12 BIOTECHNOLOGY STUDENTS' CONFERENCE

BOOK OF ABSTRACTS

VERSION **BEFORE** EDITORIAL REVISION

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LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: THE STUDY OF THE INTERACTION BETWEEN *PECTOBACTERIUM BETAVASCULORUM* AND PLANTS

SPEAKER: MARIA BOROWSKA-BESZTA

SUPERVISOR(S): MAŁGORZATA WALERON, PHD, DSC

The genus *Pectobacterium* includes various species that cause soft bacterial rot, blackleg, and wilt as well as contribute to significant losses in many economically important crops. One of them *P. betavasculorum* is a sugar beet pathogen causing vascular necrosis, resulting in high losses in sugar production. Despite the seriousness of the damage caused by P. *betavasculorum*, very little is known about this species. Our research on this bacterium has shown that it chelates iron ions, produces auxins – plant hormones, and modifies A. thaliana roots during direct interaction. One of the reasons behind the modification may be a deficiency of nutrients, including phosphorus and iron. In addition, the latest studies showed that Extracellular Membrane Vesicles (EMVs) produced by bacteria can play an essential role in pathogenicity and during interaction with host plants. EMVs might also modulate plant immunity.

This work aimed to study the interaction between *P. betavasculorum* and monocotyledonous and dicotyledonous plants. To analyse whether the observed roots modifications are influenced by auxin synthesized by bacteria or by the iron deficiency caused by the competition, three A. thaliana lines with knock-down mutations in genes associated with auxin and Fe metabolism were used. It was also checked whether the direct interaction between plants and *P. betavasculorum* is mediated by membrane vesicles. The production of EMV by monocultures of plants and bacteria, and during their direct interaction, was investigated using transmission electron microscopy and proteomic analysis. For the production of EMVs, plants inoculated with bacteria were grown in a liquid MS medium.

The results showed that among *Pectobacterium* species, only *P. betavasculorum* is able to modify the architecture of roots of plants from different species. However, the modification manifests itself differently in dicotyledons and monocotyledons. No differences in roots modifications between wild types of A. thaliana and auxin and Fe mutants were observed. Thus, we can conclude that *P. betavasculorum* root modification on a solid medium does not depend on iron competition.

Moreover, TEM analysis has shown that EMVs are produced by both plants and bacteria. It should be noted that the inoculation of *A. thaliana* with bacteria resulted in an increase in the production of EMVs. Proteomic analysis of EMVs content showed 126 common proteins for monoculture of *A. thaliana* and *A. thaliana* inoculated with *P. betavasculorum*. What's more, 35 unique proteins were identified for the control group of non-inoculated plants only, and 394 proteins were identified in EMVs isolated from Arabidopsis plants inoculated with bacteria. Among the identified proteins, the most abundant were those related to the plant stress response as well as various transporters, while in the case of bacterial MVs, various virulence factors were detected.

The obtained results indicate that EMVs participate in the interaction between plants and bacteria and serve as virulence vesicles as their cargo consists of proteins that play a role in virulence and nutrient acquisition.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: PLASMA-INDUCED ERADICATION OF ANIMAL PATHOGENS FROM A PORCINE SKIN MODEL

SPEAKER: PRUSIŃSKI MICHAŁ

SUPERVISOR(S): WOJCIECH ŚLEDŹ, PHD

Animal skin pathogens are responsible for over 30 billion US$ losses annually. *Staphylococcus* spp. are leading causes of mastitis in milk animals, while *Dermatophilus congolensis* and Candida spp. are responsible for economic loss to leather and wool industries along with disqualifying animals like horses from races and exhibitions. There is a huge need for novel, effective methods to eradicate these multidrug-resistant microorganisms.

Thus, I aimed to evaluate the applicability of non-thermal Diaelectric Barrier Discharge (DBD) helium type plasma for eradication of pathogens responsible for skin infections in animals. Sterile porcine skin fragments were inoculated with 20 µl of 0.5 McF microbial suspension. After 24h incubation at 36°C the plasma jet was applied. Viable microbes were collected with a sterile swab and serially diluted to 10-8. The dilutions were streaked horizontally on BHI or YPG media and incubated for 24-96h at 36°C prior to counting the resultant colony-forming-units (CFUs).

