished after blocking binding between Sis1 and Ssa1. To answer that question, used Ssa1 ΔEEVD that cannot bind with Sis1 protein. BLI results indicates that mixture of Sis1 and Ssa1 ΔEEVD cannot bind with the aggregate. Together these findings shows that formation of complex Sis1-Ssa1 is essential for binding with aggregate and binding occurs mostly between Ssa1 and the aggregate.

LABORATORY OF PROTEIN BIOCHEMISTRY

TITLE: A comparison of prokaryotic and eukaryotic Nucleotide Exchange Factors in refolding of proteins from aggregates

SPEAKER: **Victoria Wiernikouskaja**

SUPERVISOR(S): *prof.* ***Krzysztof Liberek***

Chaperone proteins are essential for maintaining proteostasis. For example, the Hsp70 chaperone system can refold proteins from toxic aggregates. Hsp70 protein binds polypeptide substrates and releases them in an ATP-dependent cycle, which is regulated by two cochaperones: J-domain protein and Nucleotide Exchange Factor (NEF). NEF, by definition, promotes the transition of Hsp70 from an ADP-bound to an ATP-bound state. Prokaryotic NEF GrpE is known to be essential for the refolding activity of Hsp70. NEFs in eukaryotes can be divided into three families, including the Hsp110 family. Contrary to GrpE, the influence of Hsp110 on Hsp70-mediated protein recovery from aggregates remains uncertain. The study engages modified BLI (bio-layer interferometry) to investigate the role of Grp from Escherichia coli and the Saccharomyces cerevisiae Hsp110 family member Sse1 in the process of protein disaggregation and folding. The results suggest that GrpE is important at both the early and final stages of the process, while Sse1's effect is concentration-dependent and is more prominent at the early stage. These findings contribute to a better understanding of NEF participation in the recovery of proteins from aggregates by the Hsp70 system in bacteria and yeasts.

LABORATORY OF PROTEIN BIOCHEMISTRY

TITLE: Purification and biochemical characterization of human Rab6B protein clinical variants.

SPEAKER: ***Mikołaj Rej***

SUPERVISOR(S): ***Szymon Ziętkiewicz****; PhD, DSc;*

G protein family of small GTPases is divided into two distinct classes – small, monomeric G proteins and large heterotrimeric G proteins. Both act as molecular switches inside cells, controlling a vast amount of cellular processes. The ability of the proteins to bind a guanine nucleoside leads to conformational changes in the catalytic domain and in turn defines their activity. Presence of GTP in the active site leads to protein stabilization and activation whereas its hydrolysis to GDP deactivates the protein. The GTPase activity of G proteins is regulated by specific GAP (GTPase Activating Protein) and GEF (Guanosine Exchange Factor) proteins. The strict control of g proteins activation is necessary for proper functioning of the whole organism. Many mutations in the G proteins present severe consequences due to disruptions in the mechanisms activated by those proteins. For example, mutations in small G protein Ras often lead to deregulation of MAP kinase pathways responsible for cell survival and proliferation. A mall G protein class member Rab6B, responsible for retrograde transport of vesicles from the Golgi apparatus to the ER in the brain, was found mutated in a individual suffering from microcephaly. A novel variant - Rab6B A171E, discovered during a whole genome sequencing of the patient is thought to be the reason for the symptoms. To answer the question whether the discovered variant of Rab6B is pathogenic, I purified 3 variants of Rab6B – WT; A171E and A171V. Using tryptophan fluorescence assays and circular dichroism, structural stability of all variants were tested. The results have shown a minor difference in secondary structure of the A171E variant alongside slight destabilization visible in a lower melting point of the variant, compared to others. Additional tests regarding the susceptibility of the variants to digestion using trypsin revealed more differences between the variants. Overall, even though the experiments highlighted differences between the proteins stability and structure, it is important to understand that they may not necessarily translate to differences in cellular activity. Because of this, further experiments are required to confirm the theory.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: Assessment of the risk of co-selection against antimicrobial blue light and thermal stress in Escherichia coli.

SPEAKER: ***Patrycja Pikulik***

SUPERVISOR(S): ***Aleksandra Rapacka-Zdończyk****; PhD;*

Escherichia coli is a Gram-negative, commensal bacterium which is an integral part of the microflora of the large intestine. Although many strains of E. coli coexist with humans without harming the host, there are also pathogenic strains that cause serious intestinal and extraintestinal diseases. The presence of pathogenic intestinal strains is observed in both animal and plant food products, which constitutes a significant challenge for the food industry. The most common method of food preservation is thermal processing. However, the growing demand for minimally processed products is increasing interest in non-thermal bacterial eradication methods, such as antimicrobial blue light (aBL). The mechanism of action of aBL is based on exciting endogenous photosensitizers with light at a wavelength of 400-470 nm, which leads to the generation of reactive oxygen species that damage various cellular structures of bacteria. Despite the numerous advantages of aBL, the safe introduction of this method to the food industry requires a comprehensive understanding of its potential limitations. Studies conducted so far in our laboratory have shown that E. coli can develop tolerance to aBL, and mutants lacking the genes encoding heat shock proteins DnaJ and DnaK exhibit hypersensitivity to aBL.

The aim of this study was to obtain a population of E. coli K-12 that developed temperature tolerance and another, tolerant to aBL, and to assess the risk of co-selection for both of these factors. The second aim of this study was to assess the impact of short-term (10/20 min) and overnight (16 h) preincubation at elevated temperatures on the bacterial response to aBL.

As a result of repeated exposure of E. coli K12 to sublethal doses of the tested selection factors, populations tolerant to temperature stress and populations tolerant to aBL were obtained. The observed adaptations were confirmed to be phenotypically stable, suggesting that they result from changes in the genetic material. The results showed that populations with increased tolerance to aBL also exhibit increased tolerance to temperature, while populations tolerant to increased temperature do not show altered sensitivity to aBL. Furthermore, both short and overnight preincubation at elevated temperature significantly increased the survival of E. coli K12 when exposed to antimicrobial blue light.

SESSION 4

LABORATORY OF BIOPOLYMERS STRUCTURE

TITLE: Application of mass spectrometry for quantitative determination of cortisol in diverse biological matrices

SPEAKER: ***Julia Bilińska***

SUPERVISOR(S): ***Stanisław Ołdziej****; PhD, DSc;*

Cortisol is a key hormone that is regulated by the HPA axis and plays an important role in the stress response of the body. Accurate measurement of cortisol levels in various matrices helps to understand how the body responds to stress. Cortisol is primarily found in an inactive form bound to proteins such as globulins or albumin. Only about 5% is in an unbound, biologically active form that interacts with glucocorticoid receptors. Classical methods for assaying free cortisol use zinc sulphate for extraction, a time-consuming procedure that requires samples to be desalted prior to analysis by mass spectrometry. An alternative method, which simplifies and speeds up the process, has been investigated by protein removal from samples using centrifugal filters. Plasma, saliva, follicular fluid and urine samples were processed using centrifuge filter and then analyzed using mass spectrometry. Obtained results showed that protein removal protocol tested gives comparable results to the classical method.

Due to the low concentrations of free cortisol, derivatisation was also investigated. This was done to lower the quantification threshold and improve the analytical characteristics. Girard T reagent, previously used for derivatisation of other steroid hormones, was used. However, the obtained results were unreliable, showing much higher values than expected. Further studies with other derivatisation reagents could extend the research potential of developed method and facilitate the analysis of samples with very low cortisol concentration for example archaeological samples.

LABORATORY OF BIOPOLYMERS STRUCTURE

TITLE: Synthetic NTPases and nucleases based on Trpzip motif

SPEAKER: **Magdalena Labudda**

SUPERVISOR(S): ***Stanisław Ołdziej****; PhD, DSc;*

In current literature, scientists have only discussed the potential use of peptides with a tryptophan zipper motif as a simple model capable of binding single-stranded nucleic acids. Limited research has been conducted on the catalytic activity of peptides possessing the Trpzip structure or a closely related sequence. The common ability to bind other molecules found in enzymatic proteins can be replaced by small hairpin peptides. Properly designed peptides and environmental conditions would constitute the beginning of a new molecular evolution of proteins. Biocatalysis is one of the fastest growing areas of the chemical market, the aim of which is to conduct both safe and ecological chemical-biological processes. Simple, small molecules such as peptides would reduce purchase costs and facilitate synthesis compared to high-molecular enzymes. To validate the hypothesis, capillary electrophoresis was used, which allows obtaining high-resolution results in a short time. Studies have shown that short peptides containing a tryptophan zipper motif have the ability to hydrolyze nucleic acids by interacting with their structure and inducing degradation processes. The hydrolysis process also depends on the pH of the environment, incubation time and temperature, as well as the concentration of the reagents. Further research may contribute to expanding knowledge about the interactions between peptides and nucleic acids.

LABORATORY OF EXPERIMENTAL AND TRANSLATIONAL IMMUNOLOGY

TITLE: The distribution of CD1a molecule on antigen presenting cells upon exposure to permissive and non-permissive ligands.

SPEAKER: ***Wiktor Jarecki***

SUPERVISOR(S): ***Danuta Gutowska-Owsiak****; PhD, DSc;*

Extracellular vesicles (EVs) are a heterogeneous population of phospholipid membrane organelles that are secreted by all living cells and take part in intercellular communication. Differences in their origin directly affect their size, surface and cargo content; the cargo of extracellular vesicles is protected by a lipid bilayer. EVs carry biological molecules such as proteins, lipids, nucleic acids and small molecular mediators from parent cells to recipient cells. Exosomes are the smallest type of the extracellular vesicles, their size varies between 50 and 150 nm and they are composed mainly of lipids.

