13 BIOTECHNOLOGY STUDENTS' CONFERENCE

BOOK OF ABSTRACTS

WORKING VERSION

PREPARED BY: *PAWEŁ SERAFIN*

TABLE OF CONTENTS

INTRODUCTION

<Preface by the Dean>

HONORARY SPEAKER

"Small extracellular vesicles drive aberrant responses of T lymphocytes to lipid antigens in atopic skin"

Danuta Gutowska-Owsiak, M.D., PhD, D. Sc.

Professor at the University of Gdańsk

Danuta Gutowska-Owsiak graduated from the Medical University in Gdańsk, Poland, and then moved to the UK, where she undertook doctoral training at the University of Liverpool. In 2009 she joined the University of Oxford and the MRC Human Immunology Unit (group of Prof. Graham Ogg) at the prestigious Weatherall Institute of Molecular Medicine. Danuta was distinguished with the "Young Investigator Award 2017" by the British Society for Investigative Dermatology for her substantial contribution to the dermatology research. She was awarded the prestigious National Science Centre -EU Marie Skłodowska-Curie COFUND fellowship POLONEZ and First TEAM startup grant from the Foundation for Polish Science which allowed her to initiate a programme of immunological research at the Intercollegiate Faculty of Biotechnology UG-MUG in Gdańsk. Following this, Danuta received NCN Sonata BIS and NCN Opus funding to consolidate her line of research.

Danuta has a strong interest in the immunity of the skin and epidermal barrier formation. Together with her team, she investigates allergic inflammation as well as basic immunological mechanisms of T cell biology, including more translational angle.

TUESDAY 6TH JUNE

SESSION 1

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Role of CD73 (ecto-5'-nucleotidase) in the regulation of the tumour microenvironment in breast cancer progression

SPEAKER: ***Paweł Serafin***

SUPERVISOR(S): ***Patrycja Koszałka****, PhD, DSc;* ***Paweł Serafin****, MSc, Eng;*

Ecto-5'-nucleotidase (CD73, eNT) catalyses the conversion of nucleoside 5'-monophosphates to nucleosides, particularly converting 5'-AMP to extracellular adenosine (eAdo), which leads to various physiological and pathological responses. In the context of solid tumour growth, the development of hypoxia and inflammation triggers an increase in CD73 expression and the accumulation of interstitial adenosine. It is primarily associated with the suppression of the anti-tumour immune response by regulatory T cells, which hinders the activity of CD8+ T cells.

Experimental studies using murine cancer models, either through genetic alterations or chemical inhibition of CD73, have shown that reducing CD73 levels decreases tumour growth and metastasis in various types of cancer. This includes breast cancer (BC), which is the most diagnosed cancer type among women with high mortality. However, BC progression and development are complicated due to its heterogeneity and specific molecular stratification according to the expression of estrogen (ER) and progesterone (PR) receptors, and overexpression of human epidermal growth factor receptor type 2 (HER2). While several clinical studies have shown a correlation between high CD73 expression and shorter overall survival in BC patients, there are conflicting data regarding its dependence on the status of ER, PR or HER2. Some studies have even suggested that elevated CD73 expression may predict a good prognosis in stage I-III BC patients. It was suggested in meta-analyses, that the correlation between CD73 expression and clinical outcome may depend on other factors, such as age, specific population studied, and previous treatments received.

While orthotopic syngeneic breast cancer models can partially overcome the heterogeneity issue, they may not fully reflect the multistage process involved in breast cancer progression. The changes that occur within the tumour microenvironment during the initial stages of primary tumour development seem to play a significant role in the dissemination of cancer cells in breast cancer. To address these complexities, a multistage breast cancer model induced by a carcinogen/promoter combination that closely resembles the developmental pathway of human breast cancer was utilised. Preliminary research indicates a complex relationship between CD73 role in BC initiation and progression and the expression of PR, ER and the tumour burden.

As tumour microenvironment is a key factor in cancer progression, this part of the project concentrates on the role of CD73 in the regulation of the changes in immune cell types, EMT and stem cell markers in the microenvironment of the primary and secondary mammary gland tumours in the chemically-induced murine BC model. The data will be correlated with the expression of main clinical biomarkers of breast cancer, such as ER, PR and HER2, as well as with the tumour burden. Extracellular adenosine metabolism in the regulation of cancer cell stemness will be additionally analysed in vitro using mammosphere cultures.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Supplementation of Obinutuzumab with gain-of-function variant of C2 protein improves therapeutic efficiency

SPEAKER: ***Patryk Szynkowski***

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

One possible way of treating blood cancers is immunotherapy. Along with chemotherapy, monoclonal antibodies are administered to patients' bloodstream, to eradicate malignant cells with the help of the immune system. These antibodies target the CD20 antigen to which they bind, activating one of four pathways that lead to cell death. However, none of the currently available monoclonal antibodies can effectively utilize all four ways to maximize cytocidal efficiency, and due to the nature of these pathways, it is almost impossible to manufacture antibodies that could do so. That is the reason why some of the researchers change the approach to improving immunotherapies based on anti-CD20 monoclonal antibodies. Instead of trying to create an omnipotent one, they try to improve their effectiveness by utilizing mutations found within the complement system and supplementing serum with variants of C2 containing these mutations. One of the said variants of C2 was a subject of my project.

The aim of the research was to prove the gain-of-function properties of a quadruple mutant of C2 and its impact on the effectiveness of the type II anti-CD20 monoclonal antibody – Obinutuzumab. By performing two distinctive assays – CDC and ADCC with supplementation of serum with wild type and quadruple mutant of C2. It allowed us to directly measure the impact of the C2 variant on two out of four pathways directly (Complement Dependent Cytotoxicity and Antibody-Dependent Cellular Cytotoxicity) and third indirectly (Programmed Cell Death) in in vitro environment. The results confirmed the positive impact of the gain-of-function variant on the cytocidal effects of Obinutuzumab on CD20-positive cells. Additionally, effector cells used for ADCC assays were NK cells isolated from freshly acquired units of blood, legitimizing received results by usage of normal, wild-type NK cells instead of immortalized NK-derived cell line.

Further analysis is needed, however, current data shows that utilization of mutations within the complement system is a valid way to improve the therapeutic effectiveness of anti-cancer drugs such as Obinutuzumab and in the future may become a standard procedure in blood cancer therapies.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Mutations in genes encoding complement proteins found in rare autodegenerative diseases

SPEAKER: ***Agata Żukowska***

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

Complement system is one of the essential elements of an innate immune response. It is a complex of dozens of proteins present in plasma, as well as in other body fluids, along with associated receptors and regulators. It works by activating the enzyme cascade, leading to a number of phenomena that are important in the course of the immune response. The kidneys are particularly sensitive to complement abnormalities due to strong blood flow and the delicate structure of the filtration barrier. Kidney disease caused by a malfunction of the complement system includes atypical hemolytic uremic syndrome (aHUS). 70% of aHUS cases are caused by mutations in genes encoding regulatory proteins and alternative pathway inhibitors. The remaining 30% remains unexamined. However, it is suspected that their cause may be abnormalities associated with the classical pathway, which wasn't taken into account during disease diagnosis. Due to the high degree of similarity in the structure and amino acid sequence between the C2 protein present in the classical pathway and factor B from the alternative pathway, there is a high probability that they will have analogous mutations, which is why it is extremely important to analyze the C2 protein mutations occurring in patients suffering from aHUS.

The aim of my master's thesis was to analyze the function of the T422A and R355Q variants of the C2 protein previously identified in patients with aHUS.

To obtain mutant variants of the C2 protein, site-directed mutagenesis was conducted. Then, the DH5α E. coli cells were transformed using mutant DNA and the presence of the mutated gene was checked by sequencing. Eukaryotic cells were transfected and the expressed protein was purified using the affinity chromatography setup connected to ÄKTA Start system. Finally, obtained mutants were checked for their activity.

The results of the Western Blot analysis showed that the protein was obtained in a degraded form. Further studies are needed and the expression of the protein in ExpiCHO system under reduced temperature conditions may be required.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Anti-C4d antibodies as a potential immunotherapeutics

SPEAKER: ***Jacek Kuryło***

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

The limited effectiveness of cancer therapy is one of the main problems of modern medicine. It is a very common scenario that cancer cells develop resistance. After administering the initial doses of dedicated therapeutics, each subsequent dose is ineffective. Some of the therapeutic antibodies activate the complement system, which leads to the osmotic lysis of the target cell. However, tumor cells counteract this cytotoxic activity by overexpression of complement inhibitors that cleave the active complement component C4b to the inactive C4d fragment. Then, cells that have been treated but not destroyed by the complement system are labeled with covalently-bound C4d. Therefore, a neoepitope on C4d is an ideal target for the second-wave antibodies aimed to eliminate cells that become resistant to previous therapy. The potential of this strategy gives hope for the creation of a universal anti-cancer therapy in the future.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: FGFR4-dependent response to PARP Inhibitors in Triple Negative Breast Cancer cells

SPEAKER: ***Patrycja Kawa***

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

BACKGROUND:

Breast cancer (BC) is the leading cause of cancer death among women. It has been recognized as a clinically and molecularly heterogeneous disease with multiple subtypes. Triple negative breast cancer (TNBC) is characterized by the lack of expression of estrogen, progesterone and human epidermal growth factor receptor 2 and accounts for up to 20% of all BC cases. The treatment landscape for this subtype consists of chemotherapy, with very limited options for targeted therapies. One of approved targeted therapies for TNBC is based on PARP inhibitors, but it is available only for patients with mutations in BRACA1/2 genes.

It is well acknowledged that tumor microenvironment (TME) affects progression and response of BC to therapies. Members of the Fibroblast Growth Factor Receptors (FGFR) family are one of the key mediators recognizing signals from the fibroblast-rich tumor microenvironment. FGFR4, a structurally unique member of the above family, has been shown to be overexpressed in many cancers (including BC) and involved in resistance to anti-cancer therapies.

AIM OF THE STUDY:

Analysis of FGFR4 involvement in response of TNBC cells to PARP inhibitors.

RESULTS:

The study showed that FGFR4 plays an important role in growth and proliferation of TNBC cells. Usage of a specific FGFR4 inhibitor reduced growth of TNBC cells in 2D and 3D cultures in Matrigel. The FGF19, FGF21 and FGF23 stimulations resulted in significant growth promotion of TNBC cells and abolished Talazoparib (PARP inhibitor) mediated inhibition of TNBC colonies size. Surprisingly, FGFR4 knock-down diminished response of TNBC cells to Talazoparib (PARP inhibitor) and FGF19, FGF21 and FGF23 treatment enhanced observed effect - protective effect against PARP inhibitors was shown in both, parental and FGFR4-negative cells.

CONCLUSION:

The results demonstrate a crucial role of FGFR4 in proliferation, growth and response of TNBC cells to targeted therapies. The study showed for the first time that FGFR4 sensitize cells to PARP inhibitors whereas resistance to anti-PARP treatment might be mediated by other FGFR family member. Moreover, these findings highlight the possible clinical value of FGFR4 as a predictive factor of TNBC patient's response to PARP inhibitors.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: Interdependence between FGFR2 signaling and the IRS-1 in response to anti-estrogen therapies in luminal breast cancer

SPEAKER: ***Martyna Ochocińska***

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

Introduction

Breast cancer is one of the most common cancers among women. The most frequent subtype is the luminal subtype, which accounts for 70% of all breast cancer cases. Despite the availability of effective hormonal therapies (e.i. tamoxifen, fulvestrant) the acquisition of resistance to treatment has become a serious and growing problem in recent years. The emergence of resistance among patients has been linked to alternative activation of the ER through signals originating from the tumor microenvironment, including fibroblast growth factor receptor (FGFR) signaling. Furthermore, a correlation has been found between the level of FGFR2 protein and the expression of IRS-1 gene in tumor from patients with ER+ breast cancer.

IRS proteins constitute a family of adaptor proteins mainly involved in glucose metabolism, growth, and proliferation. It is well-acknowledged that besides the classical pathway for IRS-1, which involves signaling from the insulin-like growth factor 1 receptor (IGF-1R), IRS-1 can also form a complex with the estrogen receptor. Therefore, there has been increasing interest in the interplay between the FGF7/FGFR2 signaling pathway and IRS-1 protein, which could be a mechanism involved in the acquisition of resistance to anti-ER drugs.

Project Aim

Investigation of the FGF7/FGFR2 signaling pathway impact on the regulation of IRS-1 protein and study how the interplay between FGF7/FGFR2 signaling and IRS-1 affects the response to anti-ER therapy in luminal breast cancers.

Materials and Methods

Western blot analysis was used to examine the impact of the IRS-1 protein inhibitor, NT157 on the level and phosphorylation of selected proteins, as well as to investigate the influence of FGF7 cells stimulation on the phosphorylation level of IRS-1 protein. The same method was used to examine the interdependence between Jun family proteins and IRS-1 protein. Lentiviral system was used to derive luminal breast cancer cell lines with silenced expression and overexpression of IRS-1. The derived cell line variants and the wild-type variant, were used to conduct anchorage-independent growth assay in soft agar to observe how the level of IRS-1 protein expression affects the response to tamoxifen and fulvestrant, and to investigate whether the interplay between FGFR2 signaling pathway and IRS-1 affects the response to selected therapies.

Results

The Western blot analysis revealed the following results: the IRS-1 protein inhibitor not only affected its degradation but also induced changes in ER, PR and Jun proteins level. Furthermore, FGFR2 signaling affected the phosphorylation level of IRS-1 and IGF-1R. The interdependence between Jun family proteins and IRS-1 protein was demonstrated. The 3D cultures showed that overexpression of IRS-1 protein is associated with a poorer response to tamoxifen and fulvestrant, and FGF7 cell stimulation in the presence of drugs exacerbates the resistance effect.

Summary

The presented results demonstrate that active FGF7/FGFR2 signaling pathway affects IRS-1 by modulation of its phosphorylation level. Furthermore, luminal breast cancer cell lines overexpressing IRS-1 demonstrated poor response to anti-ER therapies, and theFGFR2 signaling exacerbates this effect. Additionally, the levels of IRS-1 protein and Jun family proteins are interdependent, which may also contribute to development of resistance to anti-ER drugs.

SESSION 2

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: FGFR2 activity in regulation of AP1/ER interplay in luminal breast cancer

SPEAKER: ***Alan Warszawski***

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

Breast cancer (BCa) is the most common cancer among women worldwide and the leading cause of cancer death. The most common subtype is luminal BCa, characterized by the expression of receptors for steroid hormones, i.e. ER (estrogen receptor) and PR (progesterone receptor). The gold standard treatment of luminal BCa is anti-ER endocrine therapy with inhibitors of ER activity such as tamoxifen or fulvestrant. However, oncologists are facing a growing problem of resistance to such drugs. Almost 45% of patients do not respond to anti-ER therapies from the beginning (de novo resistance), and resistance often evolves during the therapy (acquired resistance).

One of the main reasons of resistance to anti-ER treatment is signaling from the tumor microenvironment (TME), including fibroblasts growth factor/fibroblasts growth factor receptor (FGF/FGFR) signaling. FGFR can activate ER independently of estradiol binding. It has been also proved that AP-1 (activator protein 1) transcription factor interacts with ER redirecting its binding to DNA (tethering mechanisms) which could lead to endocrine resistance. Our laboratory's latest work showed a correlation between expression of AP-1 transcription factor (JunB/c-Jun) and FGFR2. We have also proved that FGFR2 signaling induces JunB expression. Hence, my project aims to verify the involvement of FGFR2 in regulation of AP-1/ER interplay in luminal BCa.

My results show that FGFR2 JunB axis is responsible for abrogation of negative effect of tamoxifen on BCa cell growth which involves JNK and GSK3 activity. Obtained results suggest that FGFR signaling impairs turnover of JunB. These might contribute to endocrine therapy resistance in BCa. That hypothesis however needs to be verified by further investigation.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: The role of chaperone-mediated autophagy in SARS-CoV-2 infection

SPEAKER: ***Maria Borysiak***

SUPERVISOR(S): ***Andrea Lipińska****; PhD;* ***Marcin Lubocki****, MSc;*

SARS-CoV-2 is an enveloped, positive sense single-stranded RNA virus. It is a novel pathogen that emerged in 2019, causing a worldwide pandemic of COVID-19. Its molecular biology is yet to be fully understood. The knowledge of SARS-CoV-2 and its viral protein-host protein interactions are substantial for creating any strategy for fighting off the infection. SARS-CoV-2 replication and translation occurs in the cytoplasm. The previous findings indicate that RNA viruses, such as Zika or HCV, can utilize autophagy for their pathogenesis. Chaperon-mediated autophagy (CMA) belongs to cellular autophagy strategies, dedicated to selective degradation of cytosolic proteins.. The CMA substrates contain the KFERQ motif, a pentapeptide composed of amino acid residues with unique characteristics. A protein with KFERQ is recognized by the HSC70 protein together with co-chaperons and transported to the lysosomes. In the lysosome, transmembrane protein LAMP2A, a crucial element of CMA, differentiating it from other types of autophagy, translocate the substrate to the lysosomal lumen fort degradation.