Application of the DBD plasma led to total eradication of *D. congolensis* ATCC 14637, and significant reduction in CFUs of S. aureus ATCC 25923, S. epidermidis ATCC 14990, and C. albicans ATCC 90028 cells due to plasma-assisted generation of reactive oxygen and nitrogen species as well as UV radiation.

This method shows great potential as a non-chemical treatment, which is very important in light of ever-increasing pollution and antibiotic resistances. I expect implementation of DBD-based plasma treatments in animal husbandry and veterinary sectors in the future.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: ANALYSIS OF THE POTENTIAL ROLE OF 2-OXOGLUTARATE-DEPENDENT DIOXYGENASES IN PLANT RESPONSES TO ABIOTIC STRESSES

SPEAKER: JANUCHOWSKA ALICJA

SUPERVISOR(S): ANNA IHNATOWICZ, PHD

In their natural environment, plants are subjected to a number of stress factors, therefore they have evolved strategies to combat them. Among them is the production of heat shock proteins, phytohormones etc. However, another key element in the protection of plants against stress factors that is currently being studied by many research groups is the production of secondary metabolites.

Secondary metabolites, such as alkaloids, flavonoids and coumarins have been utilized by humans for centuries due to their medicinal properties, such as antimicrobial, antioxidative or even anticancer. These are produced by medicinal plants, and conveniently, by a model plant *Arabidopsis thaliana*. In this study, we focused on 2-oxoglutarate-dependent dioxygenases (2OGD), which encode enzymes potentially involved in the coumarins and flavonoids metabolic pathways. The selected 2OGD enzymes are possibly a part of phenylpropanoid pathway, a crucial route of coumarin production. The recently discovered enzymes that belong to the same family and are involved in coumarin biosynthesis are F6'H1 and 2 (feruloyl-CoA 6'-hydroxylase 1 and 2) and S8H (scopoletin 8-hydroxylase). Our laboratory team selected two homologs for these genes and named corresponding enzymes F6'H5 and F6'H6. The aim of my study was to characterize Arabidopsis f6'h5 and f6'h6 mutants grown in selected stress conditions in order to reveal the biological function of F6'H5 and F6'H6 genes in plant tolerance to abiotic factors.

During my studies, I performed in silico analysis that led to (1) identification of proteins potentially interacting with F6'H5 and F6'H6, (2) and selection of a number of stressful environmental factors which might upregulate the corresponding transcripts. Then I cultivated the selected Arabidopsis knock-out mutant lines with T-DNA insertions within the F6'H6 coding sequence or the antisense sequence to F6'H5 gene, in a variety of stress conditions (in soil and hydroponics cultures). Detailed phenotypic characterization was carried out, including measurements of changes in morphological features and performing biochemical analyzes, such as the assessment of the content of chlorophyll and anthocyanins in leaves. Moreover, I performed genotyping analysis and quantified the expression levels of selected genes in plants cultivated in control and drought stress conditions.

The above results indicate that the F6'H5 gene is an important factor in the response of plants to drought stress, which confirms the results of our previous in vitro studies.

LABORATORY OF PLANT PROTECTION AND BIOTECHNOLOGY

TITLE: EFFECT OF ISOLIQUIRITIGENIN ON THE ACTIVITY OF TAMOXIFEN IN BREAST CANCER CELLS

SPEAKER: SPISACKA KLAUDIA

SUPERVISOR(S): ANNA KAWIAK, PHD

Breast cancer is the most commonly diagnosed type of cancer. Two of its most common subtypes, luminal A and luminal B express the estrogen receptor (ER) and thus respond to tamoxifen (TAM) treatment. However, tamoxifen resistance is a common occurrence, associated with the activation of many different cellular mechanisms. This study focuses on the MAPK/ERK signaling pathway, and more specifically, on the ERK protein, which is a common component in ER non-genomic and ligand-independent signaling. Its activity inhibition may help to overcome tamoxifen resistance. Isoliquiritigenin (ISL), a chalcone compound isolated from the licorice (Glycyrrhiza glabra) root extract, has a proven inhibitory effect on the MAPK/ERK signaling pathway.