Dendritic cells (DCs) are antigen-presenting cells (APCs) with a unique ability to prime naïve T cells. Their main function is to capture, process antigens and to interact with T cells; dendritic cells regulate both T cell immunity and tolerance. Tolerogenic dendritic cells (tolDCs) produce increased amounts of anti-inflammatory cytokines such as IL-10 and have low expression of costimulatory molecules on their surface. By not providing sufficient stimulatory signals to T cells, they stimulate naïve T cells to differentiate into regulatory T cell populations (Treg cells). They are also able to induce anergy of autoreactive T cells as well as their apoptosis.

While peptide antigen presentation occurs with the involvement of either MHC (Major Histocompatibility Complex) class I or MHC class II molecules, presentation of lipid antigens involves the CD1 molecules. CD1a proteins are highly expressed by Langerhans cells in the skin as well as other myeloid dendritic cell populations.

The aim of the study is to investigate whether specific lipids or lipids derived from digested sEV membranes from different cellular sources may affect the cellular distribution of CD1a molecule in tolerogenic dendritic cells, with a direct influence on the lipid-specific T cell responses.

K562 and HEK293T cells were grown in EV-depleted culture medium for 48 hours and sEVs were isolated by the serial ultracentrifugation protocol. The size and concentration of sEVs were measured by Nanoparticle Tracking Analysis (NTA) method. To assess changes in the expression level and distribution of CD1a, model antigen presenting cells (K562-CD1a and K562-empty vector) were pulsed with bee venom phospholipase A2 (PLA2), purified lipids (24:1 SM, C18:0 PC, DHA, myristic acid) or lipid antigens liberated from the sEVs membrane by digestion with PLA2. Internalization and recycling was assessed by flow cytometry and holotomography.

The results indicate that already after 10 min of incubation, PLA2 activity promotes internalization of the CD1a molecules, suggesting differences in the CD1a bound lipids; longer time points show saturation after one hour from PLA2 exposure. After internalization some of the CD1a molecules return to the surface of the K562-CD1a cells or are replaced with a newly-synthesized ones.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: Analysis of Spike Protein Gene Expression of MHV and SARS-CoV-2 Coronaviruses in Mammalian Cells.

SPEAKER: ***Adam Jałtuszewski***

SUPERVISOR(S): *prof.* ***Krystyna Bieńkowska-Szewczyk***

The ongoing COVID-19 pandemic, caused by SARS-CoV-2, has necessitated extensive research into the virus's mechanisms of infection and pathology. Both SARS-CoV-2 and the murine coronavirus, Mouse Hepatitis Virus (MHV), are Betacoronaviruses and share structural and functional similarities, particularly in their spike (S) proteins which mediate cell entry and syncytia formation. Understanding the expression and impact of these spike proteins in mammalian cells can provide insights into viral pathogenesis and potential therapeutic targets.

In this study, I transfected various human (e.g. HEK293, A549) and mouse (e.g. LR7) cell lines with plasmids encoding the spike proteins of different variants of SARS-CoV-2 and MHV. I aimed to assess the production of spike proteins, their influence on cell morphology, and their capability to induce syncytia formation. Protein expression levels were evaluated using Western blotting, while immunofluorescence was employed to observe cellular localization and morphological changes. Additionally, syncytia formation was assessed by microscopic examination.

Western blot analysis confirmed the expression of SARS-CoV-2 and MHV spike proteins in transfected cell lines. The spike protein bands were detected at the expected molecular weights, indicating successful protein production. Immunofluorescence staining revealed distinct localization patterns of the spike proteins, with both SARS-CoV-2 and MHV spikes predominantly localizing to the cell membrane, consistent with their role in mediating cell-cell fusion.

Morphological examination showed significant changes in cell shape and structure post-transfection in some cell lines. Notably, cells expressing the SARS-CoV-2 spike protein exhibited pronounced syncytia formation, characterized by large, multinucleated cells. This effect was particularly evident in the HEK293 and VeroE6 cell lines.

My results demonstrate that the spike proteins of both SARS-CoV-2 and MHV are not only expressed effectively in mammalian cell lines but also induce significant morphological changes, including syncytia formation. This indicates that the spike protein alone is sufficient to drive this process, a crucial aspect of viral pathogenesis. The differential syncytia formation across cell lines suggests a potential influence of host cell factors on spike protein function and virus-cell interactions.

This study provides valuable insights into the behavior of SARS-CoV-2 and MHV spike proteins in mammalian cells, highlighting their pivotal role in syncytia formation. These findings enhance our understanding of coronavirus-induced cell fusion and could inform the development of therapeutic strategies targeting spike protein-mediated pathogenesis.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: Role of FGFR4 in proliferation and growth of Triple Negative Breast Cancer cells and their response to PARP inhibitors

SPEAKER: ***Ewelina Chodowiec***

SUPERVISOR(S): ***Dominika Piasecka****; PhD;*

Triple Negative Breast Cancer (TNBC) is the most aggressive subtype of breast cancer (BCa), which lacks expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2). TNBC commonly occurs in younger women and has the worst prognosis and limited treatment options for TNBC patients. One of few valid targeted therapies are poly(ADP-ribose) polymerase (PARP) inhibitors, which function is associated with defects in homologous recombination repair genes-BRCA1/2. Mutations in these genes occur in almost 20% of TNBC patients, but there are growing evidence that PARP inhibitors can be also effective treatment for patients without mutations of these genes due to BRCAness phenotype. However, there is an urgent need for finding other molecular markers and new targets for TNBC treatment.

The attractive target seems to be the Fibroblast Growth Factor Receptor 4 (FGFR4), which is part of Tyrosine Kinase Receptor (RTKs) family along with FGFR1-3. FGFR4 shares the least homology and contains different amino acid in kinase domain, the C552 residue. This unique receptor is overexpressed in many cancers including BCa where its involved in metastasis and resistance to anti-HER2 therapies. However, there is significant lack of knowledge about FGFR4 role in TNBC.

Taking into account all this information, the aim of the study was to evaluate the role of FGFR4 in proliferation, growth and response to PARP inhibitors of TNBC cells.

The obtained results showed that FGFR4 plays important role in growth and colony formation of TNBC cells but does not influence 2D proliferation. It has been also established that TNBC cells are sensitive to PARP inhibitors and this sensitiveness is due to FGFR4 expression. Knockdown of FGFR4 led to acquisition of the resistance to anti-PARP treatment of TNBC cells.

Our study confirmed that FGFR4 could be a prognostic marker of response to PARP inhibitors by TNBC cells and that FGFR4 signalling is important in growth and colony formation of triple negative breast cancer cells.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: The Influence of FGFR4 on the Response of LAR TNBC Cells to CDK4/6 Inhibitors

SPEAKER: ***Wojciech Sadowski***

SUPERVISOR(S): ***Dominika Piasecka****; PhD;*

Breast cancer remains the most prevalent cancer among women globally, with a substantial number of cases exhibiting triple-negative breast cancer (TNBC) characteristics. TNBC, defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 amplification, presents a unique challenge due to its aggressive nature and limited treatment options. Among TNBC subtypes, the luminal androgen receptor (LAR) subtype shows distinct molecular features and therapeutic responses. CDK4/6 inhibitors have emerged as a promising treatment modality, though their efficacy can be influenced by various cellular mechanisms, including the fibroblast growth factor receptor 4 (FGFR4) signaling pathway.

Preliminary studies have indicated that FGFR4 activation may reduce the efficacy of CDK4/6 inhibitors in treating TNBC. The specific impact of FGFR4 on the responsiveness of LAR TNBC cells to these inhibitors remains poorly understood.

This study aims to elucidate the role of FGFR4 in modulating the response of LAR TNBC cells to CDK4/6 inhibitors. Understanding this interaction could reveal critical insights into resistance mechanisms and suggest potential therapeutic strategies to improve treatment outcomes.

Initial findings suggest that FGFR4 activation correlates with decreased sensitivity to CDK4/6 inhibitors in LAR TNBC cells. Conversely, pharmacological inhibition of FGFR4 diminish the effectiveness of CDK4/6 inhibition, suggesting a potential combinatory therapeutic approach.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: Impact of FGF7/FGFR2 signaling and IRS-1 protein on development of resistance to anti-ER therapies in luminal breast cancer

SPEAKER: ***Karolina Dudzic***

SUPERVISOR(S): ***Kamila Kitowska****; PhD;*

Breast cancer (BCa) is the most common cancer among woman worldwide. The most frequent subtype of breast cancer is the luminal subtype, 70% of newly diagnosed BCa cases are classified as the luminal subtype. This subtype is characterized by high expression of estrogen (ER) and progesterone (PR) receptors and the absence of HER2 receptor expression. Despite great success of anti-ER therapies (tamoxifen, fulvestrant), 45% of patients develop the resistance against those therapies. The emergence of resistance has been linked to the alternative activation of ER through fibroblast growth factor receptor (FGFR). FGFR2 which is activated by FGF7 was found to confer the resistance to anti-ER drugs. Furthermore, in ER+ BCa patients a correlation has been found between FGFR2 protein and the expression of IRS-1 gene.

IRS-1 is an adaptor protein, involved in signaling from insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R). It is well known that apart from the classical function, IRS-1can also form a complex with ER and affect its transcriptional activity. The better understanding of the FGF7/FGFR2 signaling mechanism and the role of IRS-1 protein may help to find new therapies for patients with tumors resistance to anti-ER drugs.