According to one of the studies, LAMP2A is a restriction factor for SARS-CoV-2 replication. This finding suggests that CMA may be involved in SARS-CoV-2 pathogenesis. Therefore, I have generated three human cell lines with impaired CMA by introducing shRNA-mediated LAMP2A knockdown. Then,, I screened the SARS-CoV-2 proteome for potential CMA substrates. The best candidate was ORF3a protein. Apart from containing the necessary KFERQ motif, ORF3a was co-localized with lysosomes. Through experimental analysis based transfection, I found that ORF3a levels have changed in cells with impaired CMA. My results suggest that CMA may be involved in ORF3a degradation.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: The immunomodulatory properties of the Nsp14 protein from SARS-CoV-2 virus

SPEAKER: ***Kamila Buklewska***

SUPERVISOR(S): ***Andrea Lipińska****; PhD;* ***Michalina Michalska****, MSc;*

Coronaviruses are a large family of RNA viruses that can cause disease in both animals and humans. They have recently gained special public attention due to the COVID-19 pandemic, caused by the SARS-CoV-2 virus, which began in late 2019 in China. Infection with SARS-CoV-2 produces a variety of symptoms varying in severity, from mild flu-like disease to severe pneumonia and respiratory failure. The COVID-19 pandemic caused serious consequences, not only for public health care but also social and economic. A key element in the fight against SARS-CoV-2 and the development of new therapies is to learn virus biology and interactions with the host cell in detail.

The genome of SARS-CoV-2 resembles the genomes of other coronaviruses, and it encodes 5 structural proteins and 16 non-structural proteins that play important roles in its pathogenesis. One of these proteins is the nonstructural protein 14 with a high level of conservation within the range of the coronavirus family. The structure of the NSP14 protein consists of several domains, including an N-terminal methyltransferase (MTase) domain and a C-terminal exonuclease (ExoN) domain. These two domains perform separate but crucial functions during the virus infectious cycle.

This enzyme is suggested to act as a host shut-off factor, hampering cellular mRNA maturation and export. My project's main hypothesis is that by inhibiting host cell gene expression, Nsp14 would be involved in inhibiting the immune response in infected host cells.

I focus on a deeper understanding of the function of the Nsp14 protein, by analyzing its effects on human 293T cells. For this purpose, a plasmid carrying the Nsp14 protein gene was constructed and used to transfect human cells. Lysates from the expressing cells were then analyzed for the levels of individual proteins from the antigen presentation pathway. The results suggest that the production of Nsp14 protein in the cells leads to reduced levels of immunologically important proteins such as antigen transporter-forming TAP1 and TAP2, and antigen-displaying MHC class I. In addition, cytometric and Western blot analysis showed that the production of Nsp14 protein in cells also reduces the level of GFP marker protein, which was encoded by the same transcript, following the internal ribosome binding site.

Ongoing research assesses the Nsp14 protein's impact on cellular translation using fluorescent OP-PURO labeling of newly synthesized proteins.

The results indicate that further research on the Nsp14 protein is crucial for understanding its functions in coronavirus pathogenesis and exploring its potential as a target for antiviral therapies. The unique properties of the Nsp14 protein will make it an ideal potential target for the development of antiviral drugs. Moreover, given that this protein is highly conserved in coronaviruses, a detailed understanding of the structural and functional features of the Nsp14 protein may help develop novel therapeutic agents against emerging RNA viruses.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: Analysis of humoral response against HCV induced by DNA vaccination

SPEAKER: ***Maksymilian Chmielewski***

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

About 70% of people afflicted with acute hepatitis C virus (HCV) infections will develop a chronic HCV infection, which carries an increased risk of liver cirrhosis and cancer. An estimated 58 million people suffer from chronic HCV infections, with about 1.5 million new cases annually. As there is currently no vaccine and diagnostic rate remains low, the search for a vaccine continues.

E1 and E2 glycoproteins of HCV form a heterodimer on its surface which reacts with host cell receptor facilitating viral binding and entry. As such, they were deemed an important target for neutralizing antibodies. Both glycoproteins contribute to the high genetic variability of HCV leading to an emergence of at least 90 subtypes. Epitope I (412-423) however, is a highly conserved fragment of the E2 protein and remains mostly unchanged across various genotypes.

Hepatitis B virus small surface antigen (sHBsAg) is able to self-assemble into virus-like particles (VLPs), which have the ability to carry and expose foreign antigens. sHBsAg VLPs carrying the 412-425 epitope of HCV E2 protein were shown to elicit a strong cross-neutralizing antibody response but not T-cell response against HCV. As a result of this finding, a DNA vaccine based on minicircles (MCs) technology was proposed. To engage both arms of adaptive immune response against HCV three minicircles carrying DNA sequences coding for sHBsAg_412-425 VLPs, NS3/4A HCV protease and NS5B polymerase were designed.

The aim of this project is to analyse the humoral response in mice immunized with DNA constructs and assess the effect of a combination of sHBsAg_412-425 construct with NS3/4A or NS5B sequences. To determine this, a series of ELISA experiments analysing binding of sera to E1E2 heterodimers of several HCV genotypes and 412-425 synthetic peptide were performed. Results obtained show that sera from mice immunized with sHBsAg_412-425 construct bind E1E2 of all tested genotypes. Additionally, the combined DNA immunization with sHBsAg_412-425 and NS3/4A or NS5B induced a stronger humoral response than sHBsAg_412-425 alone. This finding may lead to further development and testing of a potential vaccine able to elicit both humoral and cellular response against HCV.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: Studying Omicron mutations in structural proteins of SARS-CoV-2

SPEAKER: ***Martyna Misztal***

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

SARS-CoV-2 is a pathogen causing COVID-19. Studying its infection with a live virus requires Biosafety level 3 laboratories. To accelerate research on this pathogen, alternative approaches to studying SARS-CoV-2 in lower biosafety levels have been established. Among these are virus-like particles (VLPs), which do not carry infectious genetic material, compared to a wild type virion. Another way of studying SARS-CoV-2 proteins is expressing them independently in mammalian cell lines, to evaluate their properties. Omicron is a clinically significant SARS-CoV-2 variant, as it heavily evades the immune response from the antibodies generated after vaccination or previous disease. It was first identified in 2021 and is still the most commonly circulating variant.

In this work, Omicron mutations in all four structural proteins (Spike, Nucleocapsid, Membrane, and Envelope) of SARS-CoV-2 were evaluated using the VLP system transferring a luciferase construct. VLPs were generated in human 293T cells. In the second part of the project, to study the Omicron mutation in the Envelope protein, stable cell lines were generated, using retrovirus vectors. Both versions of the Envelope gene - ancestral and Omicron, were successfully introduced to human lung carcinoma cell lines, achieving stable expression. Omicron VLPs were found to be more efficient at transducing cell lines expressing SARS-CoV-2 entry factors. Whether this is a consequence of more efficient entry or improved packaging, remains to be established.

LABORATORY OF RECOMBINANT VACCINES

TITLE: Generation of stable cell lines capable of production of pigeon paramyxovirus type 1 (PPMV-1) proteins

SPEAKER: ***Dzmitry Dauhalevich***

SUPERVISOR(S): ***Łukasz Rąbalski*** *PhD;* ***Aurelia Schweda****, MSc;*

Pigeon paramyxovirus type 1 (PPMV-1) is an enveloped negative-stranded RNA virus that belongs to the Paramyxoviridae family. It infects both domestic and wild pigeons, causing symptoms similar to Newcastle disease. PPMV-1 can be used as a foundation for the production of virus-like particles (VLPs), which are self-assembled, non-replicating structures that mimic native virions. PPMV-1 based VLPs have the potential to be utilized in various therapeutic fields of biomedicine. The current study is part of an extensive project that focuses on the production of PPMV-1 based VLPs for use as components in therapeutic vaccines for cancer immunotherapy. The production of VLPs requires the simultaneous transfection of producer cells with plasmids carrying viral genes, which involves a laborious and challenging optimization process. The production of VLPs can be facilitated by creating stable cell lines that express specific viral proteins, which can then be transfected with additional viral or non-viral proteins to produce complex VLPs. This Master's project aimed to generate stable cell lines that produce PPMV-1 proteins: matrix (M) and nucleoprotein (NP), or hemagglutinin-neuraminidase (HN) and fusion (F) proteins. The lentiviral expression system was utilized to introduce the target genes into the genome of a 293T cell line. Two viral genes were expressed from single plasmids, made possible by the presence of a P2A self-cleaving peptide coding sequence separating the genes. The gene inserts were cloned into lentiviral transfer vectors, which, along with other components of the system, were used to generate stable cell lines. Additionally, the resulting cell line expressing M and NP proteins was examined for the production of VLPs formed by the viral proteins, considering that the M protein alone is sufficient for VLPs formation. The results indicated that the stable cell lines produced M, NP and HN proteins, with M and NP expressed at higher levels than HN protein. The F protein could not be detected due to the unavailability of antibodies specific to this antigenic variant. Other results showed that the medium from the cell line producing M and NP proteins contained VLP-like structures with a diameter of 80-100 nm. Considering the aforementioned, the obtained cell line stably expressing M and NP proteins can be a promising candidate for the production of VLPs, taking into account a comparably high level of viral protein expression and potential to form basic virus-like particles.

LABORATORY OF RECOMBINANT VACCINES

TITLE: Production of TBEV virus-like particles with stabilized E protein dimers as a potential vaccine antigen

SPEAKER: ***Klaudia Miszke***

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

Tick-borne encephalitis virus (TBEV) is a pathogen transmitted mainly by ticks. It has been proven that infection might also be caused by the consumption of unpasteurized milk and dairy products from infected animals. TBEV causes non-specific symptoms, such as fever, malaise, headache, vomiting, and myalgias. However, in some cases, infection may lead to meningitis or encephalitis. Vaccination is the most effective way of protection against TBEV. Vaccines based on inactivated virus available on the market are highly effective and safe, although expensive.

For this reason, it is desirable to find an alternative vaccine that could be used as a potential affordable veterinary vaccine to protect animals from infection and virus transmission to humans.

Glycoprotein E is the main component of mature TBEV virion. It plays a pivotal role in the viral entry and fusion with the host's cell, and it is the primary target for antibodies. It forms heterodimers on the surface of a mature virion. It has been proven that dimer conformations of E protein prevent from cross-reactivity of antibodies and their presence might be used in the development of a highly effective recombinant vaccine. Furthermore, protein E seems to take part in the formation of virus-like particles (VLPs). The features above make it a target of research in the development of a new vaccine against TBEV.

The aim of the project was the production of TBEV virus-like particles with stabilized E protein dimers in mammalian cells and their characterization. The VLPs produced during the project could be used as a potential vaccine or diagnostic antigen.

The first part of the project was cloning of constructs, encoding recombinant E and prM proteins of TBEV into an expression vector. Mutations had been introduced to places where disulfide bonds are likely to form and stabilize dimer conformations. Next the transfection of mammalian cells with recombinant plasmid vectors was performed. Western Blotting analysis confirmed the production of recombinant proteins. In order to maximize protein production efficiency, transfection conditions were optimized.

ELISA test demonstrated that introduced mutations made the recombinant proteins less recognized by used antibodies, compared to wild type proteins. N-glycosylation analysis confirmed that recombinant proteins undergo this process correctly. The ability to form VLPs was proven with sucrose gradient ultracentrifugation and electron microscopy observation. Higher protein conformations were present in fractions of 28 and 36% of sucrose. In the TEM analysis VLPs were clearly visible, which confirmed that introduced mutations do not disturb the process of their formation.

The production and characterization of recombinant TBEV VLPs was achieved successfully in mammalian expression system. Introduced mutations lead to the efficient stabilization of E protein dimers, however, their presence is probably responsible for the impaired availability of epitopes for particular antibodies. It is possible that the mutations caused the formation of new epitopes, making the protein a good candidate for a vaccine antigen. Modified proteins are correctly glycosylated and form VLPs, which are secreted into the medium.

LABORATORY OF RECOMBINANT VACCINES

TITLE: Nucleic acid binding protein complexes as potential antiviral vaccines

SPEAKER: ***Natalia Paluch***

SUPERVISOR(S): *Prof.* ***Bogusław Szewczyk****;* ***Karolina Gackowska****, MSc;*

Influenza A virus causes annual epidemics around the world. Due to the rapid mutation rate of the influenza genome, seasonal vaccine development for the currently circulating viral strain is essential. This work focuses on influenza virus nucleoprotein, which as a conserved protein, can potentially be used in the production of a universal influenza vaccine. In addition, the ability of the nucleoprotein to bind RNA makes it possible to use this protein as a platform for the transport of nucleic acids.

Using a protein expression system in insect cells, a recombinant influenza virus nucleoprotein was obtained. A nucleoprotein with the domains of the gp64 protein, responsible for binding heparin present in the membrane of mammalian cells, was also obtained. It is expected that the presence of the above domains will facilitate the binding of the resulting protein to the mammalian cell and penetration into the target cell.

The native feature of nucleoprotein is its ability to bind, stabilize and protect RNA. It has been proven that the obtained nucleoprotein can bind non-viral RNA sequences. The complexes obtained in this way may allow the immunization of organisms in order to produce immunity against the influenza virus, as well as the delivery of the RNA construct to the cell and further expression of the proteins encoded bythem.

SESSION 3

LABORATORY OF TRANSLATIONAL ONCOLOGY

TITLE: Phenotype Analysis of Prostate Cancers with BRCA1 Gene Aberrations

SPEAKER: ***Krzysztof Wierbiłowicz***

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

Prostate cancer is the second most commonly diagnosed cancer among men worldwide. Currently, screening is based on measuring prostate-specific antigen (PSA) levels in peripheral blood, and diagnosis is confirmed by histopathological evaluation of biopsy material. However, there is a lack of adequate biomarkers to assess the potential for the development of an aggressive form of the disease and distant metastases, which are the leading cause of death for prostate cancer patients. In recent years, it has been shown that small subpopulations of tumor cells with somatic loss of the gene encoding the BRCA1 protein can give rise to metastatic disease. Hence, it is important to understand the underlying molecular mechanisms causing more aggressive phenotypes in cells bearing BRCA1 gene aberrations. Here, TCGA-PRAD (The Cancer Genome Atlas - PRostate ADenocarcinoma) dataset, including Copy Number Variation (CNV), microRNA, and mRNA data from 495 prostate cancer patients was analyzed to understand what are the molecular characteristics of BRCA1-negative tumors. CNV analysis revealed that BRCA1 loss is associated with broad deletions of neighboring genes, including type I keratin cluster on the 17q21.2 locus. When corrected for those structural changes, RNA-seq pathway analysis uncovered the downregulation of glucuronidation processes and steroid hormone biosynthesis. Additionally, target analysis of miRNA with upregulated expression associated with BRCA1 loss showed enrichment for pathways involved in gene expression, cell cycle and stress responses. Overall, this study presented new insights into the biology of prostate cancer tumors bearing BRCA1 gene loss, with potential translational implications.

CORE FACILITY LABORATORIES

TITLE: Characterization of proteomic changes in plasma samples of CRC patients

SPEAKER: ***Weronika Kamysz***

SUPERVISOR(S): ***Zhi Jane Chen**, PhD, DSc; **Katarzyna Macur**, PhD; **Victor Urbiola Salvador**, MSc;*

Mass spectrometry-based plasma proteomics provides a great tool for novel biomarker discovery and finding new therapeutic targets. Plasma-derived colorectal cancer biomarkers are applicable for diagnosis, monitoring, and prognosis. In this thesis, I analyzed proteomic changes in plasma samples of 36 CRC patients in comparison to 26 healthy controls as well as in relation to cancer progression and in the presence of cancer-related inflammation. The analysis identified 323 protein groups across all samples at 1% FDR. Out of those 323 proteins, 137 were quantified. 43 differentially expressed proteins were found between the CRC patients and healthy control groups, many of which were already researched as CRC biomarkers. Many of the differentially expressed proteins were related to immune response. Plasma levels of complement components C5, C9, C4BPA, C1QB, and C4B were significantly increased. Some of these proteins were also related to lipid metabolism, including apolipoproteins APOA2 and APOA4, which were both downregulated in the patient group. Moreover, 11 differentially expressed proteins were found between groups of patients with inflammation and patients without inflammation, and 4 differentially expressed proteins were found between early and late stage patients.

The results propose several plasma proteins that could have a significant role in CRC development and should be further researched to be applied as CRC biomarkers. Additionally, some of those proteins are mediators of inflammation, highlighting their role in the development and progression of cancer. These findings have the potential to help establish new ways of CRC diagnosis, monitoring and treatment.