The aim of this study was to determine the effects of ISL on tamoxifen-resistant ER+ breast cancer cells. The effects of ISL on ER+ breast cancer cells were examined. The influence of ERK protein overexpression on resistance to tamoxifen was established and the sensitivity of ISL-treated cells to tamoxifen was determined. Two breast cancer cell lines were used for the study, MCF-7 and T-47D. To examine the effects of ISL on both cell lines MTT assay and flow cytometry were used. Results from these experiments show that ISL reduces viability and induces apoptosis in ER+ cells. Based on the results of the MTT assay with a concentration gradient of TAM, the optimal incubation time and concentration of TAM for further testing were established. To acquire cells overexpressing ERK, first the CRISPR ERK Activation Plasmid Transfection was used, and then with Alpha Screen SureFire ERK ½ Assay, the levels of ERK were measured. Finally, with cell viability tests, it was established that cells with ERK overexpression are less sensitive to TAM, however, ISL sensitizes cells in both the control group and the ERK-overexpressing group to TAM. In conclusion, ISL increases the activity of TAM in ER+ breast cancer cells, indicating its potential in the resensitization of ER+ breast cancer cells to TAM.

CORE FACILITY LABORATORIES

TITLE: PROTEOMIC ANALYSIS OF BIOLOGICALLY ACTIVE FRACTION FROM COELOMIC FLUID, ISOLATION OF PROTEIN COMPONENTS

SPEAKER: SZPIECH AGATA

SUPERVISOR(S): PAULINA CZAPLEWSKA, PHD, DSC

Coelomic fluid fills up body cavity of earthworms and contains lots of biologically active proteins and peptides that may serve as anticancer, antifungal or antibacterial agents. One of the protein component of the coelomic fluid is lysenin that has an ability to specifically recognize and bind to sphingomyelin. This study is conducted in cooperation with scientists from Lublin who developed a protocol for preparation of coelomic fluid from Dendrobaena veneta, which include heating of coelomic fluid for 10 min in 70°C. Processed coelomic fluid has an activity against Candida albicans, non-small cell lung carcinoma and colon adenocarcinoma, but is not toxic for nomal human cells. During studies processed and unprocessed coelomic fluid was characterized using nano differential scanning fluorimetry technique and its interaction with lipids was examined with surface plasmon resonance. Additional haemolytic properties of coelomic fluid were tested and proteomic analysis using mass spectrometer was performed. Results show that there are differences between processed and unprocessed coelomic fluid proteins conformation and its interaction with lipids. Processed coelomic fluid doesn't induce hemolysis while, unprocessed does. During studies proteins from coelomic fluid were identified and lysenins sequences from Dendrobaena veneta were determined.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: SEARCH FOR PHAGE-ENCODED LYSIS GENES WITHIN CLINICAL ISOLATES OF CLOSTRIDIOIDES DIFFICILE

SPEAKER: AGACIŃSKA MARTA

SUPERVISOR(S): ADAM IWANICKI, PHD

Clostridioides difficile is becoming an increasing both health and economic burden. Clostridium difficile infection (CDI) is a toxin-mediated disease with symptoms ranging from mild to severe diarrhea, which can develop into life-threatening pseudomembranous colitis or toxic megacolon. Increasing antibiotic resistance, the ability to sporulate, and toxin production make C. difficile the leading cause of nosocomial infections. Although treatment of CDI is available, limitations such as antibiotic resistance, the possibility of pathogens and toxin gene transmissions, and the high possibility of reinfection make treatment insufficient. All abovementioned make us seek alternative treatment methods. One of the most promising therapy is using of bacteriophage-derived endolysins. Endolysins are proteins expressed at the end of the phage lytic cycle, and their function is phage progeny release made via degradation of the bacterial cell wall. Multiple studies have shown that the external application of endolysins is an effective way of destroying bacterium. The main advantages of using endolysins are high specificity, activity against multidrug-resistant strains, extremely low possibility of resistance developing, and no impact on commensal microbiota. Additionally, with new methods of protein engineering emerging, soon we should be able to develop endolysins of desired properties. To make it happen we need more endolysin sequences in databases. Endolysin is a rather evolutionary conserved protein, but changes in amino acid sequence can alter their biological activity, which may result in finding a protein with better lytic properties.