In this research we checked if IRS1 protein is involved in FGF7/FGFR2-mediated protective effect from anti-ER therapies in luminal breast cancer. First, we performed the 3D test in Matrigel® for luminal BCa cell line (wilde type vs. IRS-1 overexpression) with fulvestrant and showed that the IRS1 overexpression induced anti-ER drug resistance. Next experiment in 3D culture of cells grown in the presence of FGF7 and/or NT219 (IRS-1 inhibitor), fulvestrant and/or tamoxifen revealed that FGF7-medtaited impairment of anti-ER therapies is reduced when IRS-1 is degraded. Moreover Western Blott analysis showed that IRS1 protein was necessary for estrogen receptor phosphorylation and activity.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: The role of the interplay between FGF/FGFR signaling and DUSP activity in resistance to luminal breast cancer therapies

SPEAKER: ***Gabriela Mišta***

SUPERVISOR(S): *prof.* ***Rafał Sądej***

Breast cancer (BCa) is the most common cancer among women worldwide. The luminal subtype, which is characterized by the expression of estrogen and progesterone receptors, is the most frequently diagnosed among all BCa. Currently, there are several effective targeted therapies against breast cancer such as tamoxifen which inhibits estrogen receptor activity. However, their effectiveness is limited by intrinsic or acquired resistance, which patients develop over the time of treatment. The mechanism of acquired resistance might be based on BCa cell stimulation originating from the tumor microenvironment. This might involve initiation of fibroblasts growth factor/fibroblasts growth factor receptor (FGF/FGFR) signaling which can activate estrogen receptor independently of estradiol binding. Moreover, it has been shown that DUSP proteins, which are MAP kinase phosphatases, are potentially involved in drug resistance. Our team found that FGF7/FGFR2 signalling promotes an increase in expression of DUSP4 and DUSP6 genes. Hence, the aim of my project is to investigate the interplay between DUSP4/6 and FGF/FGFR signaling in the context of resistance to anticancer therapies. It has been confirmed that stimulation of MCF7, luminal BCa cells, with FGF7 at the presence of tamoxifen induced expression of both DUSP4 and DUSP6. This prompted us to derive MCF7 cells with overexpression of DUSP4 and DUSP6. Adhesion-independent growth analyses indicated that overexpression of DUSP4 or DUSP6 does not affect cell response to anticancer drugs. The next test was conducted using BCI, which is a specific inhibitor of DUSP proteins activity. The results showed that inhibition of DUSP proteins abrogates the protective effect of FGF7 towards tamoxifen and cisplatin. Although overexpressions of DUSP4 and DUSP6 genes in luminal BCa don't result in development of resistance itself, it seems that DUSP proteins play a role in reducing the cell sensitivity to anticancer drugs. That concept however needs to be verified by further investigation.

WEDNESDAY 7TH JUNE

SESSION 5

LABORATORY OF TRANSLATIONAL ONCOLOGY

TITLE: Detection and significance of extracellular neutrophil traps in prostate cancer progression

SPEAKER: ***Aleksandra Parteka***

SUPERVISOR(S): ***Natalia Bednarz-Knoll****, PhD;*

INTRODUCTION

Variable external signals might affect neutrophils and lead to production of neutrophil extracellular traps (NETs), described mainly in sepsa or cardiovascular diseases, but hypothesised also to be protumorigenic or even prometastatic. As the exact function of NETs in cancer remains unknown, their detection and comparison to patients' outcome may expand our knowledge on cancer progression. AIM This study aimed to detect NETs in prostate cancer (PCa), describe their putative correlation with clinico-pathological factors, circulating tumor cells (CTCs), and assess their potential impact on patients' survival.

METHODS AND MATERIALS

Peripheral blood from 126 PCa patients undergoing radical prostatectomy and 19 healthy males was collected, and processed by density gradient centrifugation to separate PBMC fraction putatively containing NETs. PBMC fractions were stained using epithelial (pan-keratins) and mesenchymal (vimentin) markers to detect CTCs and evaluated using imaging flow cytometry. NETs were recognized in brightfield as branched and densely intertwined fibers including platelets and/or leukocytes. In parallel, to confirm the identification of NETs, NETosis was induced using phorbol 12-myristate 13-acetate (PMA) in peripheral blood from 4 healthy volunteers. The statistical analysis was conducted using IBM SPSS Statistics.

RESULTS

Identified NETs ranged 0-6724 (mean 362) and 0-2931 (mean 297) / 1 mln of DAPI+ cells, if analysed in localized and metastatic PCa, respectively. No NETs were detected in blood originated from healthy males. Presence of NET correlated only with coexisting cardiovascular disease ($p = 0.025$). There was no correlation between NETs and CTCs or NETs and progression of disease.

CONCLUSION

NETs might be co-detected with CTCs in PCa patients' blood using imaging flow cytometry. NETs occur more often in patients with cardiovascular diseases. There is no corelation between cancer progression and NETs. However, the exact clinical effect, in particular in the subcohort of patients with coexisting cardiovascular disase merits further investigation in the larger cohort of patients.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: Accumulation of various photosensitizers in human keratinocytes using model of atopic dermatitis

SPEAKER: ***Izabela Wojtalewicz***

SUPERVISOR(S): ***Joanna Nakonieczna****, PhD, DSc;*

Atopic dermatitis (AD) is a chronic, non-infectious inflammatory skin condition that is significantly more prevalent in highly developed countries and is increasingly regarded as a civilization disease of the 21st century. The etiology of AD is not entirely understood, but it is believed to involve genetic, immunological, and environmental factors. A common genetic feature in patients with AD is mutations in the gene encoding filaggrin, leading to reduced level of this protein. Filaggrin is a crucial epidermal protein that maintains the skin's barrier function by binding keratin cytoskeleton fibers. Mutations in the filaggrin gene impair this barrier function, increasing the epidermis's permeability to pathogens and allergens. Staphylococcus aureus colonizes the skin of up to 90% of AD patients. S. aureus is classified as an ESKAPE pathogen, characterized by extensive antibiotic resistance, including strains resistant to penicillin, methicillin, and vancomycin. Recent studies also indicate the intracellular presence of S. aureus. One method to combat intracellular pathogens is antimicrobial photodynamic inactivation (aPDI). This technique combines light, a photosensitizer, and an oxygen-rich environment to treat infections. Upon irradiation, photosensitizer molecules transfer energy to molecular oxygen within the cell, generating reactive oxygen species (ROS) such as hydrogen peroxide, singlet oxygen, and free radicals. These ROS are highly reactive and cause damage to lipids, proteins, and nucleic acids, ultimately leading to cell death. The aim of this study was to investigate the accumulation of various photosensitizing compounds in two human keratinocyte cell lines: a control line (HaCaT FLGctrl) and a line with silenced filaggrin expression (HaCaT ΔFLG). Additionally, the study aimed to assess the cytotoxicity and phototoxicity of selected compounds in both cell lines.

LABORATORY OF TRANSLATIONAL ONCOLOGY

TITLE: The effect of basal breast cancer cell phenotype on the expression of immune-related markers

SPEAKER: ***Marcin Banacki***

SUPERVISOR(S): ***Aleksandra Markiewicz,*** *PhD;*

Introduction

Epithelial-mesenchymal transition (EMT) is a source of phenotypic heterogeneity of tumour cells and is commonly observed in basal breast cancer. In basal tumours, the mesenchymal phenotype of cancer cells has been linked with a worse prognosis [1] and resistance to immune therapy [2].

Aim of the study

The aim of the study was to evaluate the effect of EMT status of basal breast cancer cell lines on the level of selected immune-related proteins.

Materials and methods

Using publicly available transcriptomic data, we have compared the expression of selected immune-related genes in 11 epithelial and 12 mesenchymal basal breast cancer cell lines. Based on the results, we have chosen 2 epithelial (EPI) cell lines to induce EMT (HCC70 and HCC1806 – treatment with TGFβ) and 2 mesenchymal (MES) cell lines to inhibit EMT (CAL-51 and CAL-120 – treatment with pyrvinium pamoate (PP)). Cell lines after EMT induction or inhibition were characterised on the transcriptome level by qPCR (EMT phenotype markers: CDH1, EPCAM, PAI1, VIM) and on the surface protein level flow cytometry (HLA-A/B/C or HLA-DR).

Results

In the transcriptomic analysis of publicly available datasets a decrease in the expression of HLA-DQB1 ($p=0.015$), immunoproteasome subunits PSMB10 ($p=0.009$) and PSMB8 ($p=0.079$), immune checkpoints CD86 ($p=0.045$), ICOSLG ($p=0.001$), VTCN1 ($p=0.003$), TNFRSF14 ($p=0.012$) and GAL9 ($p=0.00009$) has been observed in the cell lines of MES phenotype. The success of EMT induction in EPI cell lines was validated by qPCR, which has shown an upregulation of mesenchymal markers after the treatment with TGFβ: 5-fold increase in PAI1, 43-fold increase in VIM in HCC1806, and a 335-fold increase in PAI1 expression in HCC70. Moreover, a decrease in the expression of some immunoproteasome subunits in HCC1806 (PSMB8: 60% of control, PSMB9: 70% of control) and HCC70 (PSMB8: 60% of control, PSMB9: 10% of control) was observed after EMT induction. Flow cytometry has revealed a post-EMT decrease in surface level of HLA-A/B/C proteins in HCC1806 cells, and a post-EMT decrease in surface level of HLA-DR in HCC70 cells.

Conclusions

In basal cell lines, mesenchymal phenotype might be linked with decreased immunogenicity via a number of mechanisms - decreased immunoproteasome, HLA-A/B/C levels or even decrease in MHC class II expression.

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POLPHARMA BIOLOGICS SA

TITLE: Long-term Predictions of Immunoglobulin Physical and Chemical Degradation Using the Arrhenius Equation

SPEAKER: ***Marcel Letniowski***

SUPERVISOR(S): ***Zbigniew Jaremicz****, PhD;*

Nowadays, biologics are one of the most popular groups of drugs in the industry. This is because biologics are used in targeted therapies, which are increasingly desired by patients and healthcare providers. Biologics work on specific tissues or spots in the organism, making them less harmful to the patient. Additionally, due to their specificity, the efficiency of such therapies is much higher compared to typical therapies.