CORE FACILITY LABORATORIES

TITLE: High resolution quantitative proteomic characterization of T lymphocytes

SPEAKER: ***Wiktoria Bilik***

SUPERVISOR(S): ***Zhi Jane Chen****, PhD, DSc;* ***Katarzyna Macur****, PhD;*

Lymphocytes are type of white blood cells which are responsible for protecting organism against pathogens, parasites or even cancer. This thesis focuses on T cells that can be divided into subtypes with differing functions during the immunological reaction. For example, the role of cytotoxic T lymphocytes is destroying infected cells as wells as cancer cells, while helper T cells stimulate or inhibit immune responses. Infiltration of malignant tissue with immune cells can impact on cancer outcomes. Therefore, studying T lymphocytes and their roles in tumour microenvironment is crucial for prediction, diagnostics and monitoring of cancer as well as treatment development.

In the first part of this thesis, the performance of two compartment preferential enrichment kits and additional strategies for mass spectrometry analysis were tested on Jurkat cells which are an immortalized line of human T lymphocytes. Among the 4 extracted fractions (cytoplasm, membrane, nucleus, cytoskeleton), cytoskeleton was not enriched properly by any of the methods. The best performance was observed for Qproteome Cell Compartment kit combined with HiPPR detergent removal kit. The second part of this thesis focuses on proteomics analysis of colorectal cancer (CRC) tissue samples. Macrodissection combined with proteomics determined characteristic features of CRC tissue and allowed for comparison of malignant with normal adjacent matched tissues.

In conclusion, the best examined preferential enrichment method can be applied for mass spectrometry analysis of cytoplasmic, nuclear and membrane fraction of T cells. Moreover, the optimized protocol of tissue proteomic can be used for studies of CRC tissue proteomics to identify biomarkers and novel potential therapeutic targets.

TITLE: What HIIT (High-intensity interval training) induced changes in a proteome can tell us about pregnant women's health?

SPEAKER: ***Dominika Goik***

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

Pregnancy is an unusual state for an organism, and some changes might lead to an increased risk of disorders, such as preeclampsia which is an untreatable hypertensive disorder but can be prevented with physical exercise. We proposed HIIT (High-intensity interval training) as an exercise modality that might help meet physical activity guidelines during gestation. Unfortunately, there is a lack of studies on the influence of HIIT activity on changes in urinary proteome which might reflect alterations taking place on a molecular level. Our study focused on identifying molecular changes in the proposed preeclampsia biomarker induced by an 8-week HIIT program in pregnant women's urinary proteome. We showed that HIIT might protect against developing preeclampsia by reducing inflammation-induced damage in endothelial cells of blood vessels.

SESSION 4

LABORATORY OF MOLECULAR BIOLOGY

TITLE: The importance of the interaction of plasmid Rep proteins with the E. coli DnaA protein in the initiation of plasmid DNA replication

SPEAKER: ***Michał Mieczkowski***

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

RepE and TrfA proteins, belonging to the Rep proteins family, are involved in initiating F and RK2 plasmids, respectively, DNA replication in E. coli bacteria. These proteins can exist in two forms: dimeric and monomeric but only as the monomers, they act as DNA replication initiators. Both proteins, RepE and TrfA, have the ability to specifically bind to iteron sequences located near the DUE (DNA Unwinding Element) origin fragment. The DUE is the sequence where the unwinding of the double-stranded DNA and binding of proteins involved in DNA replication initiation process occurs. DnaA is a protein that initiates E. coli chromosomal DNA replication and also assists plasmid replication initiators, RepE and TrfA, in the plasmid DNA replication initiation process. Currently, it is known that some proteins from Rep family, like π and RepA, are able to interact with DnaA. However, no literature data are available for RepE-DnaA and TrfA-DnaA interactions.

The aim of this study was to analyze the interaction of DnaA protein with RepE and TrfA proteins. Based on the literature, structural and functional data, RepE and TrfA protein variants were designed. For each variant, the point mutation was introduced with site-specific mutagenesis. Obtained variants were overproduced and purified. Then, the interactions between the DnaA protein and the wild-type and V64A variants of the RepE protein, as well as between the wild-type, F222A and Y223S variants of TrfA, were analyzed using ELISA and the Bio-layer Interferometry (BLI) methods.

The results of this study indicate that not only previously mentioned π and RepA proteins can interact with DnaA, but also other proteins of the Rep family, e.g. RepE and TrfA. Interestingly, the F222A variant of TrfA showed a reduced ability to bind to DnaA. However, the precise characterization of the possible impact of point mutations on the interactions between DnaA and variants of RepE and TrfA proteins requires additional studies.

LABORATORY OF MOLECULAR BIOLOGY

TITLE: Analysis of interaction of TrfA protein with the RK2 plasmid origin of replication in Pseudomonas putida cells during stringent response

SPEAKER: ***Jakub Wołoszyn***

SUPERVISOR(S): *Prof.* ***Igor Konieczny***

Replication of genetic material begins when Origin Binding Proteins (OBP) recognise and bind to characteristic motifs found in a well-defined origin region. In iteron-containing plasmids this region is called oriV and it contains short characteristic motifs which are called iterons, that are recognised by plasmid encoded initiator Rep proteins. Representant of this plasmid family is RK2, replication of which is regulated by plasmid encoded TrfA replication initiator protein.

Stringent response is a bacterial stress signalling pathway triggered by nutrient depravation. It enables adaptation to poor environmental conditions by synthesis of alarmones, altering cells metabolism, diverting its resources from growth and division and toward amino acid synthesis.

My project investigates how stringent response elicited by amino acid starvation in Pseudomonas putida cells influences interaction between TrfA and DNA of RK2 plasmid and its mini-replicon pKD19L1. To analyse interaction between TrfA and DNA ChIP (Chromatin Immunoprecipitation) has been performed using cells containing RK2 plasmid or its mini-replicon. Samples were collected in three time points: before stress, two hours after incubation in minimal medium, and thirty minutes after recovery in optimal medium. Results of this analysis show, that during stress binding of iterons by TrfA is decreased, compared to cells in optimal medium. Shortly after recovery, the amount of immunoprecipitated oriV increases.

These results further demonstrate how tightly regulated this process is and present a potential source for interesting new inquiry about the exact mechanism behind reduced binding of replication initiator protein during stringent response.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: The effect of oleanolic acid on human neuroblastoma cell line (SH-SY5Y)

SPEAKER: ***Patrycja Markiewicz***

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

Neuroblastoma, a malignant neoplasm derived from neural crest cells, poses a significant challenge in pediatric oncology. This study aimed to evaluate the impact of oleanolic acid (OA) on neuroblastoma cells (SH-SY5Y). Series of investigations were made, including MTT assay, cell cycle assay, apoptosis assays, autophagy assay and mass spectrometry analysis.

The primary objective was to confirm the research hypothesis of the inhibitory effects of OA on neuroblastoma cells. Quantification of cellular cytotoxicity was accomplished through the MTT assay. The results substantiated the potent inhibitory effects of OA on cellular proliferation, with the determination of the half-maximal inhibitory concentration (IC50) in 715 ug/ml. The cell cycle analysis was conducted to examine the potential of oleanolic acid to disrupt the normal cell division process in neuroblastoma cells. The analysis provided that OA inhibits cell division in the S and G2/M phases of the cell cycle. Apoptosis induction was assessed using flow cytometric analysis combined with Annexin V-FITC/propidium iodide staining and independent analysis with caspase-3/7 assay. The experimental data demonstrated a statistically significant increase in the population of apoptotic and necrotic cells treated with 715 ug/ml OA. Autophagy induction has been proven by acridine orange staining and imaged by a confocal laser scanning microscope. SWATH-MS analysis allowed for the identification of 279 proteins and 9 of them exhibited significantly up-regulated or down-regulated.

This investigation substantiated the research hypothesis by demonstrating the pronounced effects of OA on neuroblastoma cells (SH-SY5Y). Future investigations are required to elucidate the precise molecular mechanisms involved.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: Analysis of the effectiveness of the photodynamic method based on a new photosensitizing compound against ESKAPE group pathogens

SPEAKER: ***Marta Kubicka***

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

Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter cloacae are six pathogens that have been classified into a single group, called ESKAPE, due to their threat to health and life caused by their ability to acquire resistance to multiple antibiotics and their high pathogenicity. Due to multi-drug resistance, there is a great need to find new therapies against infections caused by this type of bacteria. One of the solutions is photodynamic therapy, which requires three elements for its action – a photosensitive compound called a photosensitiser, light from the visible range and oxygen. The combination of these three components leads to the production of highly toxic reactive oxygen species, leading to bacterial cell death. The novelty of the study is based on a new photosensitising compound, which is a porphyrin derivative containing gallium ions. The similarity of this photosensitizer to the structure of haem allows it to invade bacteria through haem natural receptor, leading to disruption of iron metabolism. This process is called the Trojan horse strategy. The combination of light-dependent and light-independent mechanisms could potentially increase the efficacy of photodynamic therapy.

During the study, the efficacy of photodynamic treatment on ESKAPE group pathogens using a new photosensitizing compound was investigated, depending on the concentration of the compound and the light dose. The data demonstrated that photodynamic treatment was the most effective against Gram-positive ESKAPE bacteria. Acinetobacter baumannii proved to be the most vulnerable Gram-negative bacteria, while Klebsiella pneumoniae and Enterobacter cloacae were the two least vulnerable bacteria to photodynamic treatment. In the case of Pseudomonas aeruginosa, it was observed that blue light alone induced significant killing of bacteria. After modifying the experimental conditions (lowering the power density of a light source, using EDTA and washing off the excess photosensitizing compound), high efficacy of photodynamic treatment was obtained. In the next stage of the work, the accumulation of the analysed compound in bacterial cells was studied. The results of the experiment showed that the accumulation of a photosensitizer in cells is correlated with the effectiveness of photodynamic therapy.

In conclusion, photodynamic treatment based on new photosensitiser compound, GaCMP-2-3, causes significant eradication of ESKAPE pathogens, photodynamic treatment is less effective against Gram-negative bacteria, and the effectiveness of photodynamic treatment can be improved by changing the conditions of the procedure.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: Inactivation of Staphylococcus aureus biofilm using photodynamic method and new gallium ion modified porphyrin derivative

SPEAKER: ***Magdalena Król***

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

Introduction: *Staphylococcus aureus* is a pathogen causing the most deadly bacterial infections worldwide and one of the common causes of healthcare-associated infections (HAI). Staphylococcal infections are mostly problematic for three main reasons: first, development of antibiotic resistance mechanisms, which means that available medicines are unable to combat infection. Second, *S. aureus* produces a large number of virulence factors and toxins that damage the host organism.

Third, *S. aureus* has the ability to form a complex structure, named a biofilm. It is formed by the attachment of free-floating bacterial cells (planktonic form) to a surface. Then, bacteria multiply and produce the extracellular matrix that mainly consists of carbohydrates and proteins to protect biofilm from antimicrobial drugs and immunological responses. Moreover, the biofilm structure is 1,000 times more tolerant to antimicrobials compared to the planktonic form. Another problematic issue is that bacterial biofilm can be formed not only on tissues but also on abiotic surfaces such as implants which promote inflammation after surgical procedures.

The antimicrobial photodynamic inactivation (aPDI) is an method, alternative to antibiotics, to fight against multidrug-resistant pathogens such as *S. aureus*. In this method, three components are necessary: oxygen, photosensitizer (PS) and light. PS molecules accumulate inside bacterial cells. Subsequently, under exposure to light, PS converts molecular oxygen into reactive oxygen species, which causes bacterial cell death due to oxidative stress. Gallium porphyrins are known as a potential photosensitizers working in aPDI in the Trojan Horse strategy pattern.

The aim of this study was to propose antimicrobial photodynamic inactivation (aPDI) with novel gallium metalloporphyrin derivative (GaCMP-2-3) [1] as a method to combat *S. aureus* biofilm.

Materials and Methods: The Newman strain of *S.aureus* was studied. The photosensitizer used in the experiments was gallium ion-modified GaCMP-2-3 porphyrin (concentrations 0.1-25 μM) and an light emmiting diod (LED) producing blue light (λ_{max}= 409 nm) (light dose 1.56 - 24.96 J/cm^2). Biofilm cultures were formed on a plastic plate, then on polycarbonate coupons placed in a CDC reactor model that mimics the flow of fluids in the natural environment inside the host organism. Biofilm model on titanium dental implants was used to prove practical application of the studied approach. The biofilm was inactivated during a photodynamic procedure. The reduction in bacterial viability is shown numerically and by fluorescence microscopy imaging.

Results: aPDI was successfully used to inactivate **S. aureus** cultures in the planktonic form with a bacterial viability reduction of 6.1 [Log_{10} CFU/mL]. However, for the biofilm form on the plate, this value was 2.5 [Log_{10} CFU/mL], for the biofilm cultured in the flow-through system 1.3 [Log_{10} CFU/cm^2]. For implants, the reduction was 4.2 [Log_{10} CFU/implant].

Conclucions: The aPDI method based on GaCMP-2-3 and blue light can effectively reduce the survival of *Staphylococcus aureus* cells in planktonic form and more importantly in the complex biofilm structure formed in all three models.

[1] Hao Zhang et al. 'Iron-blocking antibacterial therapy with cationic heme-mimetic gallium porphyrin photosensitizer for combating antibiotic resistance and enhancing photodynamic antibacterial activity', Chemical Engineering Journal, 2023

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: Analysis of tolerance development to antimicrobial photodynamic inactivation using gallium-complexed porphyrin derivative in Staphylococcus aureus

SPEAKER: ***Kamila Kamińska***

SUPERVISOR(S): ***Mariusz Grinholc****, PhD, DSc;*

Staphylococcus aureus is an opportunistic pathogen belonging to the ESKAPE group which consists of bacteria that are particularly successful in evolving in terms of acquiring antibiotic resistance mechanisms. Because of the growing problem of multi-drug resistance of bacteria to almost all known antibiotics, there is an urgent need to administer alternative methods of treatment.

One such method is antimicrobial photodynamic inactivation (aPDI). The main feature of this method is its non-selectivity. It means affecting many molecular targets which is possible due to the formation of reactive oxygen species (ROS). This type of treatment consists of three crucial elements: an exogenously administered photosensitizer (PS), light of the appropriate wavelength, and oxygen. PS, excited with light, leads to ROS production and the outcome is microbial cell inactivation.

However, there are some studies that indicate the risk of tolerance development during the aPDI treatment. Hence, the aim of this project was to study the phenomenon with the utilization of a new derivative of porphyrin - GaCMP-2-3. The compound has a heme-mimetic structure and can be easily uptake by bacteria cells acting as a photosensitizer. Simultaneously it acts as an antimicrobial because of the presence of Ga3+ that enables the blocking of iron metabolism in bacterial cells. Then S. aureus ATCC 25923 treated with GaCMP-2-3 was illuminated with blue light (λmax 409 nm) to activate ROS production.

The sub-lethal doses of aPDI were applied to the S. aureus and the experiment was repeated in 15 successive cycles (exposure – regrowth – exposure). The assessment of GaCMP-2-3-aPDI tolerance development revealed S. aureus, with the utilization of higher light doses, was less susceptible to the treatment in the 5th, 10th and 15th cycles in comparison to the control. However, the phenotypic stability test of gained tolerance, performed with the sample from the 10th cycle and 5 additional passages with no aPDI exposure, indicated no stability of the feature. The hemolysis ability in all samples was examined during this study and in all of them the ability was preserved. It may be one of the confirmations of no genetic alterations that influence aPDI tolerance in this study. Also, the intracellular uptake of photosensitizer of samples from cycles: 1, 5, 10, and 15 showed no decreased uptake of GaCMP-2-3 during tolerance development. That indicates that examined S. aureus strain didn't develop a mechanism to omit accumulation of PS.

The obtained results indicate that multiple sub-lethal aPDI treatment with GaCMP-2-3 leads to tolerance development in S. aureus ATCC 25923 and GaCMP-2-3-aPDI treatment carries a low risk of stable tolerance/resistance. As a consequence, GaCMP-2-3 can be regarded as a good candidate for future research in antimicrobial treatment.

WEDNESDAY 7TH JUNE

SESSION 5

LABORATORY OF PROTEIN BIOCHEMISTRY

TITLE: Comparison of biochemical properties of wild type human glutamine synthetase and Δn17

SPEAKER: ***Karolina Milcz***

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

Glutamine synthetase (GS, GLUL) catalyzes the first step in which nitrogen is introduced into cellular metabolism: glutamate + ATP + NH3 → glutamine + ADP + phosphate. GS is found mainly in the brain, where it primarily provides a mechanism for ammonia assimilation and detoxification, as well as a mechanism for glutamine biosynthesis and neurotransmitter signal termination. The GS forms a decamer in eukaryotes and has 10 active sites, each located at the junction of two adjacent subunits in the pentameric ring.

A newly discovered mutation of human glutamine synthetase, which has not yet been described in the literature, has a deletion covering the first 17 amino acids from the N-terminus of the original sequence. Structurally, this corresponds to an α-helix facing the center of the pentamer ring.