The aim of this work was to search for prophage-encoded endolysin genes within different clinical isolates of Clostridioides difficile. During the course of this work, 3 previously unknown endolysin (from strains 21, 34/19, 226) genes were found, amplified, cloned, and sequenced. Sequencing results revealed that from the 4 strains tested, sequences of endolysin were exactly the same for strains 34śr and 21, and almost the same for strains 34/19 and 226, with one proline to serine substitution. The changes in activity between endolysin from strain 34śr and 34/19 remain to be tested.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: CONSTRUCTION OF EXPRESSION VECTOR FOR THE PRODUCTION OF PHICDKH01 BACTERIOPHAGE ENDOLYSIN

SPEAKER: KUBACKA AGATA

SUPERVISOR(S): ADAM IWANICKI, PHD

CDAD (Clostridium Difficile Associated Disease) is an infection of the colon caused by the bacteria Clostridium difficile. It occurs in patients whose normal bowel flora have been disrupted, commonly by recent antibiotic use. Clostridium difficile is a leading cause of healthcare-associated infections and an important public health threat. Current treatments include antibiotic therapy, which eradicates healthy gut microbiota as well - leading to reoccurring infections. Endolysins are bacteriophage-derived proteins that could offer an effective solution. As enzymes, they have the ability to hydrolyse bacteria cell walls. The aim of this project was to construct an expression vector for the production of phiCDKH01 bacteriophage endolysin and to study its biological activity on different bacteria strains.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: CONSTRUCTION OF EXPRESSION VECTOR FOR THE PRODUCTION OF PHICDKH01 BACTERIOPHAGE ENDOLYSIN

SPEAKER: ARENDT MAGDALENA

SUPERVISOR(S): KRZYSZTOF HINC, PHD

Bacteriophages, or simply phages, are viruses infecting bacteria. They outnumber bacteria in biosphere by a factor of at least 10 and they influence the evolution of most bacterial species. "Temperate" phages have the ability to integrate into the chromosome of their host upon infection, where they can reside "dormant" as prophages until conditions favor their reactivation and cause viral replication and cell destruction. Phages can encode powerful toxins that can be transferred to bacteria through infection. Genes encoding these toxins can be expressed and boost virulence of some bacterial pathogens. Clostridioides difficile is gram-positive, anaerobic bacteria that produces toxins. It is human pathogen that causes gastrointestinal infections. Under stress conditions, the bacteria produce spores which are not susceptible to antibiotics and therefore C. difficile infections are often recurrent. Prophage has been found in genome of the clinical strain of C. difficile. The bioinformatic analysis revealed that it contains over 50 open reading frames, two of which encode toxins from the ADP-ribosyltransferase family. ADP-ribosyltransferase toxins consists of two domains that are expressed from two separate genes. They cause inhibition of actin polymerization and destruction of the actin cytoskeleton in human intestinal epithelial cells. The aim of my project is to produce toxin proteins encoded by the prophage, check their biological activity and study the expression of their genes in C. difficile. If the genes of the prophage are expressed and the toxins are active, the presence of the prophage in the genome of the bacterium may boost its virulence. Knowing this, we will be more aware of which strains of C. difficile are more dangerous to humans.

LABORATORY OF MOLECULAR BACTERIOLOGY

TITLE: CONSTRUCTION OF RECOMBINANT SPORES CAPABLE OF BINDING ZINC IONS

SPEAKER: KALINOWSKA KATARZYNA

SUPERVISOR(S): ALESSANDRO NEGRI, PHD

Due to the various industrial operations heavy metals, such as zinc, nickel, copper, cobalt or iron, are released and accumulated in natural ecosystems. In higher concentrations heavy metals may have toxic effects on plants and animals. There are several chemical methods that can be used to treat heavy metal polluted ecosystems. However, all of them have major drawbacks, which led to the development of biological purification methods. One of them is the use of engineered microorganisms displaying heterologous proteins as biosorbents. Endospores, also known as spores, are dormant and non-reproductive entities of some bacterial species that are formed in presence of unfavorable environmental conditions. Spores have remarkable resistance properties, which include resistance to extreme heat, lack of nutrients, ultraviolet radiation and exposition to various chemicals. One of the bacterial species able to produce spores is Bacillus subtilis, a rod-shaped bacterium, found in soil and in gastrointestinal tracts of humans and ruminants. This species is classified as generally recognized as safe (GRAS) by The United States Food and Drug Administration (FDA), which gives it a potential to be used in, for example, food or medical industries. In this project selected B. subtilis spore coat proteins have been engineered to express heterologous zinc-binding motifs on the surface of the spores, so they could serve as a potential bioremediation tool for contaminated ecosystems.