Despite their high efficacy and multiple benefits for patients, biologics are not widely used due to their high therapy cost compared to small molecule drugs. This high cost is attributed to the complex production process and the lengthy, expensive development of biologics products. As a result, there is a recent trend towards increasing the utilization of informatics tools to reduce the number of required experiments and development time without compromising the overall quality of the solution. This shift not only improves product understanding but also leads to shorter time to market and reduced therapy costs, making it more accessible for patients.

In my master's thesis, well-known Arrhenius equation was used to predict the changes in content of dimers and oxidized species during long-term storage of biologic drug. Based on data from 6 months storage at 25 °C (accelerated studies) and 40 °C (stressed studies), the project aimed to achieve accurate predictions for biologic stored at 5 °C for 2-4 years. During the study stability data generated for 10 batches of humanized monoclonal IgG4, provided by Polpharma Biologics S.A., were used for model development and its further verification.

The developed model allowed for accurate predictions of changes in aggregate content, but in the case of oxidation, the predictions did not match the experimental data, most likely due to the high variability of the analytical method used for determining the content of oxidized species. Further model optimization is possible to enable more accurate predictions. Future studies should include data generated at additional intermediate temperatures (e.g., 30 °C) to enable such optimization. Studies should also be extended to other biologics to determine whether the results are a tendency or an exception to the rule. Ultimately, a step-by-step protocol providing guidance on data analysis was created during the course of model development.

POLPHARMA BIOLOGICS SA

TITLE: Specificity characterization of anti-HCP antibodies used in host cell proteins impurities analysis of chosen biological drugs. Application of mass spectrometry and proteomics methods.

SPEAKER: ***Natalia Kowcun***

SUPERVISOR(S): ***Tomasz Goździewicz****, PhD;*

Biological drugs play crucial role in various diseases treatment. They are based on large biological molecules produced by genetically modified living organisms. As the structure and mechanism of action of biopharmaceuticals are much more complicated than in case of classical small-molecule drugs, their production is strictly regulated. However, just as traditional low-molecular-weight medications have generic counterparts, the pharmaceutical industry continues to develop biosimilars for biological drugs. One of the requirements for pharmaceuticals is the absence of manufacturing-related impurities. For biologic drugs Host Cell Proteins (HCPs) belong to such contaminants. Those are proteins synthesized by the cells used in the production process, besides the targeted molecule. As they might be able to affect the efficacy and safety of the drug, it is critical to monitor their presence and quantity. This is routinely accomplished by the use of enzyme-linked immunosorbent assays (ELISA). While those tests are considered as a golden standard method for HCPs monitoring, certain limitations of ELISA have to be noted. This is why more and more recent studies focus on liquid chromatography-mass spectrometry-based methods (LC-MS) as an orthogonal approach for HCPs characterization in biological drugs manufacturing processes. LC-MS methods can be also used to support ELISA critical reagents characterization what was done in this research. The purpose of this thesis was to study the exploitation of the mass spectrometry techniques for characterization of the specificity of the polyclonal antibodies reagents used in ELISA assays during biosimilar drugs development in Polpharma Biologics S.A. The process of samples preparation was optimized. Then, the amount of proteins identified in the HCP standard purified by affinity chromatography with the usage of anti-HCPs antibodies was compared with the amount of proteins identified in the unpurified standard. The final antigen coverage of the antibodies used in the ELISA assays, when compared to the rate of identification obtained by the standard mass spectrometry technique, was established as 51% and 57% for captured and detection antibodies respectively and 43% for both reagents. The results should be treated with caution The impact of the research on the received outcome is discussed. However, to confirm the antigen coverage of the process specific antibodies by LC-MS methods for the particular cell line, additional investigations with improved analyte purification strategies are required.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: Surface presentation of cold-active β-galactosidases on Bacillus subtilis spores

SPEAKER: ***Kacper Boguszewski***

SUPERVISOR(S): *Prof.* ***Michał Obuchowski***

Spore surface display is a biomolecule presentation technique, that permits the use of otherwise unstable or fragile in extreme environments. It is a technique widely used in medicine, industry and environment reclamation. This study focuses on displaying cold-active β-galactosidases on Bacillus subtilis spores. These enzymes, which reportedly function efficiently at low temperatures, could be used to detect temperature changes above zero. Bacillus subtilis spores were selected due to their robustness, and how well known the sporulation process is in this model organism. Naturally occurring spore coat proteins of B. subtilis serve as excellent scaffolds for enzyme display, enhancing enzyme stability and activity. Cold-active β-galactosidase genes from Arthrobacter psychrolactophilus strain F2 and Rahnella sp. strain R3 were synthetized, cloned, and expressed in B. subtilis. Fusion constructs were confirmed via PCR. Enzyme activity of enzymes produced in B. subtilis in near-zero temperatures is currently being tested. If viable, chosen β-galactosidases will be grafted onto spores and undergo further tests concerning enzyme activity and stability in various conditions.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: The role of the Xre protein in maintaining the lysogeny state of phage phiCDKH01 infecting clinical isolates of Clostridioides difficile

SPEAKER: ***Małgorzata Roskwitalska***

SUPERVISOR(S): ***Adam Iwanicki****, PhD, DSc;*

Clostridioides difficile is an anaerobic, pathogenic bacillus that is one of the most commonly recognized causes of diarrhea in hospitalized patients. Antibiotics used against CDI are often ineffective and lead to recurrence of the infection, creating a need for more effective treatment methods. An alternative to the currently used antibiotics may be phage therapy, which, thanks to the use of bacteriophages specific to C. difficile, helps to avoid exacerbation of intestinal dysbiosis. The problem is that all currently known phages infecting this bacterium are temperate phages, which means that instead of immediate cell lysis, they can integrate into the host genome or replicate episomally. The subject of the study was the recently characterized C. difficile bacteriophage phiCDKH01, in whose genome a gene encoding a transcription regulator from the XRE family was identified. This regulator in other bacteriophages is responsible, among other things, for controlling the lytic/lysogenic cycle, which is why its role in the phiCDKH01 phage was investigated. The study presented the binding site of the Xre protein with a fragment of the phage genome and the impact of this binding on the transcriptional activity of the phage's putative promoters. The research showed that the Xre protein may act as a repressor and controls the transcription of genes encoding the phage's putative antirepressors, which are involved in regulating lytic development. In this way, the Xre protein may be responsible for maintaining the lysogenic state and could be a target for developing effective phage therapy, as the removal of the repressor gene would increase the lysis of pathogenic C. difficile cells.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: Bacillus subtilis spores useful in maintaining the homeostasis of oral flora

SPEAKER: ***Natalia Trochowska***

SUPERVISOR(S): ***Alessandro Negri****, PhD;*

During my presentation I will raise the problem of oral diseases, mainly dental caries, which are related to disruption of homeostasis in oral flora. I will be speaking about potential solution to this issue – spores of Bacillus subtilis, which will display ArcA protein on their surface. This protein catalyzes the conversion of arginine to citrulline and ammonia, which might act antagonistically to fermenting bacteria found in the biofilm in mouth, due to its ability to increase pH level in the environment. The display of ArcA protein on the spore surface could be a significantly cheaper solution than standard production, which makes it promising and possibly available to a larger group of people. I will show my current progress in my research and present what else am I going to do.

SESSION 6

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Design, production, purification and functional analysis of S94N and R205L gain-of-function variants of the complement C2 protein

SPEAKER: ***Jakub Buniewicz***

SUPERVISOR(S): ***Marcin Okrój****, PhD, DSc;*

The complement system is a crucial element of the innate immune response against pathogens. It can be activated by one of the three pathways: classical, lectin and alternative. They differ in the initial stages of activation, but all of them lead to lysis of the target cells and release of the proinflammatory molecules. Dysregulation of the complement caused by the gain-of-function (GoF) mutations in complement genes can lead to chronic inflammation and autoimmune response.

Mutations in factor B of the alternative pathway, which shares 39% amino acid identity with the classical pathway component C2, are often associated with complement hyperactivity. The goal of my work is to translate two GoF mutations observed in factor B to protein C2 sequence and to assess activity of the obtained variants.

Plasmids with wild-type C2 sequence were modified using site-specific mutagenesis to generate S94N and R205L variants of the C2. Then, the transfection of ExpiCHO cell line was performed. Expressed C2 variants were purified with affinity chromatography, but only R205L mutant was successfully obtained. Lastly, the activity of R205L variant was tested.

Protein C2 is involved in early stages of the classical pathway activation. It is composed of two subunits: C2a and C2b. Part C2a, together with the C4b, forms the central complex of the complement system called C3 convertase that converts C3 into an active component C3b. The C2b subunit takes part in the complex formation, but it dissociates afterward.

All of the GoF mutations in C2 are located in the C2a subunit. My research for the first time identified GoF mutation located in the C2b part of the C2. The R205L C2 variant enhanced cytolytic activity of the classical pathway, suggesting that this mutation improves binding of C2 to C4b at the stage of C3 convertase formation.

This discovery is the first step to designing a competitive inhibitor of the C2 protein that binds C4b and displaces wild-type protein but would be devoid of enzymatic activity. Such an inhibitor would block the classical pathway at the early stage of its activation before pro-inflammatory molecules are released.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Expression of recombinant C2 gain-of-function complement protein in a prokaryotic system.

SPEAKER: ***Marta Greglewska***

SUPERVISOR(S): ***Marcin Okrój****, PhD, DSc;*

Complement system is a crucial element of innate immunity and its activation is one of the effector mechanisms of the therapeutical antibodies. C2 protein plays a key role in the initial stages of the classical pathway activation of the complement system. The gain-of-function variant of C2 (C2 GoF) was created by the introduction of known mutations originating from factor B occurring in patients with rare kidney diseases, based on the homology between these two protein. It has been proven that supplementation of serum with recombinant C2 GoF variants enhances the cytocidal effect of type I anti-CD20 monoclonal antibodies.