To biochemically characterize the Δn17 form and compare it with wild-type glutamine synthetase, both proteins were overproduced in a bacterial system and purified using size-exclusion and ion-exchange chromatography. Novel GS activity assay was proposed based on spectrophotometric ATPase assay. There was no significant difference in activity between the GS Δn17 variant and the GS WT variant. However, differences in the stability of the structure of both proteins were found. Thermostability was tested at disparate pH points and with the use of urea and guanidine hydrochloride. In addition, the quaternary structure of both forms was examined by molecular filtration.

LABORATORY OF PROTEIN BIOCHEMISTRY

TITLE: GLUL ΔN17 - from acquisition to testing of the heterogenic mixture of human GLUL protein and its pathologic variant expressed in the same cell.

SPEAKER: ***Marek Bulczak***

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

Glutamine Synthetase is a dual-function enzyme present in the human body, specifically in the brain, liver, muscles and endothelial cells. Main function of this protein is detoxification of the neural, hepatic and muscle tissue from ammonia, by de novo synthesis of glutamine. Second activity can be observed in the endothelial cells, where it evinces palmitoyl transferase capabilities, attaching RhoJ to membrane thereby playing a major role in angiogenesis. Loss of function in this protein is lethal and yields with prenatal death.

Newly discovered mutation of the GLUL gene encoding glutamine synthetase results with deletion of the first 17 aminoacids from the N terminal of the original sequence. The patient is a carrier of heterozygous Δ17 mutation evincing symptoms of neurological disorders.

Establishing a procedure for co-expression in the same organism is essential for examining and understanding of the effects of heterozygotous mutation in human.

Choice of the most optimal model organism, vast optimisation of the overproduction and purification protocols were achieved. Using electroporation and chemoporation protocols E.coli BL21(DE3) transformants were created. After IPTG induced overproduction purification protocol was optimized, using French Press, FPLC size-exclusion and ion-exchange chromatography. Acquisition of the wild-type glutamine synthetase, Δ17 glutamine synthetase and same-organism origin mix of them enabled comparative testing.

Thermolability of samples was measured via capillary fluorimetry. Activity differences and its correlation with time and temperature between samples were studied using real-time spectrophotometry.

LABORATORY OF EVOLUTIONARY BIOCHEMISTRY

TITLE: Examination of the mechanism of amyloid-β42 recognition in the family of J-domain proteins using molecular dynamics

SPEAKER: ***Przemysław Domański***

SUPERVISOR(S): ***Bartłomiej Tomiczek****; PhD, Eng;*

Amyloid β42 (Aβ42) is a peptide composed of 42 amino acids. Aβ42 molecules are characterized by a tendency to aggregate. Mature amyloid fibrils are the main component of insoluble amyloid plaques, the formation of which in the brain is a characteristic feature of people with Alzheimer's disease, which is the most common type of dementia. Along with other pathological changes, aggregation of Aβ42 peptides contributes to neurodegeneration.

The J-domain protein family is one of the largest and the most diverse groups of proteins. Among the J-domain proteins, based on their structure, we can distinguish 3 classes: A, B and C. A common feature of the J-domain proteins is the presence of a conserved J-domain, which stimulates the ATPase activity of HSP70. Together with HSP70 proteins and nucleotide exchange factors (NEFs), J-domain proteins form functional networks that have the ability to prevent aggregation of proteins associated with neurodegenerative diseases. The human J-domain protein-DNAJA2, was previously identified as a suppressor of tau protein aggregation, the aggregation of which is also associated with neurodegenerative diseases. Also, DNAJB1 protein, in cooperation with HSP70, exhibits the disaggregation activity of α-synuclein, a protein present mainly in neurons, which aggregation is characteristic of Parkinson's disease.

J-domain proteins have been identified as potentially important in the recognition, disaggregation and inhibition of Aβ42 peptides aggregation. However, the exact mechanism of such activity has not been described to date.

The purpose of my thesis was to investigate, using molecular dynamics techniques, whether J-domain proteins interact with amyloid β42 and what is the mechanism of such potential interaction.

During the presentation of my thesis results, I will demonstrate how structural differences between class A and class B J-domain proteins affect the interaction with amyloid β42, as well as which amino acid residues have been identified as crucial for the J-domain protein-amyloid β42 interaction.

LABORATORY OF EVOLUTIONARY BIOCHEMISTRY

TITLE: An ancient tale of two JDPs: The evolutionary journey from protein folding to preventing Alzheimer's disease

SPEAKER: ***Dominik Purzycki***

SUPERVISOR(S): ***Bartłomiej Tomiczek****; PhD, Eng;*

Two classes of proteins (A and B) can be distinguished within the J protein family (JDP). As co-chaperones, they cooperate with the heat shock protein Hsp70, and together play critical roles in protein homeostasis in all living organisms. This system has many functions in the cell, but its key function is to break down protein aggregates that are formed from misfolded proteins in stress conditions. JDPs deliver the substrate to Hsp70 and stimulate ATPase activity of Hsp70, which makes them a molecular guide for the Hsp70 protein.

Class B J-proteins are distinguished from Class A by their ability to break down amyloid substrates. Amyloids are fibrillar aggregates, which are responsible for disease-associated toxicity in nerve cells. Their presence in brain tissues is associated with high levels of neuropathology during neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's. Interestingly, it is still puzzling what differences between Class A and B proteins contributed to the acquisition of this unique ability to disassemble amyloid structures.

My research aims to understand the molecular basis behind the functionalization of Class B JDPs and to identify evolutionary changes that lead to gaining the ability to break down amyloid structures. For this purpose, I used Maximum Likelihood reconstruction of Class A and B JDPs last common ancestor (AncAB) and ancestor of all Class B JDPs from cytosol (AncB). First the genes coding AncAB and AncB were synthesized and its phenotype was verified in vivo in Saccharomycess cerevisiae in the absence of native JDPs. Next, I overexpressed genes coding AncAB and AncB in E.coli, and purified the ancestor proteins using affinity chromatography and size exclusion chromatography, and investigated the interaction of purified proteins with aggregated substrates using biolayer interferometry (BLI).

The obtained results indicate the specialization of Class B JDPs allows an efficient recruitment of Hsp70 machinery to beta amyloid fibrils. I observe similar efficient recruitment of Hsp70s with modern day Class B protein (DNAJB1) and ancestral protein AncB, but not with Class A proteins. Both Class B protein (DNAJB1) and ancestral protein AncB much more efficiently load Hsp70 machinery onto the amyloid aggregates than to amorphous aggregates. The results indicate that there was a functional specialization between JDP classes and that class B has a better ability to process amyloid substrates, while Class A JDPs have a better interact with unfolded substrates. This study will help to better understanding the evolution of JDPs, and natural defence mechanisms against pathogenic amyloid aggregates, which are still a huge trouble for today's medicine, with over 50 million people currently suffering from Alzheimer's disease.

LABORATORY OF PROTEIN BIOCHEMISTRY

TITLE: The role of Hsp90 chaperone in protein refolding from aggregates

SPEAKER: ***Jacek Sobczak***

SUPERVISOR(S): *Prof.* ***Krzysztof Liberek****;* ***Agnieszka Kłosowska****, PhD;*

Proteins are vital macromolecules that perform numerous essential functions within cells. under certain conditions, such as exposure to stress, mutations, or aging, proteins misfold and form clumps or aggregates. These aggregates impair cellular processes, disrupt protein homeostasis, and ultimately lead to cellular dysfunction and diseases.

Fortunately, cells possess an intricate network of molecular chaperones known as heat shock proteins that actively combat protein aggregation. Hsp40, Hsp70, Hsp90 and Hsp100 chaperones are among the most prominent players in this defense system. Hsp70 acts as a molecular machine that recognizes and binds to misfolded or partially folded proteins, preventing their aggregation and aiding their refolding. It also collaborates with various co-chaperones to orchestrate protein quality control and ensure the proper folding of nascent proteins.

Hsp90 which has wide range of functions involving the maturation and stabilization of a vast array of client proteins in final parts of protein folding. Hsp90 interacts with its clients in a highly regulated manner, ensuring their proper folding, conformational stability, and functional integrity. In cases of stress or protein damage, Hsp90 also have a part in preventing aggregation by facilitating the refolding of misfolded proteins.

In my master project I analyzed the role of Hsp90 and cooperation with Hsp70 system in protein refolding from aggregates. I purified active paralogs of yeast Hsp90 (Hsp82 and Hsc82) which positively influenced the refolding of aggregated luciferase by Hsp70 system. My results also suggest that yeast Hsp90 effectively bind to protein aggregates through Hsp70 system when class B J-domain protein delivers Hsp70 to aggregates. Such binding is not observed when J-domain protein from class A is responsible for Hsp70 binding to aggregates.

LABORATORY OF BIOPOLYMERS STRUCTURE

TITLE: Analysis of human follicular fluid in search of protein markers of oocyte developmental potential

SPEAKER: ***Ewa Gapińska***

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

Human follicular fluid fills the cavity of the ovarian follicle and it is the environment for oocyte maturation. In my project, I am examining fluid samples from patients who are characterized by low anti-mullerian hormone (AMH) level. Low level of indicate is connected with a low ovarian reserve what is a reason to start in vitro fertilization (IVF) treatment. In my thesis, I am looking for protein biomarkers of oocyte potential development in those follicular fluids. Finding those protein markers could help choose proper oocytes for this procedure what could increase the chances of success of the IVF procedure and consequently, less zygotes could be produced what could decrease social concerns connected with this procedure.

Protein concentrations in samples are measured using a TripleTOF mass spectrometer coupled with LC with the usage of the SWATH methodology. Thanks to the statistical analysis I could make conclusions about the presence of protein biomarkers in follicular fluid.

I observed that every follicular fluid, even those which come from the same patient, has different levels of proteins, which confirms it is a good material to search for markers of oocyte development. I assume clusterin could be a marker of oocyte potential development because it is present with a significant difference in comparison to the control group in every follicular fluid from which oocyte developed to healthy blastocyst ready to transfer or to cryopreservation.

LABORATORY OF BIOPOLYMERS STRUCTURE

TITLE: Optimization of reduced capillary electrophoresis sodium dodecyl sulfate (rCE-SDS) for monoclonal antibody analysis

SPEAKER: ***Filip Lewandowski***

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

Capillary electophoresis is commonly used for analyzing several characteristics of antibodies. The presentetion will introduce the audience to process of optimizing the method for the particular protein. The begining consists of short introduction about usage of monoclonal recombinant antibodies as biosimilar therapeutics, as well as law regulations to register them as pharmaceutical products. After presenting range of methods used to analyze different antibodies' attributes, which are significant for registration process, an emphasis on capillary electrophoresis is put, which is used to determine levels of N-glycosylation, purity, low and high molecular weight impurities of antibodies. Basic method's mechanisms and possible obtained results are followed by general scheme of reduced capillary electrophoresis sodium dedecyl sulfate (rCE-SDS) procedure. Next, all significant steps are presented one by one to show which parameters could be optimized and what different options have been tested. Using exemplary results, ways of choosing final conditions in rCE-SDS analysis are explained. Last part of the presentation focuses on innovative part of the project - gel buffer preparation attempt, using detergents which consist of longer hydrocarbon tails than SDS, such as sodium tetradecyl or hexadecyl sulfate. This novel application for capillary electrophoresis is currently in development process. However, preliminary results are shown to point out the impact of the implementation on significant improvement of results quality.

LABORATORY OF BIOPOLYMERS STRUCTURE

TITLE: Peptidome composition of human saliva.

SPEAKER: ***Konrad Stankiewicz***

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

Salivary gland tumors encompass a heterogeneous group of neoplasms that present diagnostic challenges and require invasive procedures, such as biopsy, for accurate diagnosis. This study highlights the urgent need for enhanced diagnostics in salivary tumor cancer by focusing on the utilization of saliva samples as a non-invasive source for early tumorigenesis markers. By employing advanced molecular and proteomic techniques, the study aims to build a peptidome library that will help identify and validate potential biomarkers aiding in the early detection of salivary gland tumors. The successful implementation of saliva-based diagnostics would provide a non-invasive and patient-friendly alternative to biopsy, leading to improved patient experience, a reduced number of misdiagnosed cases, and earlier interventions.

Mass spectrometry-based analysis has previously been employed to identify and quantify peptides in saliva. However, the complexity and dynamic range of the salivary peptidome present significant challenges for comprehensive analysis. This research investigates the impact of proper prefractionation techniques on the quality and accuracy of peptidome analysis of saliva by mass spectrometry. Through a comparative analysis of saliva samples processed with simpler and more complex prefractionation methods, this study demonstrates that the omission or simplification of prefractionation leads to poorer results in terms of identifying salivary peptides. The research focuses on implementing techniques using a three-column high-performance liquid chromatography (HPLC) system consisting of high pH, Proteo C18, and strong cation exchange (SCX) columns. By employing this comprehensive prefractionation strategy, the study shows improvements in results compared to traditional mass spectrometry analysis that used simpler prefractionation methods.

LABORATORY OF BIOPHYSICS

TITLE: Idarubicin – platinum nanoparticles interactions and their possible biological influence

SPEAKER: ***Konrad Gancarz***

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

Cancer is being prognosed to be one of the biggest threats in medicine in the future. The high birthrate in some countries, and the aging population in the others, will most likely lead to an increase in global spending on healthcare. Due to that fact, a lot of new strategies are in development to improve already existing ones and make them more available to the wider population. Nanomedicine is one of the promising choices. Platinum nanoparticles demonstrate a number of properties that might be beneficial in that matter. For example, they are able to directly interact with various drugs. Idarubicin is the second-generation anthracycline used widely in treatment of leukemias (i.e. acute myeloid leukemia). Despite high efficiency, it is known for cardiotoxic side effects. In this study I examine the possible interactions between the drug and platinum nanoparticles and their possible influence on the biological action of examined compounds.

LABORATORY OF PHYSICAL BIOCHEMISTRY

TITLE: Development and validation of a method indicating stability of carbocisteine in gastrointestinal tract and liver conditions.

SPEAKER: ***Anna Kowalczyk***

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

Carbocisteine is an organic compound derived from cysteine. It serves as a valuable mucoactive agent for the treatment of respiratory tract disorders characterized by excessive and viscous mucus. Carbocisteine can undergo degradation, resulting in the formation of carbocysteine lactam. This degradation product is primarily generated under acidic pH conditions and thermal stress. The current available method described by the European Pharmacopoeia for the analysis of carbocisteine content and its related substances is limited to thin-layer chromatography (TLC).

Therefore, the objective of this study was to develop a more automated and efficient method using high-pressure liquid chromatography (HPLC) and to investigate the impact of conditions in the gastrointestinal tract and liver on carbocisteine stability.

Elution of carbocisteine from the HPLC column needed to be performed at a high content of organic solution in the mobile phase, which posed issues in selecting other method parameters. To optimize the method conditions, a mathematical model of the experiment was built using the design of experiment (DoE) methodology. Statistical analysis of the model allowed determining its significance and indicating the importance of each factor. Subsequently, model-based optimization permitted the selection of the most suitable chromatographic conditions for obtaining the expected results. The method was successfully validated for qualitative and quantitative analysis of carbocysteine. The degradation of carbocysteine to lactam was observed at elevated temperatures, regardless of the pH conditions. However, degradation was more pronounced under acidic pH. Furthermore, the enzymatic activity of liver enzymes was found to facilitate the conversion of carbocysteine to lactam. It was also observed that lactam could undergo limited conversion back to carbocysteine under alkaline conditions and at higher temperatures.

SESSION 6

LABORATORY OF PLANT BIOCHEMISTRY

TITLE: Effect of overexpression of the gene encoding phospholipid:diacylglycerol acyltransferase (PDAT) on the content of fats, proteins and sugars in Arabidopsis thaliana plants

SPEAKER: ***Wiktoria Korczakow***

SUPERVISOR(S): *Prof.* ***Antoni Banaś****;*

Arabidopsis thaliana is a model plant. Due to it small sizes, short life cycle and morphological characteristics it is widely used in plant biology as a model organism.

In this study an influence of overexpression of Phospolipid:diacylglycerol acyltransferase (PDAT) encoding gene in Arabidopsis thaliana was tested. PDAT is an enzyme which plays important role in biosynthesis of triacylglicerols- main component of plants oils. Analysis was performed to check if the overexpression of this gene influences content of protein, sugars and lipid in plants. For this a control line was compared with two lines with overexpression of this gene.

Plants were grown in natural conditions in soil. Selection was performed and plants on different stages of growth was used for analysis. Tests were performed for the whole plants, rosettes, stems and seeds.

To study sugar content colorimetric analysis with GOPOD reagent was performed to check the amount of free glucose and starch in tested lines.

Results show a significant differences in content of sugar between control and tested lines.

To analyze protein content BCA assay and gel electrophoresis were performed.

Results of tests show that overexpression of PDAT gene influences content of protein.

Lipid content and composition was tested by extraction of lipids from plants and further gas chromatography analysis.

Obtained results show a big differences in plant development and protein, sugar content, same as differences in lipid composition.

This research can provide knowledge about metabolism of plant lipids,proteins and sugars.