LABORATORY OF TRANSLATIONAL ONCOLOGY

TITLE: IDENTIFICATION AND CLINICAL RELEVANCE OF ANDROGEN RECEPTOR AND EPIDERMAL GROWTH FACTOR RECEPTOR EVALUATED IN CIRCULATING TUMOR CELLS OF BREAST CANCER PATIENTS

SPEAKER: ŚCIŃSKA WIKTORIA

SUPERVISOR(S): PROF. ANNA ŻACZEK, NATALIA BEDNARZ-KNOLL, PHD

Analysis of circulating tumor cells (CTCs), as an example of liquid biopsy, has been shown to be a valuable, non-invasive method of prognostic evaluation in breast cancer. Unfortunately, biomarkers that have been extensively analysed to date, don't always give satisfactory results when it comes to precision medicine and targeted therapy as breast cancer is a very complex and phenotypically heterogenous disease. There is a rapidly growing body of evidence demonstrating that androgen receptor (AR) and epidermal growth factor receptor (EGFR) may be clinically relevant biomarkers of breast cancer prognosis, especially in the triple negative subtype (TNBC). Given these already available implications, current study aimed to investigate presence of these biomarkers in CTCs isolated from patients with breast cancer of different molecular subtypes using a novel method – imaging flow cytometry which combines multiparametric flow cytometry and fluorescence microscopy allowing for more precise assessment than in the basic method.

CTCs were isolated from peripheral blood (7,5ml, EDTA tubes) from 70 breast cancer patients (including 39 luminal, 4 HER2+ and 27 TNBC). Samples were stained for AR, EGFR, keratins (K), CD45 and CD31 using an immunocytochemistry method. CTCs were detected in 16 (23%) of the 70 samples out which 7 were characterized by the luminal subtype and 9 were characterized by TNBC subtype. In total, 18% of luminal BC and 33% of TNBC patients were positive for CTCs of different phenotypes: EGFR+/AR- (n=1), EGFR-/AR+ (n=59) and EGFR+/AR+ (n=10), both in K+ and K- status. When compared to clinical data, there was a significant correlation between the luminal subtype and absence of metastasis in the CTC- subgroup of patients. Interestingly, in terms of therapy response, the patient with EGFR+AR- CTC showed progressive disease and lymph node involvement.

This study shows that breast cancer CTCs can be detected and phenotyped using the novel imaging flow cytometry method and have the potential to become markers of aggressive breast cancer. Clinical relevance of this observation needs to be evaluated in a large cohort study.

LABORATORY OF TRANSLATIONAL ONCOLOGY

TITLE: IMMUNOTHERAPY OF ROS1 REARRANGEMENT POSITIVE NON-SMALL CELL LUNG CANCERS

SPEAKER: MELCHERT MATEUSZ

SUPERVISOR(S): PROF. ANNA ŻACZEK

Introduction

Lung cancer is the leading cause of cancer-related death worldwide. Non-small cell lung cancer (NSCLC) constitutes the majority of lung cancer, while ROS1 rearrangement positive cancer accounts for 1-2% of NSCLC. ROS1 positive lung cancers can be treated with ROS1 inhibitors (e.g. crizotinib), but there is evidence of quickly acquired resistance to these drugs. Literature data demonstrates that ROS1 rearrangement increases expression of PD-L1 (programmed death ligand 1) which is target of therapeutic antibody, atezolizumab. PD-L1 which can be present on the surface of tumor cell interacts with PD-1 (programmed cell death protein 1) expressed on T-cells. PD-1/PD-L1 interaction stops the anticancer effect of cytotoxic lymphocytes. Earlier study done on PD-L1 positive breast cancer cell line showed that atezolizumab (anti-PD-L1 antibody) presents anticancer effect also without presence of cytotoxic lymphocytes, but there are no data regarding ROS1 positive lung cancer in this context.

Aim of the project

Therefore, current study aimed to assess the effect of atezolizumab in NSCLC cell lines with ROS1 rearrangement. Cytotoxic effect of atezolizumab and effect on migration of treated cells were examined.

Materials and methods

Three NSCLC cell line were used: A549 (ROS1 rearrangement negative, PD-L1-negative, negative control); H1975 (ROS1 rearrangement negative, PD-L1-positive, positive control); HCC-78 (ROS1 rearrangement positive, studied cell line). Expression of ROS1 and PD-L1 was evaluated with imaging flow cytometry (Amnis® ImageStream®X Mk II, Luminex). MTT assay was used in order to determine the cytotoxic effect of atezolizumab. Downregulation of migration was assessed with wound healing assay.