The expression of eukaryotic proteins in a prokaryotic system is a challenging task due to the presence of post-translational modifications, such as disulfide bond formation and glycosylation. However, it carries many advantages, such as rapid and efficient expression, which translates into a lower cost of protein production. Expression of the C2 GoF protein in a prokaryotic system would facilitate its therapeutic application and increase the commercial attractiveness of this product.

The aim of my project was an expression of recombinant C2 GoF complement protein in the E. coli prokaryotic system. Therefore I designed vectors carrying a sequence coding for wild-type or C2 GoF protein with His Tag with six different signal peptides: DsbA, Jei24, MalE, OmpA, PelB, PhoA. The use of signal peptides was necessary to allow the transport of the translation product to the periplasm, where disulfide bonds can be formed. DNA fragments of vector pRSET, C2 WT and C2 GoF genes, as well as signal sequences, were amplified by PCR and used for cloning by the Gibson assembly method. The correctness of the cloning was confirmed by sequencing. After the transformation of the plasmids into the E. coli BL21(DE3)pLysS strain, overproduction was induced by the addition of IPTG. The bacteria were cultured overnight at 20º C with shaking and afterwards four fractions were obtained: culture medium, periplasm, cytoplasm and insoluble fraction. All collected fractions were subjected to SDS-PAGE analysis with Coomassie brilliant blue staining and Western blot analysis using anti-HisTag monoclonal antibodies.

Analysis of the Coomassie-stained gel showed no additional band indicating the presence of overproduced proteins at the expected height of approximately 80 kDa (approximate mass of C2 protein without glycosylation). This result was confirmed by Western blot analysis, where also no specific band appeared. The obtained results suggest that the C2 WT and C2 GoF proteins are not expressed or are produced at a very low level, which is why they cannot be detected by antibodies. Therefore, future plans include purification of the protein on a Ni-NTA Agarose beads to finally verify whether the target proteins are expressed.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Assessment of complement receptor 1(CR1) function by detection of C4d fragment on human erythrocytes

SPEAKER: ***Lion Ivaniushyn***

SUPERVISOR(S): ***Marcin Okrój****, PhD, DSc;*

Complement is a complex system composed of soluble proteins that eliminate pathogens. Such a potent tool is strictly controlled by complement inhibitors of both soluble and membrane-bound nature. Preventing excessive activation of the complement system protects patients from tissue damage and autoimmune conditions. Meanwhile, excessive suppression of complement in cancer patients by overexpressing convertase regulators is associated with a poorer prognosis. There are many rare genetic variants of complement inhibitors in the human population, but the prediction of their phenotype based on molecular modeling and bioinformatical tools is not always accurate. Therefore, there is a need for functional assays that would delineate the character of given mutations in complement genes.

One such complement inhibitor, for which there is no functional assay available, is complement receptor 1 (CR1). It is a membrane-bound protein that provides cofactor activity for proteolytic cleavage of active complement components C3b and C4b. It is a relatively big protein (250 kDa) with multiple subunits, so the production of recombinant mutant proteins would be problematic. The assessment of the cofactor activity of CR1 on human cells is also challenging due to the presence of other, redundant inhibitors. Erythrocytes are an exception to this rule, as they express solely CR1 as a membrane-bound inhibitor that provides cofactor activity. Therefore, monitoring of C3b and C4b degradation products on erythrocytes seems to be a reliable strategy to assess the CR1 function.

As the antibody that specifically recognizes the end degradation product of C4b, namely C4d, is available, this work aimed to provide a proof of principle that monitoring of C4d produced during complement activation on erythrocytes is a way to measure the activity of CR1. The project embraced the isolation of erythrocytes from human blood, and their sensitization with complement-activating antibodies followed by incubation with serum or purified complement components +/- anti-CR1 antibody of defined function-blocking capacity. Monitoring of C4d increase in fluid phase was performed by sandwich ELISA and the detection of cell-bound C4d was performed by flow cytometry.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Antineoplastic Effect of Nutraceuticals and their Combinations with Chemotherapeutics on Leukemia and Breast Cancer Cell Lines

SPEAKER: ***Fabian Trzciński***

SUPERVISOR(S): ***Patrycja Koszałka****, PhD, DSc;*

Combinational chemotherapy has been proven to present multiple advantages over classical monotherapy. By targeting different mechanisms active simultaneously in the cell, various compounds present in the combination can enhance the overall anti-tumor effect, reduce toxicity towards healthy cells, and help overcome resistance to individual chemotherapeutics. Various nutraceuticals present in daily-use food products, in addition to exhibiting a variety of properties beneficial to cancer treatment (e.g., antioxidant, anti-inflammatory, anti-angiogenic), have been shown to exhibit an active anticancerous effect, as well as an ability to modulate the performance of compounds used in chemotherapy in favor of increasing their therapeutic efficacy. The research conducted within the framework of the presented project was aimed at investigating the presence of cooperative anticancerous effects exhibited against acute lymphoblastic leukemia, Burkitt lymphoma, and triple-negative breast cancer cell lines by various combinations of nutraceuticals and chemotherapeutics, and determining their optimal therapeutic concentrations. Such approach would allow the formulation of a new strategy helpful in the treatment of three clinically significant neoplastic diseases.

LABORATORY OF RECOMBINANT VACCINES

TITLE: Development of purification method of virus-like particles based on capsid protein of human norovirus

SPEAKER: ***Anna Ziółkowska***

SUPERVISOR(S): *Prof.* ***Bogusław Szewczyk***

Virus-like particles (VLPs) morphologically resemble viruses but are non-infectious because they do not possess genetic material. This study uses anion exchange chromatography to develop a purification method of VLPs based on human norovirus VP1 capsid protein. The aim is to establish a scalable method of protein purification that is comparable to ultracentrifugation methods that are used in small-scale purifications. Norovirus structural protein VP1, which self-assembles into VLPs, was obtained in two expression systems – in insect cells using a baculovirus expression system and in mammalian cells Expi293, which can produce a high yield of the expressed protein. In the former, a bacmid construct was available prior to this study and was used to transfect Sf-9 insect cells. After achieving a high enough baculovirus titer, the infection dynamic was examined, and the obtained results of MOI=1 on day 5 of infection were used in VP1 protein production. In Expi293 mammalian cells, using a commercially available transfection kit, two plasmids harboring the vp1 gene (pcDNA 3.1(+) and pcDNA 3.4-TOPO) were used to compare the effectiveness of transfection and protein yield. VP1 proteins obtained in cell culture media of both systems were precipitated with PEG 6000 and sodium chloride and further purified using anion exchange chromatography, where the desirable protein affinity to the column was achieved by changing the pH of buffers used. Samples containing VP1 protein and chromatography fractions were analyzed by SDS-PAGE stained with Coomassie Brilliant blue and Western blotting using anti-VP1 protein primary antibodies. Additionally, selected elution fractions were examined under transmission electron microscopy.

VP1 protein was efficiently secreted to the medium in both insect and mammalian systems. Mammalian cell transfection with plasmid pcDNA 3.4-TOPO harboring vp1 gene resulted in higher VP1 protein yield than with pcDNA 3.1(+) vector. Ion exchange chromatography could be an alternative to the ultracentrifugation purification method of VLPs based on human norovirus capsid protein. Samples containing protein were screened under electron microscopy. They demonstrated characteristic virus-like particle structures in pH 7,0, pH 6,0, and pH 9,0, but no virus-like particles in elution with a buffer of pH 3,0. Furthermore, pH 6,0 of anion exchange chromatography buffers established slightly better protein affinity to the resin and immediate disassociation in step elution.

LABORATORY OF RECOMBINANT VACCINES

TITLE: Analysis of the effect of glycosylation inhibitors on the SARS-CoV-2 virus structural proteins production

SPEAKER: ***Ewa Raszewska***

SUPERVISOR(S): ***Ewelina Król****, PhD, DSc;*

The SARS-CoV-2 virus, despite its low mortality rate, contributes significantly to the health and quality of the population's life. Moreover, it has an ability to adapt quickly to the environment as well as evade immune system responses. It means that the virus is and will be present in populations causing new epidemics and pandemics. Despite commercially available vaccines and drugs against this coronavirus, further research is needed to better understand its structure and life cycle.

The main goal of this master project was to analyze the glycosylation process of SARS-CoV-2 virus structural proteins. Treatment with commercially available compounds that inhibit the N-glycosylation process was used to test the effect on SARS-CoV-2 protein production. Furthermore, in the case of protein S, it was examined how the tested inhibitors affect its ability to bind to the ACE2 receptor.

The research showed that in silico designed and then created in vitro plasmids with gene constructs allow for efficient expression of E, M, N proteins. Attached HiBiT tag to the genes encoding for E, M, N proteins enables their identification by specific antibodies or by interaction with the complementary LgBiT domain, resulting in luminescence. In addition, it has been shown that structural N and S proteins are secreted into the medium. The presence of N-glycosylation sites for N protein was not confirmed. Partial inhibition of N-glycosylation after tunicamycin treatment was demonstrated for monomers of proteins E and M. It has been shown that in case of S protein, tested compounds inhibited N-glycosylation, with tunicamycin and NGI-1 showing the best efficacy. This was also associated with a decrease in the ability of S protein to interact with ACE2 receptor. The demonstrated cytotoxicity of the tested compounds in most cases was at a low level, confirming that the above results were not due to the low protein expression resulting from the poor condition of the cells. In conclusion, in the present study we have obtained expression plasmids with constructs containing structural proteins E, M, N of SARS-CoV-2 virus, which allow their efficient production and easy identification. Analysis of structural proteins - E, M, N, S allows us to determine the presence or absence of N-glycans on their surface, analyze the effect of compounds inhibiting the process of N-glycosylation on the properties of the proteins, and the ability to bind to the ACE2 receptor in the case of S protein. Therefore, the above results can be used for further studies focusing on other aspects of such a broad issue as glycosylation.