LABORATORY OF PLANT BIOCHEMISTRY

TITLE: The impact of knock-out and overexpression of genes encoding acyl-CoA:lysophosphatidylethanolamine on the development of Arabidopsis plants under salt stress conditions

SPEAKER: ***Lizaveta Rusakovich***

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

Human intervention has led to the formation of several problems, including secondary soil salinization. Such farming technics as irrigation affect soil used specifically for growing crops, which causes a decrease in non-salinized arable land. High salt concentration prompts diminished water availability and disturbance of water homeostasis in plants, resulting in the inhibition of cells and root growth. This leads to lower yields, which is highly undesirable for the economy and countries with the problem of hunger in the population.

Salt stress is a very complex problem, every mutation, that somehow changes the metabolism of the cell is worth exploiting at the angle of stress resistance. Mutant plants of Arabidopsis thaliana at our laboratory are the ones with knock-out and overexpression of the genes responsible for acyltransferase activity, more precisely Acyl-CoA:lysophosphatidylethanolamine acyltransferases (LPEAT) overexpressors and knock-outs. The cell uses those acyltransferases to transport fatty acids from acyl-CoAs to lysophospholipids in the Lands cycle. PLA2s (phospholipases A2) are responsible for the conversion of phospholipids into corresponding lysophospholipids with the simultaneous release of fatty acids. On the contrary LPLAT (lysophospholipid acyltransferas) family members are responsible for the reverse reaction. As a result, the cell can produce different phospholipids, which perform a wide range of functions from being the main component of the cell membrane to signaling particles. Naturally, this raises a question, will these mutations affect plant resistance to salt stress?

Several experiments were performed to check and verify whether mutants react any differently to salt stress. First, through morphological observations were established appropriate salt concentration and the most resistant mutant line. The data on general lipid content were collected with the use of gas chromatography. Enzyme assays were performed to measure acyltransferase activity in non-treated and salt-stressed plants. Also, differences in the pigment content of the leaves were checked.

In this study only the LPEAT1 overexpressor line was investigated fully, further research is needed to get the full picture. However, the following can already be said: overexpressor LPEAT1 copes better with salt stress conditions and salt stress decreases the activity of the LPEAT in both wild-type plants and overexpressor, but mutants show higher levels of activity than wild-type plants.

LABORATORY OF PLANT MICROBIOLOGY

TITLE: Identification of molecular determinants of bacterial adhesion to abiotic surfaces in Ochrobactrum anthropi

SPEAKER: ***Aleksandra Karpińska***

SUPERVISOR(S): ***Sylwia Jafra****, PhD, DSc;*

Ochrobactrum anthropi is a human opportunistic pathogen that has relatively low virulence. However, it is resistant to antimicrobials, particularly from β-lactam group. Another interesting feature of. O. anthropi is its ability to adhere and form biofilms on the abiotic surfaces, including medical devices, such as catherers and protheses. In case of infection that includes colonisation of catherers or protheses, these devices need to be removed and antibiotic therapy must be applied. Even though O. anthropi is human pathogen, it can also colonise biotic surfaces, such as plant roots. The aim of this study is to identify genes associated with biofilm formation on abiotic surfaces using transposon mutagenesis. The mariner himar1 transposon inserts randomly into bacterial genome, leading to inactivation of random gene. Mutants are then screened by testing their biofilm formation ability on plastic 96-well plates . So far, 300 of 400 obtained mutants were tested on three different media: LB, TSB and 0,1 TSB. 125 of tested mutants produced more biofilm than the wild type and 93 produced less of biofilm in at least one of tested medium. These mutants will be then further tested. The experiments include swimming and swarming motility assay and biofilm formation on glass, plastic and medical tubes. Then the mutants will be sequenced in order to identify the genes responsible for surface colonisation.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: Characterization of Arabidopsis T-DNA mutants and overexpression lines of the CAX4 gene encoding the metal transporter, cultured under selected micronutrient deficiencies

SPEAKER: ***Alicja Adrjańska***

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

Microelements are necessary for plants for proper growth and development. Plants use them, among others, for photosynthesis, respiration, protection against pathogens. Many microelements act as enzyme cofactors. The exact processes of uptake, transport and homeostasis of many micronutrients are still not fully understood and require further research. Currently, many studies are being carried out to thoroughly understand the nutrient transporters that are responsible for the transport of cations in plants. One family of such transporters is the CAX family (from CAtion eXchanger). Members of this polygenic family can be found in many organisms (including Arabidopsis, rice and yeast).

Here, we performed a functional analysis of the Arabidopsis CAX4 gene by performing phenotypic characterization of a set of lines: (1) two independent T-DNA insertion mutants (cax4-1, cax4-2), (2) two overexpression lines (CAX4::cax4, CAX4::Col-0), and (3) wild-type control (Col-0) plants. We studied the effect of iron (Fe), zinc (Zn) and manganese (Mn) deficiency on the growth and development of the above plant genotypes growing in different hydroponic cultures. We also investigated the biochemical traits as the chlorophyll and carotenoids accumulation, as well as the variation in flowering time (FT) which was quantified by counting rosette leaf numbers (RLN) of plants grown both in soil and in various hydroponics. We also performed an in silico analysis to collect information about the CAX4 deposited in available databases. These analyzes showed that the CAX4 gene is expressed in the vacuolar membrane and root apical tissue, and also showed an interesting protein network with other proteins such as CAX11, HMA3 or SSE1.

We showed that both cax4 mutant lines when grown under Zn and Mn deficiency, had higher leaf and root fresh weight (FW) compared to wild-type plants. Interestingly, under Fe-deficient conditions, the opposite effect was observed, where cax4 mutants had lower FW of roots and rosette leaves. At the same time, under these conditions of Fe deficiency, the content of ChlA and ChlB in cax4 mutant plants increased. Additionally, we observed that cax4 mutants had a slight delay in flowering time (FT) compared to wild-type plants when grown in soil. Overexpressing lines showed intermediate phenotypes (between mutants and wild-type plants) for most of the growth- and biochemical-related traits studied here (except for the flowering time of soil-grown plants). Zn deficiency caused the strongest reduction in fresh weight among all genotypes, while Mn deficiency caused the greatest accumulation of carotenoids. The detailed function of CAX4 in the transport and maintenance of Fe, Mn and other metal homeostasis in the plant cell requires further research.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: Examination of the applicability of MALDI-TOF MS for identification of soft rot Pectobacteriaceae in environmental analysis.

SPEAKER: ***Filip Korolkiewicz***

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

Soft rot Pectobacteriaceae (SRP) belong to a group of phytopathogens with pectinolytic properties that can infect many different plant species including crops and vegetables. Because of their high prevalence they heavily affect food production around the world. As there are no effective methods for eradication of these phytopathogens, the most common control methods rely on prevention, involving monitoring of the plantations for the presence of SRP and removal of infected plants. One of the most important staple crops is potato, which is particularly prone to soft rot and blackleg diseases caused by SRP. It is therefore of vital importance to quickly and accurately identify any SRP pathogens present in potato fields. The goal of this work was to look at the applicability of MALDI-TOF MS for identification of SRP. This method has been so far mainly applied to detect human pathogens and the reference spectra for plant pathogens are underrepresented in the Bruker's database. By recording spectra for well-identified strains, the local library enlarges, allowing for better identification of the environmental isolates. Accuracy and sensitivity of this method should be compared with the existing alternatives and examined on environmental samples.

Here, pectinolytic bacterial isolates collected during countrywide monitoring of Polish potato fields in 2022 were identified to the species level by molecular diagnostics tools including single PCRs, multiplex PCRs, as well as sequencing of housekeeping genes and the collected results have been juxtaposed to the MALDI-TOF MS-based identification. Out of 34 bacterial isolates analysed with MALDI-TOF MS, 31 were successfully identified. The results were also largely congruent with those obtained using PCR tests with 28 out of 31 identifying within the same group and the remaining 3 as Psudomonas spp., previously unidentified with PCR tests aimed at SRP. Furthermore MALDI-TOF MS was able to identify isolates with species-level accuracy, classifying 9 of them as D. solani where previously with multiplex PCR they were simply identified as Dickeya spp. Isolates belonging to P. carotovorum, P. parmentieri and P. versatile were also undifferentiated by multiplex PCR whereas MALDI-TOF MS was able to distinguish between them. Therefore, judging by these results, it seems that after building a well-represented local database of the reference spectra, some species of SRP could be accurately identified to the species level with MALDI-TOF MS in less than 30 min. Such decrease of time and labour needed for identification would be highly beneficial to the efforts of preventing the spread of SRP.

Soft rot Pectobacteriaceae (SRP) belong to a group of phytopathogens with pectinolytic properties that can infect many different plant species including crops and vegetables. Because of their high prevalence they heavily affect food production around the world. As there are no effective methods for eradication of these phytopathogens, the most common control methods rely on prevention, involving monitoring of the plantations for the presence of SRP and removal of infected plants. One of the most important staple crops is potato, which is particularly prone to soft rot and blackleg diseases caused by SRP. It is therefore of

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: Comparative phenotypic and genetic characterization of Pectobacterium strains isolated from mono- and dicot plants.

SPEAKER: ***Aleksander Ostrowierch***

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

Bacteria from the genus Pectobacterium are widespread in nature and have been isolated on all continents, mostly from the vast range of crops and ornamental plants. Those bacteria are phytopathogens capable of causing diseases like soft rot, blackleg and wilt on various plant species. Among twenty-one species described within the Pectobacterium genus, only three recently described species, P. aroidearum, P. colocasium and P. zantedeschiae are unique because of their ability to infect both monocots and dicots. Worldwide increased temperatures accompanied by extensive plant trading are favouring the spread of phytopathogens, which cause huge losses in agriculture. Thus, the main aim of the study was to learn about the biology of three – P. aroidearum, P. colocasium and P. zantedeschiae.

The study was performed on 38 Pectobacterium strains isolated from various countries. Genetic characterization of the strains was determined with recA gene sequencing and ERIC-PCR. While the bioinformatic tool OrthoVenn2 was used for genomic comparison. Pathogenicity assays on chosen monocots and dicots were performed for the determination of phenotype.

Obtained results show that investigated species are extremely diverse. The observed genotypes were unrelated to the geographic origin of analysed strains except for strains from the Netherlands and Israel that seem clonal. All strains were virulent on monocots and dicots, showing no host specificity, yet they were indistinguishable phenotypically.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: Genomic and phenotypic profiling of Pectobacteriaceae strains isolated from various water sources

SPEAKER: ***Kacper Smorawiński***

SUPERVISOR(S): *Prof.* ***Ewa Łojkowska****;*

Bacteria from Pectobacterium and Dickeya genus are important plant pathogens that cause soft rot and blackleg diseases leading to significant crop losses. They are also found in waterways and thus present a risk of infecting farmlands during irrigation. For this reason in several countries projects devoted to monitoring of the bacterial presence and identification in regional water sources were performed.

Materials for the presented research included 46 bacterial strains isolated from Polish water that have to be identified and 70 pectinolytic bacterial strains isolated from water in Poland and different countries that were already identified as: Dickeya aquatica, Dickeya chrysanthemi, Dickeya zeae, Pectobacterium aquaticum and Pectobacterium quasiaquaticum.

The aim of this project was to recognize whether various waterways are important sources of pectinolytic bacteria, asses the virulence of isolated bacterial strains belonging to Pectobacterium and Dickeya spp. and evaluate their biodiversity on the levels of phenotype and genotype. For this goal, pectinolytic strains isolated from water were subjected to: 1) phylogenetic analysis with the use of recA and dnaX genes; 2) genomic profiling with the use of BOX and ERIC PCR and; 3) phenotypic characterisation which included evaluation of the ability to macerate potato tuber tissue and enzymatic activities of plant cell wall degrading enzymes: pectinases, cellulases and proteases.

Evaluation of 46 unidentified strains allowed to identyify 4 pectinolytic strains belonging to Pectobacterium genus. Analysis of recA and dnaX genes indicated that all 4 newly identified pectinolytic strains belong to Pectobacterium versatile species. Comparison of housekeeping genes' sequences showed high similarity within strains from most species, with the exception of D. zeae, which appear very heterogenous. It also allowed for reclassification of the first P. aquaticum strain isolated earlier in Poland to P. quasiaquaticum species.

The phenotypic studies indicated that there are virulent strains within D. zeae and D. aquatica strains isolated from Polish, Finnish, British and Israeli waters. Moreover D. zeae and D. aquatica strains exhibited high activities of pectinases and cellulases. Proteases of all analysed strains showed lower activity than those of reference strain Dickeya solani IFB0099 isolated from infected potato plants. Obtained results indicated that waterways can be important source of plant pathogenic bacteria.

LABORATORY OF BIOLOGICALLY ACTIVE COMPOUNDS

TITLE: TaqMan PCR-based method for quantitative detection of phages from Viunalikevirus species

SPEAKER: ***Robert Burzyński***

SUPERVISOR(S): ***Robert Czajkowski****, PhD, DSc;*

Bacteriophages (phages) were discovered about 100 years ago. It is estimated that there are more than 10^31 bacteriophages on the planet. In the first decades after their discovery, studies focused on phages were quite common, but after antibiotics discovery, they have been forgotten for some time. In recent years, growing interest in phage research have been observed, mainly due to increasing problem of antibiotic resistant bacteria. In relation to growing interest in phage research, there is a growing need for phage detection methods. Currently known methods greatly rely on knowledge about the phage-host interactions in advance and due to that additional steps in research are needed. Furthermore, methods which are very specific require expensive equipment, what might be an obstacle for some researchers. On the other hand, cheaper methods often lack sensitivity. With that problems in mind, I focused on establishing new phage detection method, which would be cheap, sensitive and easy to carry out. My method is based on TaqMan PCR, which allows for real time, quantitative detection of specific species of bacteriophages. In my work, I designed different primers and TaqMan probes, which were tested with different phage DNA concentrations (10 – 100 ng/µl). Finally, I chose 2 best combinations of primers and probes for TaqMan PCR, which were able to effectively detect and quantify isolated DNA, even in low concentrations such as 10 ng/µl and about 1000 virions per µl. The simplicity and affordability of this method will provide new options in phage associated researches.

LABORATORY OF BIOLOGICALLY ACTIVE COMPOUNDS

TITLE: Toxicity of secondary metabolite mixtures from Drosera gigantea and identification of antimicrobial constituents

SPEAKER: ***Martyna Muszyńska***

SUPERVISOR(S): *Prof.* ***Aleksandra Królicka****;* ***Marta Krychowiak-Maśnicka****, PhD;*

Carnivorous plants from Droseraceae family have been used in traditional medicine for ages, e.g., to treat infections. Currently it is known that medicinal properties of their extracts are attributed to secondary metabolites (SMs), i.e., products of secondary metabolism which are not necessary for survival but might be advantageous to the plant. Naphthoquinones are the main group of antimicrobial compounds found in carnivorous plant tissues, however extracts containing those metabolites are cytotoxic to eukaryotic cells. Extracts from Drosera gigantea, i.e., one of the species from the family of carnivorous sundews, also exhibit antimicrobial activity, but they have been found to be non-toxic to eukaryotic organisms. Metabolite composition of D. gigantea was only partially determined and it remains unknown which metabolites are responsible for antimicrobial activity. The goal of this project was to isolate and identify antimicrobial SMs of D. gigantea and investigate toxicity of their mixtures. Due to D. gigantea being endemic, in vitro plant cultures were used to obtain plant tissue. Water extracts from D. gigantea tissue were purified on C18 columns and hydrophilic fraction was used for further experiments as it exhibited the highest antimicrobial potential. When the fraction was separated using thin-layer chromatography (TLC), seven bands were distinguished and isolated to initially identify them by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Preliminary results showed that five constituents of the fraction are naphthoquinone glycosides. The rest of the chemicals remained unknown. Antibacterial activity of separated SMs was tested by agar overlay TLC-bioautography against Staphylococcus aureus ATCC 25922, Escherichia coli ATCC 25923 and Pseudomonas aeruginosa ATCC 27853. Among all separated SMs only one metabolite inhibited growth of S. aureus and E. coli. Unfortunately, HPLC-MS analyses of the metabolite were inconclusive and it could not be identified. An efficient method of its isolation was developed and its structure will be analyzed with nuclear magnetic resonance. Toxicity of the fraction was tested in BALBc mice by administering it orally every 24 hours for five days. By the end of the experiment all the animals were alive. Body weight, organ weight, blood count and serum biochemistry were compared between experimental and control groups. No significant differences were found, showing that the fraction of D. gigantea extract is non-toxic in tested dose. In conclusion, only one secondary metabolite with antibacterial activity was found in D. gigantea and its identification is still in progress. Additionally, active fraction containing this metabolite was found to be non-toxic to mice.

LABORATORY OF BIOLOGICALLY ACTIVE COMPOUNDS

TITLE: Cultivation of selected species of Drosera in Plantform elicited, periodic-flooded cultures to eradicate Pseudomonas aeruginosa.