LABORATORY OF RECOMBINANT VACCINES

TITLE: Optimizing Tick-Borne Encephalitis Virus sequencing procedure: a study on polish voles reservoirs.

SPEAKER: ***Bartosz Mundt***

SUPERVISOR(S): ***Łukasz Rąbalski****, PhD;*

Introduction

My master's thesis is focused on the Tick-borne encephalitis virus (TBEV), which belongs to the Flaviviridae family. According to the WHO, TBEV is responsible for 10,000-12,000 cases of disease annually worldwide, but about 70% of cases are not reported due to flu-like symptoms. It may infect arthropods such as ticks, humans, and rodents, which are important reservoirs of the virus. Rodents are widespread in most ecosystems and are highly infested by ticks and their larvae, which makes them a great target for TBEV research.

Aim

The aim of the work is to update PCR primer pools that are able to perform whole TBEV genome sequencing on Polish virus isolates and phylogenetic analysis of TBEV from rodent samples from northeastern Poland. An important goal of the work is also to demonstrate the transmission of the virus from an infected mother to her fetus.

Materials and methods

The genetic material of the virus came from the Military Institute of Health and Epidemiology(MIHE) in Puławy and from the Medical University of Gdańsk. The first source provided different strains of TBEV cultured in various cell cultures, which served as a positive control in initial primer pool tests. The second source provided samples from rodents and their fetuses, previously identified as positive by the nested PCR method at MUG. In my project, I used previously designed primers for the TBEV virus to see if they could be successfully used in sequencing a virus infecting rodents. The result of using these primers should be tailed amplicons. The sequencing method I used was nanopore sequencing, because it is relatively inexpensive, fast, and has previously been used successfully in SARS-CoV-2 sequencing.

Results

I am currently unable to perform phylogenetic analysis due to the fact that I have not been able to obtain complete genomes from rodent samples so far. I have used several approaches using different pools of primers, but the results obtained are still not satisfactory.

Discussion and conclusion

The lack of full genome sequences may be due to several reasons. There is a high possibility that regions for designed primers possess SNPs that are unable to tailed amplicon procedure. Also, rodent samples may have

contained a low titer of the virus possess SNP's that are unable to tail amplicon procedure. Also, rodent samples may have contained a low titer of the virus, and, in addition, the RNA from these samples may have been highly degraded. That's why further research is needed to design new primers that will make it possible to sequence the entire genome of Tick-borne encephalitis virus.

LABORATORY OF EVOLUTIONAL BIOCHEMISTRY

TITLE: Functional and structural analysis of the Hsp70 protein which is the common ancestor of the yeast proteins Ssq1 and Ssc1

SPEAKER: ***Kamila Stanek***

SUPERVISOR(S): ***Rafał Dutkiewicz****, PhD, DSc;*

Heat-shock protein 70 (Hsp70) is a protein that plays a crucial role in cell and has many various functions. One of them is taking part in the biogenesis of iron-sulphur clusters. Iron-sulphur clusters are prosthetic groups of many important proteins responsible for oxidative phosphorylation, DNA and RNA metabolism, regulation of gene expression, and many other processes. Defects in the Fe-S cluster biogenesis pathway may cause various human diseases. Hsp70 takes part in that process by interaction with scaffold protein Isu via the LPPVK motif, which enables the transfer of FeS cluster from Isu to the target protein. In the course of evolution, the gene encoding Hsp70 in yeast underwent duplication. As a result, two copies of this gene evolved into genes encoding Ssc1 and Ssq1 proteins. Ssc1 protein kept all functions typical for Hsp70, but on the other hand, Ssq1 has specialized in the biogenesis of iron-sulphur clusters. To discover what causes the difference between the specificities of those proteins, the sequence of their common ancestor AncCQ was reconstructed. In my study, I purified the substrate binding domain (SBD) of the AncCQ protein and confirmed that it binds to the LPPVK peptide by fluorescence anisotropy measurement. In the next stage of my study, I formed the SBD AncCQ-LPPVK complex, crystallized it, and determined its structure by X-ray crystallography. The result showed that the peptide binds most likely in C to N orientation, with the second proline occupying the middle position in the binding pocket.

LABORATORY OF EVOLUTIONAL BIOCHEMISTRY

TITLE: Biochemical reconstruction of the cysteine desulfurase - ferredoxin complex participating in the biogenesis of iron-sulfur clusters

SPEAKER: ***Jan Maciejko***

SUPERVISOR(S): ***Rafał Dutkiewicz****, PhD, DSc;*

Iron-sulfur (Fe-S) clusters are highly evolutionarily conserved prosthetic groups of proteins present in all living organisms. Proteins containing Fe-S clusters in their structure are involved in a number of cellular processes such as DNA repair, ribosome synthesis, electron transport and protein catalytic activity. Their presence is crucial not only for the proper functioning of the cell, but also for its survival. Dysfunctions in the biosynthesis of Fe-S clusters lead to many untreatable diseases, such as Friedrich's ataxia.

Biogenesis of Fe-S clusters requires dedicated machinery responsible for their proper formation. In prokaryotic cells, there are three systems dedicated to Fe-S cluster maturation: ISC, SUF and NIF, the former two have their counterparts also in eukaryotic cells. The ISC system, responsible for synthesizing the largest number of Fe-S clusters under physiological conditions, consists of the following components: the IscU protein, which is the scaffold on which the Fe-S is assembled, cysteine desulfurase (IscS), which provides sulfur during the assembly and ferredoxin (Fdx), which is together with its reductase responsible for transporting the electrons necessary for the reduction of sulfur to sulfide. Finally the protein complex involved in Fe-S assembly with IscU requires frataxin, which is most likely involved in the regulation of cysteine desulfurase activity. Until now, detailed structural analysis was performed only for bacterial IscS-IscU complex. However, recently there have been published results of biochemical experiments involving purified yeast proteins that in Saccharomyces cerevisiae, ferredoxin and frataxin share an evolutionary conserved interaction site on cysteine desulfurase,indicating that ferredoxin can also form stable complex with cysteine desulfurase during this process.

The main objective of my project was to determine whether it is possible, using purified bacterial proteins, to obtain stable IscS-Fdx complex in sufficient amount for structural analysis. In order to study the interaction between the proteins, first I purified both proteins, next I verified their structure, presence of prosthetic groups and their stability using circular dichroism spectrometry and finally I performed series of analysis using size exclusion chromatography in order to determine the experimental conditions to isolate IscS-Fdx complex.

SESSION 7

CORE FACILITY LABORATORIES

TITLE: Optimization of ultrasonically-assisted proteomic sample extraction and digestion

SPEAKER: ***Martyna Iwaniec***

SUPERVISOR(S): ***Katarzyna Macur****, PhD;*

Mass spectrometry (MS) is a leading technique in proteomics, allowing for the identification and quantification of proteins with high specificity. However, before MS analysis, several sample preparation steps must be completed to obtain high-quality MS spectra. In this research, we focused on optimizing ultrasonically-assisted protein extraction and digestion steps to achieve the highest coverage of protein sequences and the highest number of identified proteins and peptides.

The primary objective was to investigate whether the use of ultrasound energy would lead to improved protein extraction. Additionally, we aimed to accelerate the protein digestion process with the aid of ultrasound energy. To evaluate the effect of ultrasonically-assisted protocols on different sample types, we analyzed the proteomes of the Raoultella ornithinolytica MF1 strain and human salivary gland tissue.

For protein extraction optimization, we compared different lysis buffers and adjusted the sonication amplitude and time. For ultrasonically-assisted protein digestion, we compared MS results after 15 minutes of digestion in a sonication device to overnight digestion.

Our results suggest that the use of ultrasound energy has the potential to enhance proteomic workflows. After optimizing the protein extraction protocol for both Raoultella ornithinolytica MF1 and human salivary gland samples, we could observe an improvement in the number of identified proteins and protein sequence coverage. Furthermore, the use of ultrasound energy successfully accelerated the digestion process. The results indicate that a 15-minute digestion not only saved hours of work but also increased the number of identified proteins and peptides compared to traditional protein digestion. These results underscore the value of ultrasonically-assisted protocols in proteomics, warranting further investigation and development of such techniques to unlock their full potential.

LABORATORY OF STRUCTURAL BIOLOGY

TITLE: Structural characterization of proteins interaction involved in human mitochondrial genome repair and maintenance

SPEAKER: ***Julia Karasińska***

SUPERVISOR(S): ***Michał Szymański****, PhD, DSc;*

The mitochondrial genome operates under challenging conditions, making it particularly susceptible to oxidative damage compared to the nuclear genome. Although we have many copies of mtDNA and individual genetic alteration do not always have consequences, the accumulation of mtDNA mutations is associated with cell dysfunction and triggers pathophysiology of various medical conditions. Therefore, maintaining robust mtDNA repair is pivotal for mitochondrial function.

The base excision repair (BER) pathway is the primary biochemical repair system in mitochondria. According to the latest findings, the enzymes involved in the mitochondrial base excision repair (mtBER) cooperate with each other forming a complex known as mitochondrial repairosome. This multi–protein complex comprises DNA glycosylases, apurinic/apyrimidinic endonuclease (APE1), endo/5' – 3' exonuclease (EXOG), DNA polymerase γ (Polγ), and DNA Ligase III (Lig III). These enzymes coordinate repair process in a "passing a baton" manner, ensuring uninterrupted progression of damaged DNA substrate through the pathway. Mutations in genes encoding enzymes involved in mtBER are also attributed to the presence of various cell dysfunctions. While the biological importance of the process and the fundamental steps of mtBER are well-established, the molecular mechanism behind the complex's assembly and the way its components cooperate is still unknown. This study is focuses on elucidating the interaction between two key enzymes involved in mtBER: APE1 and EXOG.

We confirmed the formation of the APE1 – EXOG complex using ultracentrifugation in glycerol gradient and analyzed it structurally. Experimental methods, including X-ray crystallography, complemented by computational approaches like AlphaFold predictions, were employed to investigate functional interfaces within the APE1-hEXOG complex.

LABORATORY OF STRUCTURAL BIOLOGY

TITLE: Biochemical characterization of protein interaction involved in human mitochondrial genome repair and maintenance

SPEAKER: ***Katarzyna Paniak***

SUPERVISOR(S): ***Michał Szymański****, PhD, DSc;*

Maintenance of the mitochondrial genome is key for cell survival. Human mitochondrial DNA (mtDNA) exhibit higher number of oxidative DNA damages in comparison to nuclear DNA due to specific mitochondrial environment enriched in reactive oxygen species (ROS). Accumulation of damaged mtDNA leads to the development of various diseases, such as Leber hereditary optic neuropathy (LHON), Parkinson's disease and muscular dystrophy, as well as has implications in aging.

Base Excision Repair (BER) is the first line of defence allowing the cell to repair oxidation-damaged DNA sites. Mitochondrial BER (mtBER) machinery is independent from nuclear BER and consists of multiple enzymes: DNA glycosylases, apurinic/apyrimidinic endonuclease (APE1), endo/5' – 3' exonuclease (EXOG), DNA polymerase γ (Polγ) and DNA Ligase III (Lig III). It is proposed that mtBER enzymes are form a complex called repairosome which allows them to work in a 'passing-the-baton' manner at the lesion site during DNA repair. Thus, the formation of repairosome and the interactions between enzymes involved in mtBER are believed to play a role in controlling the order of reactions in the mtBER pathway. While the major elements of the mtBER pathway have been identified, our understanding of the molecular mechanisms underlying repairosome assembly and the regulation of the mtBER reaction sequence remains limited.

To examine the possible cooperation of three mtBER enzymes – APE1, EXOG and LIG III, we first purified the enzymes using different chromatography techniques. We confirmed the quality of purified enzymes with a thermal stability assay. Next, to recreate sequence of events in the mtBER pathway, we performed variety of biochemical in vitro gel assays using specially designed DNA substrates with THF (tetrahydrofuran, a synthetic analougue of abasic site), which mimic the DNA intermediate present in mtBER pathway.

Our findings revealed that the interaction between APE1 and EXOG regulates the sequence of events in mtBER. Moreover, EXOG was found to be capable of cleaving toxic intermediates resulting from failed ligation, allowing those intermediates to be processed in subsequent steps of mtBER. Together, our data provide functional evidence of the cooperation between APE1 and EXOG in regulating the DNA repair process in mitochondria.

LABORATORY OF PLANT MICROBIOLOGY

TITLE: Generating mutants of Pseudomonas donghuensis strain P482 with inactivated genes related to Type 6 Secretion System

SPEAKER: ***Ewa Izdebska***

SUPERVISOR(S): *Prof.* ***Sylwia Jafra***

Organisms interact with each other in different ways, that includes symbiotic and antagonistic dynamics. Pseudomonas spp. can populate plants providing them with nutrients, promoting their growth and protecting them from pathogens. The Pseudomonas donghuensis P482 strain, isolated from tomato rhizosphere, exhibits the abovementioned features.

Bacteria use secretion systems to transport substances through cell membranes. The sort of substances that can be transported depends on the type of secretion system used. Possessing the secretion systems allows bacteria to compete more effectively with other organisms and develop antibacterial and antifungal properties. P. donghuensis P482 possesses Type 6 Secretion System (T6SS). T6SS is a molecular mechanism discovered in 2006, present in Gram-negative bacteria. Fourteen proteins build: a complex anchored in cell membrane, base and polymeric tail with a spike. This project focuses on three proteins: VgrG – part of the spike, HcpI – monomer of the tail and ClpVI – protein taking part in the disintegration and recycling of T6SS.

Goal: The main goal was to obtain mutants of P. donghuensis P482 with inactivated genes VgrG, HcpI or ClpVI corresponding with the proteins building T6SS.

Methods: The project started with isolating P. donghuensis P482 genomic DNA, pRE112 plasmid DNA form Escherichia coli DH5α λpir and pKNOCK pDNA from E. coli S17 λpir. P. donghuensis P482 gDNA was used as a template for the amplification of fragments of genes VgrG, HcpI and ClpVI during polymerase chain reaction (PCR). Plasmids and obtained fragments were digested with enzymes: KpnI and SacI for pRE112, BamHI and XhoI for pKNOCK. Ligation led to obtaining plasmids with insert – fragment of genes VgrG, HcpI or ClpVI. During heat shock transformation, the obtained plasmids were transferred into E. coli NM522. A selective medium with antibiotic was used to confirm the presence of plasmids with a gene of resistance against the antibiotic (chloramphenicol in pRE112, Kanamycin in pKNOCK). From this step, the experiment was continued with pRE112 isolated from E. coli NM522 and used for heat shock transformation into E. coli ST18λpir (strain with genes that allow vector transfer). E. coli ST18λpir with pRE112 vector were plated on a selective medium with chloramphenicol and δ-aminolevulinic acid. The next step was conjugation: incorporating pRE112 plasmid with VgrG, HcpI or ClpVI gene fragment into P. donghuensis P482 genome, which resulted in the discontinuity of the genes responsible for coding T6SS proteins VgrG, HcpI or ClpVI and their inactivation.

Results: Mutants of P. donghuensis P482 with inactivated genes VgrG, HcpI or ClpVI which correspond with the proteins building T6SS were obtained. Growth curves, shape and size of colonies of mutants and wild-type

bacteria were compared. Inactivation of VgrG, HcpI or ClpVI genes does not affect the growth or morphology of bacteria in any significant way. pRE112 was proven to be a more effective vector compared to pKNOCK.

Further studies: Obtained mutants of P. donghuensis P482 with inactivated VgrG, HcpI or ClpVI gene can be used for researching how said mutation affects the functionality of T6SS and antibacterial and antifungal properties of the bacteria.

CORE FACILITY LABORATORIES

TITLE: Immunomodulatory Effects of Venetin-1 in Immunosuppressed Mice: A Proteomic Analysis of Spleen Tissue

SPEAKER: ***Joanna Stacevič***

SUPERVISOR(S): ***Paulina Czaplewska****, PhD, DSc;*

Venetin-1 is a protein-sugar fraction isolated from the coelomic fluid of Dendrobaena veneta earthworms. It has been previously demonstrated to possess antitumor and antifungal properties while being non-toxic to normal vertebrate cells. Recent in vitro studies on macrophages have shown that Venetin-1 enhances the secretion of very important, multifunctional cytokines, suggesting its potential use in non-specific immunotherapy.

To further investigate its safety and immunomodulatory effects, in vivo studies were conducted on mice. These studies aimed to confirm Venetin-1's ability to restore immune function following immunosuppression induced by cyclophosphamide (CP). The mice were divided into four groups of three individuals each: a control group receiving NaCl solution, a CP control group receiving CP and NaCl solution, and two Venetin-1 treatment groups. The Venetin-1 treatment groups were first immunosuppressed with CP and then administered Venetin-1 at two different dosages to stimulate immune response.

After the treatment period, proteins were isolated from the spleen cells of the mice and analyzed using mass spectrometry. The statistical protein analysis showed minimal differences between the NaCl control group and the group treated with a lower dose of Venetin-1 after immunosuppression. On the other hand, the group treated with a higher dose of Venetin-1 exhibited significant changes in protein levels. Functional protein analysis revealed that many of the upregulated proteins in the high-dose Venetin-1 group were involved in immune and proliferation processes, such as the IL-17 signaling pathway, platelet activation, and the Hippo signaling pathway.

These results support the hypothesis that Venetin-1 induces an upregulation of proteins involved in immune response activation, confirming its potential role in enhancing immune function.

TITLE: Evaluation of the influence of Venetin-1 nanoparticle on Candida auris cells

SPEAKER: ***Weronika Ścibek-Rejmontowska***

SUPERVISOR(S): ***Paulina Czaplewska****, PhD, DSc;*

Venetin-1 is a protein-carbohydrate fraction isolated from coelomic fluid of Dendrobaena veneta by a team of researchers led by prof. Marta Fiołka from Maria Curie-Skłodowska University in Lublin. So far, they have proven that Venetin-1 acts against lung cancer cells and colorectal cancer cells without harming healthy human cells. It has also been shown that Venetin-1 is effective towards the yeast Candida albicans – it targets fungal cell wall, leads to apoptosis and possible autophagy of fungal cells. Effectiveness towards this problematic pathogen causing hundreds of nosocomial infections, has led to the idea that Venetin-1 might also affect another dangerous Candida species.

Candida auris, first isolated in Japan in 2009, has been described as an urgent threat by CDC. Since 2015 the number of cases has been growing rapidly on every continent. This tendency has been especially visible during Covid-19 pandemic, because C. auris attacks immunocompromised people, with mortality rates ranging from 34 to 68%. What is more, 90% of isolates are resistant to at least one antifungal and 30% are resistant to at least two antifungals. Therefore, it is crucial to search for antifungal compounds in various sources. With evidence showcasing antifungal activity of Venetin-1, we decided to evaluate the influence of Venetin-1 on C. auris cells.