SPEAKER: ***Stanisław Rugień***

SUPERVISOR(S): *Prof.* ***Aleksandra Królicka****;*

Carnivorous plants of Drosera sp. are a source of biologically active compounds, namely secondary metabolites. They possess many interesting properties like anticancer, anti-inflammatory or antimicrobial activity. Thus, carnivorous plants extracts have a huge potential in the pharmaceutical industry, medicine and could be one of the possible solutions to the treatment of infections caused by multi-drug resistant pathogens. Extracts of Drosera sp. contain many biologically active compounds [naphthoquinones (plumbagin and ramentaceone), phenolic acids, flavonoid glucosides, flavonoids] responsible for their bactericidal properties. However, Drosera sp. extracts are less active against Gram-negative [G (-)] pathogens, naturally resistant to naphthoquinones. What is more, substances like plumbagin or ramentaceone are highly cytotoxic to eucaryotic cells, which makes them harder to use in potential treatment. Nevertheless, studies indicate that carnivorous plant extracts contain plenty of unidentified compounds that show bactericidal effect against G (-) pathogens. Therefore, we tested four different species of Drosera sp. (Drosera zigzagia, Drosera derbyensis, Drosera cayenensis and Drosera ultramafica) in in vitro culture, to define the activity of their extracts against Pseudomonas aeruginosa ATCC 27853, which is one of the most resistant to carnivorous plants' secondary metabolites G (-) human pathogen. To increase the activity of extracts from Drosera sp., plants were cultivated on ½ Murashige and Skoog medium with the addition of precursors or elicitors (L-phenylalanine, lack of nitrogen, Raoultella ornithinolytica cell lysate and co-culture with Calitriche L.). Plants were grown in liquid culture in Erlenmeyer flasks and in the periodic-flooded Plantform bioreactors. The antimicrobial activity of the methanol and water extracts from Drosera sp. cultivated in different conditions was defined by determining the minimal bactericidal concentration [MBC]. The extracts were also analyzed in terms of their composition using Thin Layer Chromatography [TLC] and High-Performance Liquid Chromatography [HPLC] to find any compounds active against P. aeruginosa. Extract from D. zigzagia was the most active against tested pathogen and, simultaneously, contained the largest amount of plumbagin. However, the extract fraction from D. zigzagia obtained with TLC, which was free from plumbagin, still was active against G (-) bacteria, including P. aeruginosa and Escherichia coli. The compounds present in this extract fraction were less cytotoxic to eukaryotic cells as tested with an aneuploid immortal keratinocyte cells (HaCaT) determined using the MTT assay.

SESSION 7

LABORATORY OF EXPERIMENTAL AND TRANSLATIONAL IMMUNOLOGY

TITLE: Small extracellular vesicles as a source of antigens for CD1a-mediated T cell responses

SPEAKER: ***Felicja Gajdowska***

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

Antigens can be presented by different specialised molecules, amongst which the most known are the MHC (Major Histocompatibility Complex) class I and II, which present peptide antigens to the T cells and trigger a response. CD1a is an MHC-like molecule, highly expressed by Langerhans cells in the skin but also present in other dendritic cell populations. Unlike MHC class I and II, CD1a presents lipid antigens to the CD1a-restricted T cells.

Extracellular vesicles (EVs) are a group of heterogeneous secreted organelles, encompassing apoptotic bodies (ABs), microvesicles (MVs) and exosomes. Distinct biogenesis pathways are reflected in their cargo (i.e., proteins, nucleic acids) and lipid composition of the phospholipid bilayer.

The role of EVs in cell-to-cell communication has been identified in recent years, and more and more studies investigate the mechanisms underlying EVs contribution in this process. However, the information on the lipid composition of their bilayer and how it can take part in the immune responses is still limited. Previous study from the lab showed that exosome-enriched keratinocyte-derived small EVs (sEVs) may provide lipid antigens for the CD1a molecule, resulting in alteration of the immune responses.

The aim of this study is to investigate whether sEVs isolated from different cellular sources may contribute their lipids as antigens during CD1a-mediated T cell responses in the cancer setting, also in comparison to other EV types.

Seven cell lines (non-cancer HaCaT control line and cancer lines: HeLa, HEK293T, HepG2, THP-1, Jurkat and K562) were investigated. Cells were grown in EV-depleted culture medium for 48 hours and EVs were isolated by the serial ultracentrifugation protocol. The size and concentration of the EVs were measured by Nanoparticle Tracking Analysis (NTA) method. Lipid antigens were liberated from the EV membranes by digestion with bee venom phospholipase A2 (PLA2). To assess T cell reactivity, interferon-γ ELISpot experiments were carried out. To this end, model antigen presenting cells (K562-CD1a and K562-empty vector transfectants) were pulsed with the digested EVs, and then co-cultured with magnetically selected T cells from healthy blood donors. Activation of T cells was measured by enumeration of spots forming units (SFUs) indicative of activation of antigen-specific T cells and interferon-γ response. Secretion of additional cytokines by T cells activated during ELISpot experiments were investigated with the ELISA method, and three interleukins (IL-10, IL-13 and IL-17) were analysed.

The results indicate that, as a general rule, interferon-γ responses were higher to pulsed K562-CD1a cells than to K562-empty vector, indicating CD1a-restriction; responses were lower than to the unpulsed control cells for all EVs types and cellular sources. ELISA results show similar trend for IL-17 secretion while levels of other cytokines were negligeable throughout.

These findings indicate that EV-derived lipid antigens isolated from different cancer cell lines reduce CD1a-restricted T cell responses. Cancer cell EVs affecting this pathway could consist a novel immune tumour evasion strategy, thus the results warrant further investigation.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE:

SPEAKER: ***Adrian Engler***

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

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: Identifing plasmids in Clostridioides difficile

SPEAKER: ***Wiktor Krukowski***

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

Clostridioides difficile is a Gram positive, spore forming bacteria, responsible for nosocomial and community acquired infections, resulting in diarrhea, and in rare cases sepsis and possibly even death. Plasmids in Clostridioides difficile remain in the cell despite lack of selection. They are described as cryptid plasmids, as role of some of them in the cell is unknown. Despite that, it is known that infections in hospitals and epidemic outbreaks are more often associated with strains carrying plasmids. This shows that plasmids may play a role in virulence. therefore, methods for fast identification of strains carrying plasmids are necessary. In this work I try to design primers for colony PCR of Clostridioides difficile, that will confirm presence of as many plasmids as possible, with as few primers as possible. Besides that, I also try to analyze sequence of a plasmid I isolated of a clinical strain of Clostridioides difficile, in order to try and find a possible cause for its maintenance in the cell.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: Bacillus subtilis spore surface display of the tissue inhibitors of metalloproteinases

SPEAKER: ***Małgorzata Kulińska***

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

Periodontitis, which is a second most common oral disease, develops slowly, which is the biggest problem in here. Patients often downplay the initial signs of the disease, considering them as symptoms of a temporary inflammatory state or excessive brushing. It is only when later, more advanced symptoms arise that our concern is aroused, prompting us to visit a dental office. The main cause of periodontitis is improper oral health care and hygiene – it is a consequence of excessive plaque buildup, which gradually covers an increasing surface area of the tooth until it reaches under the gum line. This leads to the development of inflammation, creating an ideal environment for bacterial growth. The presence of bacteria and inflammation results in the involvement of pro-inflammatory factors such as cytokines, growth factors, and matrix metalloproteinases.

Matrix metalloproteinases (MMPs) are a group of calcium-dependent proteolytic enzymes, and their primary function is to participate in the physiological and pathological processes of remodeling and degradation of extracellular matrix components, this including embryogenesis, angiogenesis as well as degenerative diseases and tumors. Their tissue inhibitors, TIMPs, form stable and reversible complexes with MMPs, and the mechanism of inhibition involves blocking the substrate's access to the catalytic site of the metalloproteinase.

And even though bacteria initiate the inflammatory state, the greatest problem in the case of periodontitis is the activity of MMPs – or more precisely, the imbalance between matrix metalloproteinases and their tissue inhibitors. It is widely known that MMPs are involved in many degenerative diseases, this including periodontitis. This was what made us think, that the usage of their inhibitors would be a good thing to consider while trying to find a way to prevent periodontal tissue damage.

The aim of this project was to create a stable fusion between two Bacillus subtilis spore proteins – CotB and CgeA and TIMP-2 as well as TIMP-3. Results from restriction analysis showed that created plasmids contain a proper sequence coding for the fusion proteins. Their presence on the surface of the spores as well as their ability to inhibit MMP-9 activity is yet to be assessed.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: Analysis of social interactions between D.Solani and B.Subtilis mutant.

SPEAKER: ***Krzysztof Prusinowski***

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

The soil provides a habitat for one of the most, yet unknown microbial community, the soil microbiota .Many of the rules governing the interactions between organisms further remain a mystery. One species of bacteria that can be found in this environment is Dickeya solani, a Gram-negative plant pathogen that attacks a wide range of crops. The most worrisome host of this microorganism for the humankind is the potato (Solanum tuberosum). Its infections causes agricultural losses counted in millions of Euros per year, for example, in Denmark in 2007 losses caused by this bacterium amounted to €25M. Biotechnologists are turning to various strategies in an effort to find a solution to this problem.

In a previous study, the team of the Laboratory of Molecular Bacteriology managed to isolate the strain Bacillus subtilis MB73/2, a Gram-positive bacterium that interacts with IPO 2222 and IFB 102 D.solani strains. In order to determine the swarming phenotype for each strain, experiments were carried out using agar with reduced glucose, to induce swarming in bacteria. In the case of strain IPO 2222, an active escape of the inoculated colony from MB73/2 was observed, while strain IFB 102 forms a inhibition zone that prevents the contact with B.subtilis. An in situ experiment was also conducted on potato (S. tuberosum) slices. The aforementioned D.solani strains differ genetically by six regions with single nucleotide polymorphisms (SNPs) which were found in the genome of IFB 102. It is likely that the differences in the swarming pattern, as well as in the interaction with MB73/2 are due to the presence of SNPs. In order to determine which of these SNPs are responsible for the phenotypic differences between the bacteria, we decided to create mutants of the IFB strain with deletions of genes with particular SNPs. One of the genes with unknown function was the lysR gene, presumably associated with phenotypic differences. In order to check if this gene has any impact on the phenotype, an integration plasmid for replacing lysR with gentamicin resistance cassette was constructed using Gibson assembly and it was incorporated into competent IFB 102 cells by electroporation, which was then checked for the swarming pattern.

Further work related to the study of the effect of differences in specific regions having SNPs is based on the creation of strains having deletions in specific genes containing polymorphisms. The topic is very broad and research is still ongoing, although some clues on the process are being obtained, still further analyses are required.

LABORATORY OF BIOMOLECULAR SYSTEMS SIMULATIONS

TITLE: Prediction of the structure of the C-ring and the MS-ring of the *Helicobacter pylori* flagellar motor

SPEAKER: ***Kacper Kępka***

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

Helicobacter pylori is a bacterium that can be found in the stomach of approx. 50% of the human population and can cause diseases, including cancer. It is shown that there are two factors allowing it to cause diseases: survivability in a highly acidic environment and high motility. Both of those factors are linked to the flagellar motor function, as it powers the bacteria propulsion system - the flagellum, and it uses the proton gradient as fuel. The MS-ring and the C-ring are the, so called, switch complex, with the function of changing the direction of rotation of the rotor of the motor. In my project I have developed a full atom model of the structure utilizing x-ray crystallography models of certain domains of the proteins building the rings and a sub-averaging tomogram from a CRYO-EM microscopy study. I have employed homology modeling approach, protein-protein docking and symmetrical docking techniques, alongside RMSD analysis of created models to the crystallography ones. By creating this model of the whole switch complex, precise interactions of FliM and FliY proteins have been discovered as well as the location of FliF protein not only in the MS-ring as believed previously, but also in the C-ring.

LABORATORY OF BIOMOLECULAR SYSTEMS SIMULATIONS

TITLE: Kinesin movement details

SPEAKER: ***Natalia Niżnik***

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

Kinesins are molecular motor proteins, which can move along microtubules. According to their function and structure similarities, kinesins were divided into 14 families. All of them have a strongly conserved motor domain, which is responsible for ATP hydrolysis and microtubule binding. The energy coming from ATP hydrolysis is used to power protein movement. Most kinesins move toward the plus end of microtubules, but some move toward the minus end. One of these untypical minus-end directed kinesins is kinesin – 14 Ncd. It plays an important role during cell division, taking part in spindle organization and chromosome segregation. Despite many studies process of the kinesin – 14 movement is still not fully understood. There are many gaps and controversies, which should be solved.

Taking this into account the aim of the project was to explore kinesin – 14 Ncd movement details and to investigate important structural elements involved in this process. For this purpose, coarse-grained molecular dynamics simulations were used. As a result, the trajectory, visualizing atoms movement over time was obtained. It helped to identify important elements of the kinesin – 14 Ncd movement.

LABORATORY OF MOLECULAR MODELING / FOC UG

TITLE: Designing and studying physicochemical properties of peptides and peptidomimetics blocking PD-1/PD-L1 complex formation

SPEAKER: ***Michał Winnicki***

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

Cancer therapy drugs have been developed for the last few decades. Those molecules include antibodies and nanobodies targeting PD-1/PD-L1 complex formation. Many of them have gone through clinical trials and play a key role in modern cancer therapy. On the other hand, most of them show many drawbacks as very high production costs, tendencies towards immunogenicity, and poor pharmacokinetic and pharmacodynamic properties. Therefore, there is an undeniable need for novel therapeutics especially peptides and their analogs, derived from their potential target's molecular structure.

In this study, I designed peptides and peptidomimetics, acting as potential inhibitors of crucial immune checkpoint PD-1/PD-L1 formation. Designed molecules amino acid sequences were based on interaction sites of PD-1 protein. Their physicochemical properties were studied using molecular dynamics simulations with the UNited RESidue coarse-grained force field along with MM/GBSA analysis using the AMBER all-atom force field. Initially designed peptides served as a basis for the design of their synthetic analogs, peptidomimetics, which reveal increased binding strength to their molecular target, the PD-L1 protein.

Results suggest that several peptidomimetics among all the designed structures are the best candidates to act as potential inhibitors of PD-1/PD-L1 complex formation and were chosen for further study of their biophysical properties in vitro.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Functional consequences of missense mutations at position 263 in complement C2 protein

SPEAKER: ***Alicja Kuźniewska***

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

The complement system is one of the first defense lines protecting from invading pathogens. However, it may turn offensive to the body's own cells and tissues when deregulated by the presence of rare genetic variants that impair physiological regulation and/or provoke abnormal activity of key enzymatic components. Factor B and complement C2 are examples of paralogs engaged in the alternative and classical/lectin complement pathways, respectively. Pathogenic mutations in the von Willebrand factor A domain (vWA) of FB have been known for years. Despite substantial homology between two proteins and the demonstration that certain substitutions in FB translated to C2 result in analogous phenotype, there was a limited number of reports on pathogenic C2 variants in patients. Recently, we studied a cohort of patients suffering from rare kidney diseases and confirmed the existence of two gain-of-function and three loss-of-function mutations within the C2 gene sequences coding for the vWA domain (amino acids 254-452) or nearly located unstructured region (243-253). Herein, we report functional consequences of amino acid substitution of glutamine at position 263. p.Q263P variant found in a patient with C3 glomerulopathy resulted in the loss of C2 function whereas the p.Q263G variant resulted in the gain-of-function phenotype, similarly to a homologous mutation p.D279G in FB. Our results confirm that the N-terminal part of the vWA domain is a mutational hot spot crucial for the complement C2 function.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Mechanism of action of type II anti-CD20 antibodies and its modulation in the context of contemporary immunotherapy of lymphoproliferative malignancies.

SPEAKER: ***Majeranowski Alan***

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

Background

Anti-CD20 monoclonal antibodies (mAbs) are widely used in clinics for the treatment of B-cell lymphomas and leukemias. Based on their prevalent effector mechanism, anti-CD20 mAbs are divided into two types: type I (e.g., rituximab, ofatumumab) and type II (e.g., obinutuzumab). The first category efficiently activates complement and poorly induces direct cell death, whereas the latter category exhibits the opposite characteristics. These differences were recently explained by structural studies on the mAb-antigen complex, which found that type I mAbs bind in a way that favors the recruitment of additional mAb particles, eventually leading to the formation of hexamers. This binding pattern ideally matches the first component of the classical complement pathway, C1q. On the other hand, binding of the first particle of type II mAbs prevents oligomerization and results in weak complement engagement. The protein we are interested in, C2, acts downstream of the antigen-mAb complex and C1q binding. Based on the homology between the alternative and classical complement pathway components, specifically factor B and C2, we created a gain-of-function (GoF) variant of the C2 protein, resulting in the formation of a hyperactive complement convertase.

Methods

To elucidate the modulation of obinutuzumab cytotoxicity by GoF C2 protein we performed complement-dependent cytotoxicity (CDC) and whole blood CDC assays. We also analyzed activity profiles of C3 and C5 convertases on obinutuzumab-sensitized lymphoma cells.