In the microbiological part of research, we examined the influence of 25, 50, 100 µg/ml of Venetin-1 on the C. auris by monitoring the growth curve for 48 hours and observed morphological changes under the microscope. Then, we tried to determine minimum inhibitory concentration (MIC) of Venetin-1. We also established C. auris's susceptibility profile. In the proteomics part, we analysed changes in C. auris' proteome after incubation with 50 and 100 µg/ml of Venetin-1 through quantitative SWATH-MS analysis.

Moreover, since evidence suggests presence of lysozyme in the coelomic fluid of D. veneta, we conducted immunoprecipitation on Sepharose columns in order to isolate and identify lysozyme. Additionally, we conducted retrospective analysis of previous samples of coelomic fluid and Venetin-1 separated on SageELF and 2-D gel electrophoresis. The search was carried out in an Annelida protein database expanded with lysozyme sequences. This type of analysis allowed to determine if lysozyme was present in the coelomic fluid.

Preliminary results suggest that Venetin-1, while acting against C. albicans, is not effective towards C. auris. Currently, there is still little known about C. auris biology. Therefore, more research needs to be done to understand this yeast and its resistance mechanisms against known and potential antifungals. Results from immunoprecipitation on Sepharose columns and retrospective analysis confirm that lysozyme is present in coelomic fluid of D. veneta. Presence of lysozyme also suggests that this enzyme might be one of the components of Venetin-1 which affects C. albicans cells.

DEPARTMENT OF INORGANIC CHEMISTRY, GUMED

TITLE: In silico selection and in vivo verification of activity of novel tregitopes - polypeptides with tolerogenic activity

SPEAKER: ***Kamil Rykała***

SUPERVISOR(S): *Prof.* ***Tomasz Grabowski***

Tregitopes are a class of regulatory T-cell (Treg) epitopes that have been shown to induce and expand Tregs, a subset of T cells responsible for maintaining immune tolerance and preventing autoimmune responses. These polypeptides possess the unique ability to modulate the immune system, making them promising candidates for addressing various immunological disorders and challenges.

Autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and type 1 diabetes, are characterized by an aberrant immune response against self-antigens, leading to tissue damage and organ dysfunction. Additionally, the development of anti-drug antibodies (ADAs) against monoclonal antibody therapies can compromise their efficacy and safety. Allergies and adverse reactions to vaccines also pose significant challenges in healthcare. Tregitopes offer a potential solution to these issues by harnessing the body's natural tolerance mechanisms.

In this research, my supervisor and I have employed in silico techniques to identify and select novel tregitope candidates with the potential to induce and expand Tregs. These computationally predicted tregitopes have been subjected to vitro screening and validation using cellular assays. The presentation will highlight the execution of in vivo verification of the most promising tregitope candidates.

By leveraging the tolerogenic properties of tregitopes, this research aims to help to develop novel therapeutic strategies for autoimmune diseases, mitigate the formation of ADAs against monoclonal antibody therapies, and enhance the safety and efficacy of vaccines. The successful identification and validation of novel tregitopes hold significant potential for improving patient outcomes and advancing the field of immunomodulatory therapies.

LABORATORY OF BIOLOGICALY ACTIVE COMPOUNDS

TITLE: Molecular analysis of dickeyocin P2D1 production by Dickeya dadantii 3937 under the influence of environmental factors.

SPEAKER: ***Marta Sobolewska***

SUPERVISOR(S): *Prof.* ***Robert Czajkowski***

Phage tail-like particles (tailocins) are bactericidal nanomolecular structures that resemble bacteriophage tails, encoded in bacterial genomes, and released via bacterial lysis. Their name stems from the fusion of "tail" and "bacteriocin," the term "tail" in their name originates from their structural similarity to bacteriophage tails. In contrast, the suffix "ocin" draws a parallel to bacteriocins—molecules released by bacteria to compete with others by inhibiting growth or causing death. This study focuses on the production dynamics of dickeyocin P2D1 by Dickeya dadantii 3937, a pectinolytic bacterium known for causing bacterial diseases in various plant species.

The primary objective was to elucidate the conditions and quantify the production of dickeyocin P2D1 under different environmental factors. Tailocin production was induced in D. dadantii cultures using different inducers, such as antibiotics and hydrogen peroxide. The tailocins were subsequently purified, and their levels were assessed using the semiquantitative spot test method.

Results indicated that the production of dickeyocin P2D1 is significantly influenced by antibiotics. Mitomycin C, norfloxacin, and ciprofloxacin induced tailocin production, whereas ampicillin and chloramphenicol reduced it compared to the control. Hydrogen peroxide (1 µM/ml), serving as a natural inducer, was found to be as effective as mitomycin C (1µg/ml). What is more, temporal analysis revealed that the highest tailocin production occurred six hours post-induction, with stable levels maintained thereafter.

Gene expression analysis was conducted to investigate the regulation of tailocin production. Primers specific to the tailocin genes in D. dadantii 3937 were designed, and RT-qPCR was used to monitor gene expression regulation. The results showed that the expression of structural genes peaked two hours after the addition of mitomycin C, correlating with the initial surge in tailocin production and subsequent bacterial lysis.

In conclusion, this study not only provides significant insights into the molecular mechanisms regulating the production of dickeyocin P2D1 by D. dadantii 3937 but also offers practical implications. Understanding these processes could facilitate the development of novel biocontrol strategies against phytopathogenic bacteria, potentially mitigating agricultural losses caused by bacterial soft rot.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: The role of FGFR and ETV4-Hippo pathway in resistance to therapies of triple positive breast cancer.

SPEAKER: ***Zofia Rynkowska***

SUPERVISOR(S): ***Monika Górska-Arcisz****, PhD*

Breast cancer is the most commonly diagnosed cancer among women worldwide. There are four main breast cancer subtypes: luminal A, luminal B, HER2-positive and triple-negative. They are categorized based on expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth receptor 2 (HER2). Among these subtypes is triple-positive breast cancer (TPBC) subtype, which is characterised by overexpression of all three receptors. TPBC patients receive a worse prognosis than HER2-negative ones and quickly develop drug resistance. TPBC is currently treated with anti-HER2 agents and chemotherapy. It has been shown that simultaneous use of anti-ER and anti-HER2 therapies, e.g. tamoxifen and lapatinib, improves disease-free survival. Developement of resistance to therapy in breast cancer is a common event. Around 40% of patiens show an innate resistance to tamoxifen and the rest of them develope it during treatment. It is widely known that resistance to anti-cancer therapies is mediated by tumor microenvironment. It has been proven that fibroblast growth factor/fibroblast growth factor receptor (FGF/FGFR) signalling can activate ER in a ligand-independent way, thus making cancer cells resistant to anti-ER drugs. Therefore, identification of molecular mechanisms of resistance is crucial to improving patients outcome. First aim of my project was to verify the mechanism of double resistance to lapatinib and tamoxifen in a TPBC cell line resistant to these inhibitors (BT-474 LTR).

The growing evidence indicates that the dysregulated Hippo pathway, especially YAP/TAZ proteins (downstream Hippo transcriptional coactivators), is involved in breast cancer progression, invasion and drug resistance. Some studies indicate that ETV4 activity is regulated by FGFR, and that ETV4 potentiates YAP retention in nucleus. A preliminary study conducted by our team showed an increase of ETV4 transcription factor mRNA in TPBC cells treated with FGF2 (an FGFR1 ligand) alone or in combination with tamoxifen and lapatinib. Hence, the second aim of my project was to verify the role of FGFR-ETV4-Hippo axis in developing resistance of TPBC cells to anti-ER therapies.

We observed increased expression level of FGFR4 in BT-474 LTR cell line. Moreover, we demonstrated a simultaneous increase in YAP and decrease in TAZ expression levels, which might be responsible for cells resistance. We also observed an increase in ETV4 expression level upon stimulation of BT-474 with FGF2. However, FGF2-mediated stimulation of BT474 cell line resulted in increased YAP phosphorylation, responsible for it translocation to cytoplasm, which suggests that upon activation of FGFR signalling, the activity of YAP/TAZ is independent of Hippo pathway. 3D growth assay in Matrigel showed that inhibition of YAP with verteporfin abolished FGFR-dependent resistance to tamoxifen. Observed results demonstrated that FGFR signalling affects YAP/TAZ and ETV4 protein expression levels. Moreover, 3D growth analyses suggested the potential application of YAP inhibitors in a

combination with anti-ER therapy. Despite conducted experiments, the role of FGFR-ETV4-Hippo axis in breast cancer remains unclear and needs to be further investigated.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: Isolation and characterization bacteriophages from hospital strains of Clostridioides difficile.

SPEAKER: ***Aleksandra Sidoruk***

SUPERVISOR(S): ***Krzysztof Hinc****, PhD, DSc;*

Clostridium difficile is an important pathogen responsible for severe gastrointestinal infections, often leading to chronic enteritis. The increase in the number of antibiotic-resistant strains requires the search for alternative therapeutic strategies, among which therapy using phage proteins is extremely promising. Studying the relationship between the virus and the host is crucial to understanding evolutionary relationships, as well as to the possibility of developing the use of bacteriophages and their derivatives in therapy. This presentation discusses the identification and characterization of bacteriophages isolated from hospital strains of C. difficile.

Preliminary findings reveal trends in the bacteriophage population associated with C. difficile. The vast majority belong to the Mayoviridae family. Genetic analysis made it possible to visualize the presence of non-functional prophages in the genome of the majority of clinical bacterial strains analysed.

These studies are part of the mapping of the population of bacteriophages that infect Clostridium difficile. Knowledge about evolutionary interactions between bacteria and bacteriophages constitutes the basis for creating bactericidal preparations based on natural antagonisms in the virus-bacteria relationship.