Results & Coclusions

Addition of serum supplemented with the GoF C2 variant to obinutuzumab-sensitized CD20-positive cells resulted in elevated CDC, which was comparable or higher than that observed for rituximab-sensitized cells. Moreover, we observed that the addition of the C2 GoF variant to lymphoma cells treated with obinutuzumab resulted in the increase of C3 and C5 convertase activity, which is hardly perceptible in the absence of hyperactive C2. Convertases formed in the presence of the GoF variant and obinutuzumab reached their Tmax value later than convertases formed upon the presence of type-I anti-CD20 antibodies. Our results indicate that obinutuzumab may gain type I characteristics when acts in tandem with hyperactive complement convertases and therefore the efficacy of complement activation by type II anti-CD20 mAbs does not rely exclusively on mAb-antigen interaction.Surprisingly, upon addition of the GoF C2 variant we observed enhancement of CDC initiated by obinutuzumab in whole blood, which outperformed the analogous increase observed in serum, whereas the CDC ratio in rituximab-treated cells was similar in whole blood and serum. This result suggests that for a short-term cytotoxicity obinutuzumab benefits from complement as its additional effector mechanisms in the physiological environment of whole blood. To complete this thread we dediced to examine the collaboration of CDC with the ADCC mechanism initiated by obinutuzumab. Our results indicate that obinutuzumab may gain type I characteristics when acts in tandem with hyperactive complement convertases and therefore the efficacy of complement activation by type II anti-CD20 mAbs does not rely exclusively on mAb-antigen interaction.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: Concept of the universal antibody targeting tumor cells resistant to the first-line immunotherapeutics

SPEAKER: ***Daria Kowalska***

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

The complement system plays an important role as part of innate immunity. Its classical pathway can be activated by certain isotypes of antibodies leading to the lysis of targeted cells. Complement activity is regulated by complement inhibitors expressed on the surface of various cells, including cancer cells as well as present in a soluble form. We distinguish two types of complement inhibitors – with cofactor activity, which supports proteolytic degradation of the active cascade components, e.g. C3b and C4b by Factor I and decay acceleration factors disabling convertases (enzymatic complexes crucial for each activation route). Inhibition at this stage leads to unproductive depletion of early complement components and exhaustion of the cascade potential. Such a mechanism of protection is utilized by cancer cells that overexpress complement inhibitors and hereby gain resistance to anticancer antibodies. Moreover, with anticancer antibodies administered in repetitive high doses, cancer cells avoid the complement attack by internalization or shedding of molecular targets, creating populations resistant to the therapy. These phenomena generate a large number of patients not responding to treatment and can even contribute to the relapse of the disease.

To maximize the effect of therapeutic antibodies, I decided to focus on a classical complement pathway protein, C4d. It is the product of proteolytic degradation of early classical pathway component C4b by complement inhibitors. C4d binds covalently to the cell surface at the place of the complement activation, with the yield proportional to the number of complement inhibitors engaged by tumor cells. Thus, the use of anti-C4d antibodies will not only initiate a second wave of activation exactly at the place of cancer cells treated by first-line immunotherapeutics but also enable to exploit of the potential of complement present in patients' serum since C4d is deposited independently of the molecular target of the previously used antibody.

The aim of the study is therefore to create an anti-C4d antibody recognizing surface-bound C4d, which can be used as a universal trigger of a second wave of complement activation.

The first step was to choose the optimal epitope for immunization that will lead to the generation of antibodies specific to C4d but not C4b nor C4 and have the longest possible sequence while maintaining high specificity and functionality. For this purpose, I have tested polyclonal antibodies purified from the anti-sera of ten immunized rabbits and have chosen 7aa and 9aa long C-terminal epitopes for the creation of monoclonal antibodies. After testing all of the received clones and confirming their functionality, I have selected the three most effective ones for the chimeric rabbit-human antibody generation in order to obtain an antibody fully compatible with human effector mechanisms. Additionally, I have introduced two point mutations shown to improve complement activation. I plan to test their potential in a two-step complement-dependent cytotoxicity assay to confirm their possible use as second-line immunotherapeutics. Independently of this, I have also tested the use of anti-C4d antibodies in immunohistochemistry.

INTERNATIONAL CENTER FOR CANCER VACCINE SCIENCES (ICCVS)

TITLE: CARMEN: A pan-HLA and pan-cancer proteogenomic database on antigen presentation to support cancer immunotherapy

SPEAKER: ***Ashwin Adrian Kallor***

SUPERVISOR(S): ***Karol Połom****, PhD, DSc;* ***Javier Alfaro****, DSc;*

Cancer immunotherapy has greatly improved the quality of life of cancer patients and it hinges on the discovery of novel cancer antigens that could be targeted to improve disease outcomes. The creation of databases such as IEDB, SysteMHC, TANTIGEN, caAtlas, HLA Ligand Atlas, Cancer Antigenic Peptide Database, SPENCER and IEAtlas support the immunopeptidomics community in understanding the landscape of antigen presentation. We have developed a pan-cancer, pan-HLA, and pan-tissue database containing immunopeptidomics data mapped to transcriptomic, genomic, immunological and biochemical data. The database was generated from 77 different publicly available immunopeptidomics mass spectrometry datasets collected between 2015-2022 (73 cancer and 4 normal datasets), covering 15 different types of cancers and 152 different HLA-I alleles. The peptides contained in our database were obtained by a combination of closed, open and de novo searches using an in-house developed computational pipeline. Following rigorous false discovery rate estimation at 1% and a second-round search to eliminate any false signals that may not have been detected in the previous round of FDR estimation, we obtained a list of 11.2 million peptide-HLA combinations comprising both coding and non-coding regions of the genome as well as bacterial peptides. These peptides have been mapped to chromosomal coordinates to facilitate adoption by the genomics community of this useful resource on antigen presentation. Pathway/biochemical analysis of each peptide was performed using the rWikiPathways package. Finally, mutations associated with each peptide were annotated using COSMIC and dbSNP resources. Our database includes a FAIR knowledge graph which contextualizes and enriches the data to enable clinicians to take effective therapeutic decisions on the appropriate form of treatment for cancer immunotherapy with the case study of clear cell renal cell carcinoma (ccRCC). We will continue to expand our database with new data over the next two years and expand the scope of its applications to facilitate uptake by the larger scientific community.

CORE FACILITY LABORATORIES

TITLE: Discovery of serum biomarker to differentiate ischemic stroke patients and identify patients that are risk-free treatable with rtPA, using a proteomic and peptidomic approach

SPEAKER: ***Marc Müller***

SUPERVISOR(S): *Prof.* ***Bartosz Karaszewski****;* ***Paulina Czaplewska****, PhD, DSc;*

Cancer therapy drugs have been developed for the last few decades. Those molecules include antibodies and nanobodies targeting PD-1/PD-L1 complex formation. Many of them have gone through clinical trials and play a key role in modern cancer therapy. On the other hand, most of them show many drawbacks as very high production costs, tendencies towards immunogenicity, and poor pharmacokinetic and pharmacodynamic properties. Therefore, there is an undeniable need for novel therapeutics especially peptides and their analogs, derived from their potential target's molecular structure.

In this study, I designed peptides and peptidomimetics, acting as potential inhibitors of crucial immune checkpoint PD-1/PD-L1 formation. Designed molecules amino acid sequences were based on interaction sites of PD-1 protein. Their physicochemical properties were studied using molecular dynamics simulations with the UNited RESidue coarse-grained force field along with MM/GBSA analysis using the AMBER all-atom force field. Initially designed peptides served as a basis for the design of their synthetic analogs, peptidomimetics, which reveal increased binding strength to their molecular target, the PD-L1 protein.

Results suggest that several peptidomimetics among all the designed structures are the best candidates to act as potential inhibitors of PD-1/PD-L1 complex formation and were chosen for further study of their biophysical properties in vitro.

CORE FACILITY LABORATORIES

TITLE: Proteomic analysis of sialoliths - identification of potential biomarkers of deposit formation in salivary glands

SPEAKER: ***Natalia Musiał***

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

Salivary stones, also known as sialoliths, are formed in a pathological situation in the salivary glands. So far, neither the mechanism of their formation nor the factors predisposing to their formation are known despite several hypotheses. While they do not directly threaten human life, they significantly deteriorate the patient's quality of life.

The aim of the study is to characterise sialoliths and search for the biomarkers in their proteomes taking into account their division into different groups for understanding the processes leading to formation of solid deposits.

In this work, we used mass spectrometry to perform qualitative and quantitative analysis to investigate the composition and select proteins that may contribute to solid deposits in the salivary glands. Twenty sialoliths, previously characterized spectroscopically and divided into the following groups: calcified, lipid and mixed, were used for the study.

The standard set of proteins for each type of salivary stone included 13 proteins, they differed type of regulation. Proteins unique for each of the groups were found, including: for the CAL group among them, e.g. proteins from the S100 group, mucin 7, keratins, neutrophil elastase or stomatin; proteins for the LIP group - transthyretin, lactotransferrin, matrix Gla protein, submandibular gland androgen-regulated protein 3; mixed stones had only 1 unique protein, fibrinogen alpha chain. Bacterial proteins present in sialoliths have also been identified.

Thanks to the identification of common proteins in all three groups and unique proteins for each of them, the potential biomarkers leading to the solid deposits formations were found. Most such proteins were identified for the CAL type, and the MIX group was the least unique. The analysis of the results indicates the possible role of bacterial infections, disturbances in calcium metabolism and neutrophil extracellular traps (NETs) in the formation of sialoliths.

LABORATORY OF BIOPOLYMERS STRUCTURE

TITLE: Search for peptide markers of salivary gland tumors in patients' saliva samples. Pilot study.

SPEAKER: ***Michał Puchalski***

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

Introduction: Salivary gland tumours are highly diverse in clinical manifestation and histology. The World Health Organization (WHO) classification distinguishes 22 types of malignant and 11 benign tumours originating from the salivary glands. Diagnostics of salivary gland tumours are based on imaging (ultrasound, magnetic resonance imaging) and fine-needle aspiration biopsy; however, the final diagnosis is based on the histopathological examination of the removed tumour. In this pilot study, we test a new approach for identification of peptide biomarkers in saliva that can be used in diagnosing tumours of the salivary glands. As a research material for peptidomic studies, we use extracts from washing neoplastic tissues and healthy tissues (control samples). At the same time, samples of saliva from patients, as well as from healthy individuals, will be analyzed. Comparison of the peptidome composition of tissue extracts and saliva samples may allow the identification of potential peptide markers of a salivary gland tumor in patients' saliva.

Methods: We analyzed the peptidome composition extracted from 18 tumour and 18 healthy tissue samples using LC-MS tandem mass spectrometry. PBS and 0.1% trifluoroacetic acid were used to extract the peptide fraction from the tissue samples. We also investigated the peptidome compositions of patients' saliva (11 samples) and healthy individuals (8 samples).

Results: We selected a group of peptides (109 peptides) that were present only in extracts from tumour tissues and in samples of the patient's saliva. Some of the identified peptides come from proteins that were previously indicated as potential biomarkers of salivary gland tumours (ANXA1, BPIFA2, FGB, GAPDH, HSPB1, IGHG1, VIM) or tumours of other tissues or organs (SERPINA1, APOA2, CSTB, GSTP1, S100A8, S100A9, TPI1). Unfortunately, in individual samples only a few peptides characteristic only of the cancer tissue and the patient's saliva appeared, which does not allow to identify a universal biomarker. Perhaps the reason for this situation is the high heterogeneity of this type of cancer. The surprising result was that extracts derived from tumor tissues did not contain peptides derived from salivary gland specific proteins (STATH, SMR3B, HTN1, HTN3). These results suggest that the developing tumor suppresses the production of proteins that are essential components of saliva, however, confirmation of this hypothesis requires further additional research.

LABORATORY OF EXPERIMENTAL AND TRANSLATIONAL IMMUNOLOGY

TITLE: New approaches to characterize Th17/Treg cell balance contributing to anti-tumour immune responses

SPEAKER: ***Dominika Miroszewska***

SUPERVISOR(S): ***Zhi Jane Chen****, PhD,;*

CD4+ T lymphocytes are critical for effective immune response. Th17 cells are the main producers of pro-inflammatory cytokine IL-17, which promotes intestinal tumorigenesis. On the other hand, T regulatory (Tregs) cells are mainly involved in immune self-tolerance and homeostasis. In the recent years, Th17 and Tregs and the balance between them has been proposed as one of the regulating factors in cancer development. Globally, colorectal cancer (CRC) is the third most common type of cancer. The role of Th17 and Tregs in CRC progression is still poorly understood. Therefore, the aim of the project is characterization and identification of the heterogeneity of tumor infiltrating T cells, especially Th17/Treg cells in CRC patients and mice models of inflammatory bowel disease (IBD) and CRC.

FFPE tissues samples from mouse model of IBD and CRC and human CRC were stained with hematoxylin & eosin to visualize and analyze the histology of the tumors and to assess the inflammation degree. The samples were also stained with immunofluorescence to detect particular T cells populations present. 4 human CRC and healthy, matched tissue section were analyzed using spatial transcriptomics to investigate gene expression in the tissue.

Histological evaluation of inflammation and CRC mice and human tissue indicated the highest number of lymphocytes in the tissue was observed with more severe inflammation, yet inflammation does not always occur when neoplastic changes are present. Moreover, the histological analysis of human samples further indicates the inflammation co-occurrence with the tumor depends on various factors such as grade of the tumor. Immunofluorescence experiments in mice models showed that despite the abundance of lymphocytes in the tissue, Th17 and Tregs remain scarce in the tissue. Ongoing spatial transcriptomics data analysis will provide more detailed information on the gene expression on mRNA level in CD4+ lymphocytes populations in the studied tissue.

LABORATORY OF EXPERIMENTAL AND TRANSLATIONAL IMMUNOLOGY

TITLE: The interdependence between exposure to an antigen and tolerance-inducing factor in time affects T cells subset characteristics and phenotype

SPEAKER: ***Kinga Panek***

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

Background: We have previously shown that the time of provision of antigen and tolerizing stimulus affects tolerogenic dendritic cells' (tolDCs) phenotype and impacts the establishment of antigen-specific responses. Here we aimed to test if the time of the DCs' exposure to an antigen and tolerance-inducing factor alters T cell line characteristics.

Methods: DCs were differentiated from monocytes by IL-4/GM-CSF and treated with a tolerizing factor (IL-10) for 24h starting on different days after which the cytokine was removed. Following, all the cells were treated with the CEFT antigen pool and lipopolysaccharide on day 5. After 7 days, 6 differently generated DCs populations were included in a co-culture with autologous T cells. After 10 days of co-culture T cells were boosted with antigens and autologous monocytes. The T cell phenotype was assessed by 15-colour flow cytometry on a day 0, 1 and 11 of the co-culture.

Results: The timing of the IL-10 provision to DCs in relation to exposure to the antigen, results in profound phenotypic differences in subsequently co-cultured T cells. The effect was observed in the central memory, naïve, effector memory and effector subsets of T helper and T cytotoxic cells and is especially profound for the activation markers CD137, CD154 and PD-1.

Conclusions: The interdependence between exposure of DCs to an antigen and tolerance-inducing factor in time contributes to the overall phenotype of the DC-interacting T cells. This finding could be important for the development of improved therapeutic approaches in the fields of autoimmune diseases, allergies and graft acceptance.

LABORATORY OF EXPERIMENTAL AND TRANSLATIONAL IMMUNOLOGY

TITLE: Proteomics characterization of immune responses in inflammation and cancer

SPEAKER: ***Victor Urbiola Salvador***

SUPERVISOR(S): ***Zhi Chen****, PhD;*

Inflammation is the most relevant contributor to several diseases including cancer. Chronic inflammatory diseases are considered the most significant cause of death and their prevalence is increasing. Also, colorectal cancer (CRC) is the third most common malignancy and the second most lethal cancer worldwide. Noteworthy, cancer-associated inflammation is a key modulator of CRC development and progression. Apart from that, the presence of abovementioned diseases in SARS-CoV-2 infected patients as comorbidities are associated with higher severity and COVID-19 death. Importantly, an imbalance between regulatory and inflammatory CD4+ T cells populations plays an essential role in these diseases. Therefore, deep understanding of pathogenic molecular mechanisms is urgently needed.

Proteomics-based approaches allow for robust protein expression profiling and can revolutionize molecular biology and clinical research. This project aims to identify and characterize helper T cells in CRC tumors as well as plasma protein changes of CRC patients and SARS-CoV-2 infected patients with/without comorbidities by proteomics approaches. two orthogonal proteomics technologies, Proximity Extension Assay (PEA) and tandem mass spectrometry coupled with liquid chromatography (LC-MS/MS), unveiled novel protein plasma changes associated with CRC development. Importantly, these plasma protein changes were linked to cytokine-cytokine receptor interactions, the complement cascade, cholesterol metabolism and signaling pathways (MAPK, Ras, NF-κB, and IL17). Moreover, we unveiled protein changes in CRC patients with cancer-associated inflammation that were related to IL17 and Th17 cell differentiation and complement among others. Noteworthy, two protein signatures were discovered that could distinguish between early and late stages of CRC and are promising biomarkers for CRC prognosis. Importantly, 6 potential biomarkers were validated in an additional CRC cohort. Additionally, plasma protein changes associated with SARS-CoV-2 infection in patients with and without comorbidities, including key immune signatures have been identified, . Moreover, protein changes linked to the generation of SARS-CoV-2-specific antibodies, long-term effects and potential association with post-COVID-19 condition were revealed. For LC-MS/MS analysis of macrodissected regions of interests from CRC and normal-matched tissue, the preliminary results with 2876 identified proteins and 208 DEPs. Further tasks include the optimization of combination of macrodissection of cancer and normal tissue with CD4 IHC staining to characterize T-cells and the application of this protocol in a CRC patient cohort as well as the downstream data analysis and integration.

The results generated from this study will facilitate biomarkers discovery as well as identification of novel regulators of T-cell-driven immune responses which can lead to novel immunotherapies design in chronic inflammation and colorectal cancer.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: Unravelling the molecular mechanism of Escherichia coli response to antimicrobial Blue Light

SPEAKER: ***Beata Kruszewska-Naczk***

SUPERVISOR(S): ***Mariusz Grinholc*** *PhD, DSc;* ***Aleksandra Rapacka-Zdończyk****, PhD;*

Antibiotic resistance is currently one of the most pressing issues of modern society. Antibiotics become ineffective and others are not discovered. Therefore, novel therapies to combat multidrug-resistant pathogens are being intensively studied. One of the most promising strategies is antimicrobial blue light (aBL) which can eradicate antibiotic-resistant pathogens. It is believed that aBL is effective because it generates reactive oxygen species that cause oxidative stress in bacterial cells and lead to cellular death. However, the detailed mechanism of aBL action and the genetic background of this process is not well understood yet. This may also be a reason why this effective method is not widely disseminated. The current research aimed to elucidate the mechanism of aBL bactericidal action, thus facilitating the widespread adoption of this method. The investigation was performed by screening KEIO 3985 of single-gene mutants of Escherichia coli K12. Of these, 64 have been identified as aBL hypersensitive, indicating that deleted genes can play a significant role in bacterial protection against aBL. The identified genes were divided into categories based on their functions. The genes are engaged in DNA repair (including SOS response), cell envelope, stress response, ATP production and cellular transport, and some others.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: Elucidating role and mechanism of regulatory network of genes encoding dioxygenases in terms of plant adaptation to land conditions

SPEAKER: ***Izabela Perkowska***

SUPERVISOR(S): *Prof.* ***Ewa Łojkowska; Anna Ihnatowicz****, PhD;*

Previous studies led us to the identification of two genes encoding dioxygenases with unknown biological function. Dioxygenases are one of the largest enzyme family in plant kingdom, including Arabidopsis thaliana. They are involved in biosynthesis of secondary metabolites – compounds that allow plants to survive in unfavorable environment. Enzymes, being the subject of my PhD (named F6'H5 and F6'H6), have interesting features from an evolutionary point of view. F6'H5 and F6'H6 arose from a whole genome duplication event, have several splicing variants and their orthologs are present in all land plants, ranging from mosses to angiosperms. Moreover, in silico analysis revealed the presence of Natural Antisense Transcript (NAT) overlapping F6'H5 which may regulate its expression level, while F6'H6 probably acquired new function via neofunctionalization process. All this taken together suggest that both may play an important role in plant adaptation to land conditions.

Using reverse genetics approach, the independent T-DNA insertional mutant lines (annotated as f6'h5 and f6'h6 knock-outs) in A. thaliana Col-0 (wild-type WT) genetic background were ordered. However, RT-PCR followed by qPCR analysis revealed that lines f6'h5-1 and f6'h5-2 had elevated F6'H5 expression level. Thus, to obtain line with non-functional F6'H5 gene the CRISPR-Cas9 genome editing approach was applied. In silico analysis suggested that osmotic, oxidative and cold stresses have the highest impact on F6'H5 and F6'H6 expression levels. Plants, WT and mutants, were grown in different types of cultures: soil, hydroponics and in vitro to perform phenotypic characterization including biochemical analysis (chlorophylls, carotenoids, anthocyanins, phenolic compounds, antioxidants), survival rate, fresh weight, root length, relative water content and electrolyte leakage determination.

The most interesting results were obtained under osmotic stress, regardless of type of culture. The f6'h5 ko mutant was less tolerant to osmotic stress (drought), while lines overexpressing F6'H5 had greater fresh weight and higher water content under drought stress, when compared to WT plants. In hydroponics, where osmotic stress was induced by 3% polyethylene glycol (PEG), F6'H5 overexpressing plants produced significantly more anthocyanins in comparison to WT. For other tested phenotypic traits, statistically significant differences were also observed in the case of mutant lines grown under oxidative and cold stresses.

Targeted and untargeted metabolomic analysis of plant extracts from roots, leaves and media after in vitro cultures are in progress. Additionally, in vitro enzymatic activity assays with potential substrates for F6'H5 and F6'H6 derived from A. thaliana and their orthologs from tomato and tobacco will be performed. Transient expression in Nicotiana benthamiana will enable us to determine F6'H5 and F6'H6 function in vivo. It is also planned to perform transcriptome profiling (RNA-Seq) of selected lines grown under osmotic stress, which will allow for a better understanding of the transcription regulation mechanisms of tested genes.

All these findings taken together suggest the importance of metabolites synthetized by F6'H5 and F6'H6 in plant stress response. Discovering their biological function may contribute to a better understanding of the plant defense mechanisms against unfavorable environment, and in the future will enable developing an effective method of protecting economically important crops.

LABORATORY OF RECOMBINANT VACCINES

TITLE: Multi-Component PPMV-1 VLPs as a Promising Platform for Targeted Cancer Immunotherapy

SPEAKER: ***Aurelia Schweda***

SUPERVISOR(S): *Prof.* ***Boguslaw Szewczyk****;* ***Łukasz Rąbalski****, PhD;*

Virus-like particles (VLPs) are non-infectious protein complexes that mimic the structure of native viruses and have shown great potential as a platform for developing anti-cancer vaccines. So far, several advantages have been demonstrated in the use of NDV VLPs, including antigen specificity, immune system activation, efficient production, stability, safety, and the ability to be used in the treatment of various types of tumors. Pigeon paramyxovirus type 1 (PPMV-1), belonging to the Paramyxoviridae family, similar to its antigenic variant, Newcastle disease virus, generates VLPs that can be further modified to express tumor antigens on their surface. This study focused on the initial and crucial stage of confirming the production of PPMV-1 based VLPs, consisting of four native viral proteins. In subsequent stages, the aim is to modify the resulting PPMV-VLPs to enable the presentation of two tumor antigens, including the epidermal growth factor receptor (HER2) or a mimetic peptide to ganglioside 2 (mGD2). We expect that the developed platform will be more effective compared to the currently used anti-cancer immunotherapies employed in the treatment of patients with HER2-positive and GD2-positive tumors.

The production of PPMV-VLPs was performed in Sf9 insect cell line using the baculovirus expression system (Bac-to-Bac). For this purpose, vectors containing two genes encoding the structural proteins of PPMV-1 were constructed, allowing for their simultaneous expression. Genes encoding the M or NP proteins were cloned under the p10 promoter, while genes encoding the F or HN glycoproteins were cloned under the polyhedrin promoter. The obtained recombinant baculoviruses were used to infect Sf9 cells. After 120 hours of production, the released PPMV-VLPs were purified and concentrated using ultracentrifugation in a sucrose gradient. After identification of some of the proteins that are components of PPMV-VLPs by Western Blot, their observation was performed. The purified PPMV-VLPs exhibited circular structures resembling native viruses, with visible spikes made of glycoproteins on their surface, as observed through transmission electron microscopy (TEM).

In conclusion, the results demonstrated the successful production of PPMV-VLPs composed of four structural viral proteins in the Sf9 insect cell line using the baculovirus expression system. The ability to modify PPMV-VLPs to express tumor antigens opens up possibilities for targeted cancer immunotherapy. The study identified two specific tumor antigens, HER2 and mGD2, which can be presented on the surface of modified PPMV-VLPs. These modifications have the potential to enhance the immune response and generate a long-lasting protective effect against HER2-positive and GD2-positive tumors.

LABORATORY OF RECOMBINANT VACCINES

TITLE: A novel purification method of virus-like particles based on sHBsAg

SPEAKER: ***Karolina Gackowska***

SUPERVISOR(S): *Prof.* ***Boguslaw Szewczyk****;*

Vaccines based on virus-like particles (VLPs) has been known and used for a long time – e.g., against human papilloma virus, hepatitis B virus or influenza A viruses. Many protocols and methods for their production have been developed but considering the expression system and type of expressed VLP, there is no universal protocol and sometimes to obtain pure VLPs, many laborious and time-consuming steps are needed. Purification of particles based on small surface antigen of HBV (sHBsAg) requires many intermediate steps in order to obtain pure product. Addition of a tag on one of the terminus could speed-up the purification process and reduce number of steps and also improve total yield of VLPs. Coding sequence for sHBsAg sequence was fused with a Twin-Strep-tag, used for construction of baculovirus expression vector in insect cells and then the protein purified on a StrepTactin column. The method was compared with two other methods: ultracentrifugation and PEG precipitation followed by ion-exchange chromatography.

LABORATORY OF RECOMBINANT VACCINES

TITLE: Development of a Potential Vaccine Against Tick-Borne Encephalitis Virus Based on Synthetic Nucleic Acids

SPEAKER: ***Klaudia Barańska***

SUPERVISOR(S): *Prof.* ***Boguslaw Szewczyk****;*

The tick-borne encephalitis virus (TBEV) was first isolated in 1937 in the Soviet Union. So far, its presence has been demonstrated in as many as 28 countries in Central and Eastern Europe, Scandinavia and selected regions of Asia. Every year, new outbreaks of infections are recorded in many countries, including in Finland, Denmark, Norway, Austria, Germany and Poland. The frequency of TBEV infections has increased more than 4-fold in the last 20 years, making tick-borne encephalitis the second, after Lyme disease, a disease most often transmitted by ticks. This may be related to the prolonged period of tick activity and their increasing number as a result of climate change in the areas of their occurrence. Despite the availability of 2 vaccine variants against this pathogen on the European market, there is still a great need to develop a safer and more immunogenic version for both humans and animals. In the case of TBEV, available preparations contain inactivated viruses, which may, for example, pose a risk of incomplete inactivation. Available vaccines require administration of several doses, the total cost of which is very high, therefore vaccination against TBEV is not mandatory, but only recommended for people at risk. Current literature reports point to mRNA vaccines as a universal solution to the problem of the spread of viral diseases. Since this approach has not been tested for tick-borne encephalitis virus so far, in this application we intend to test and compare the immunogenicity induced by TBEV mRNA encoding the E and NS1 protein genes in combination with or without virus like particles of TBEV. Ultimately, as a result of the experiment, we plan to obtain data that will contribute to the development of an effective and safe vaccine based on synthetic TBEV messenger RNA.

LABORATORY OF STRUCTURAL BIOLOGY

TITLE: Mechanistic basis for nuclease activity of EXOG, critical for human mtDNA repair

SPEAKER: ***Michal Majewski***

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

Mitochondria are the organelles in which the process of oxidative phosphorylation (OXPHOS) takes place. OXPHOS process leads to an increased concentration of reactive oxygen species (ROS) present in these organelles. This phenomenon results in a high incidence of mitochondrial DNA (mtDNA) oxidative damage. mtDNA repair system is very efficient. It is thought that the main mechanism of mtDNA repair is Base Excision Repair (BER). One of the proteins involved in this process is EXOG, a nuclease that has both endonuclease and 5'-exonuclease activity and is localized on the inner membrane of mitochondria. Cellular depletion of EXOG results in persistent single strand breaks in mtDNA, mitochondrial dysfunction and programmed cell death. What is more, EXOG is found in a complex with enzymes involved in mtDNA repair such as: apurinic/apyrimidinic endonuclease 1 (APE1), Polymerase gamma and Ligase III. During the BER process oxidized abasic site (AP) is cleaved by APE1, resulting in a 5'-end nick that is capped by a deoxyribosephosphate (dRP) or its oxidized form 2-deoxyribonolactone (dL) moiety. Both dRP and dL are resistant to the lyase activity of Polymerase gamma, while EXOG has an ability to process and remove dinucleotides from the 5'-end of DNA, including dRP and dL moiety, thus creating a substrate for Polymerase gamma to fill the gap what allows for the next steps of the repair process to occur. Structure of the EXOG. protein is needed for further in silico analysis which would allow for establishing of the binding mode of the protein with different substrates mimicking the ones present during mtDNA repair processes and further elucidate the role of this enzyme in this process. Additionally it would allow for molecular dynamics analysis in order to find the interface of interaction with other proteins of repairosome. The structure of the protein cannot be obtained with any other method than by usage of synchrotron radiation.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: In silico proteome-wide analysis of viral sequences reveals a potential for novel chaperone-mediated xenophagy pathway

SPEAKER: ***Marcin Lubocki***

SUPERVISOR(S): *Prof.* ***Krystyna Bieńkowska-Szewczyk****;* ***Andrea Lipińska****, PhD;*

The crosstalk between viruses and host autophagy is being better understood and described as clinically important. Chaperone-mediated autophagy (CMA) is a conserved mammalian cellular process that selectively degrades specific proteins in lysosomes. It only selects proteins with a degradative KFERQ motif tag in their sequence, which is bound by Hsc70 and its co-chaperones. It has been demonstrated that CMA plays a crucial part in the infection of several viruses, like HCV or Zika Virus. Although it has been noted that viruses can use CMA to cause the degradation of host proteins, no research has yet been published demonstrating that CMA can also target viral proteins.

As intracellular pathogens undergo cellular processes of their hosts, we have analyzed proteomes of human viruses to investigate if they contain KFERQ motifs and can be targets of this pathway. We developed the KFERQminer Database to explore already described KFERQ-related motifs and support true positive KFERQ findings in protein sequences. With our tool, we screened viral proteomes from several families. Our analysis of viral proteomes revealed a high prevalence of KFERQ motifs in many viral proteins. Gene ontology enrichment indicates the greatest enrichment of motifs in structural proteins and polymerases. Among the analyzed viruses, the SARS-CoV-2 proteome has the most KFERQ motifs. The analysis of genomes from the GISAID database showed that they are conserved among variants of concern. Analyzes of the structures of individual proteins indicate that a significant number of motifs may be available for recognition by chaperones.

Our observations suggest the legitimacy to extend the search for KFERQ motifs for intracellular pathogens of CMA-competent organisms, which by their nature are subjected to the cellular processes of their hosts. This suggests that CMA may play an important role in regulating the degradation of viral proteins during infection, which may have wide clinical implications.

DEPARTMENT OF HISTOLOGY, MEDICAL UNIVERSITY OF GDANSK

TITLE: PDIA3 modulates genomic response to 1,25(OH)2D3 in squamous cell carcinoma of the skin

SPEAKER: ***Joanna I. Nowak***

SUPERVISOR(S): *Prof.* ***Michał Żmijewski****;*

Protein Disulfide Isomerase Family A Member 3 (PDIA3) belongs to oxidoreductase enzyme family. PDIA3 is involved in multiple processes of protein folding, disulfide bond formation, and remodeling. It is mainly localized in the endoplasmic reticulum but may also be found in different locations including the nucleus or cell membrane. PDIA3 was also identified as an alternative membrane-associated receptor for 1,25(OH)2D3. Knockdown or overexpression of PDIA3 has an impact on broad range of physiological processes such as apoptosis, proliferation, and motility. Vitamin D directly or indirectly modulates the expression of around 3000 genes in the human genome including genes involved in cell differentiation, proliferation, and migration. Genomic activity of 1,25(OH)2D3 is mediated thought its nuclear receptor VDR, but numerous studies also describe the rapid, non-genomic activities, which involves PDIA3 protein as a membrane receptor for 1,25(OH)2D3. The aim of the study was to investigate effects of 1,25(OH)2D3 on functioning of the cell and gene expression in squamous cell carcinoma (SCC) cells line A431, in presence or absence of PDIA3 protein.

PDIA3 knockout led to changes in the expression of more than 2000 genes and modulated proliferation, cell cycle and mobility of cells, suggesting an important regulatory role of PDIA3. 1,25(OH)2D3 treatment altered genes expression profile of A431ΔPDIA3 in comparison to A431WT cells, indicating the existence of PDIA3-dependent genomic effects. Additionally, response to 1,25(OH)2D3 in cancerous A431 cells differed from immortalized HaCaT keratinocytes, used as non-cancerous control. Silencing of PDIA3 and 1,25(OH)2D3, at least partially reverse expression of cancer-related genes in A431 cells.