Results

Flow cytometry results confirmed the expression of ROS1 and PD-L1 in chosen cell lines what is consistent with literature data. MTT assay shows statistically significant differences between H1975 and HCC-78 cells treated with atezolizumab and nontreated cells, but there is no significance between cells treated with atezolizumab and cells treated with IgG. Results from wound healing assay show that atezolizumab slows closure of the wound in H1975 and HCC-78 cell lines.

Discussion

Results from MTT assay show that cytotoxic effect of atezolizumab is not statistically significant, but this study was conducted to assess atezolizumab's influence on viability of cancer cells independently of T-cell mediated cytotoxicity. Lack of cytotoxic lymphocytes in cultures was planned in order to illustrate a situation when a tumor has not been infiltrated. Wound healing assay results show atezolizumab's potential in terms of metastasis, which is leading cause of death considering lung cancer. In regards of further studies, establishment of crizotinib-resistant HCC-78 cell line is currently underway. All of experiments shown in this study will be conducted on resistant line in order to mimic a ROS1 inhibitor resistant cancer.

LABORATORY OF TRANSLATIONAL ONCOLOGY

TITLE: SINGLE CELL SEQUENCING IN LIQUID BIOPSIES DERIVED FROM OVARIAN CANCER PATIENTS

SPEAKER: KOWALCZYK IZABELA

SUPERVISOR(S): ANNA SUPERNAT, PHD

Introduction

Ovarian cancer (OC) is fifth most lethal type of cancer among women in world as for 2020. Most of cases are diagnosed in III or IV stage. The reason for such a late diagnosis lays in nonspecific symptoms therefore there is need for new method for early diagnosis. Liquid biopsies might be a great solution for that problem. They are samples of a liquid tissue, usually blood, where, in case of neoplasm patients, various cancer biomarkers can be found, e.g., circulating tumour cells (CTCs) which are present in peripheral mononuclear cells (PBMC) layer. CTCs are cells detached from primary tumour, which kept the transcriptome characteristic for the tumour subpopulation they originate from. The detachment is effect of epithelial to mesenchymal transition (EMT). After EMT CTCs escape to bloodstream, which poses a threat of them entering different, otherwise healthy, tissue and forming metastasis. Such event worsens the prognosis for patients. Currently limited information about OC CTCs is available. If we broaden our knowledge about specific markers in OC CTCs, we might be able to detect it earlier, before metastasis occur. To achieve this goal, we propose use of single cell RNA sequencing and transcriptome analysis.

Hypothesis

We hypothesise that single cell sequencing of OC patients PBMC layer allows to find CTCs among other blood cells because of their unique transcriptome. That can lead to development of a new necessary diagnostic approach.

Materials and methods

To check the hypothesis, we utilized the following workflow:

1. Blood sample collection from high grade stage III ovarian cancer patients, who were before any treatment
2. Sample preparation in four different conditions
3. Library preparation with Chromium 10X platform
4. Quality control with TapeStation
5. Sequencing with Illumina sequencer
6. Transcriptome analysis and clustering with Seurat

Results

Quality control for all tested conditions showed that libraries had quality sufficient for sequencing. Clustering led to four UMAP graphs. First plot contained 7 clusters and 867 cells with 14580 feature reads. Second one had 4 clusters and 345 cells with 12146 feature reads. Third one had 3 clusters and 175 cells with 12395 feature reads. And the last one, 8 clusters and 1425 cells with 16473 feature reads. With this method we could detect and characterize different clusters of T-cells, B-cells, NK cells, monocytes, and platelets in samples.

Conclusions

As for now, the method gives us unclear results about efficiency of finding CTCs among other blood cells using single cell sequencing. Further analysis including more samples is required as reliability of the method depends highly on cell count in each cluster. Clustering lets us distinguish subpopulations of different cells present in peripheral blood and it shows differences in cell numbers between samples. Additionally, we can see that samples without CD45+ depletion have higher cell survivability.

LABORATORY OF TRANSLATIONAL ONCOLOGY

TITLE: EVALUATION OF SCREENCELL® DEVICE FOR ISOLATION OF CIRCULATING TUMOUR CELLS AND CANCER-ASSOCIATED FIBROBLASTS FROM BLOOD OF BREAST CANCER PATIENTS

SPEAKER: ŚLIWIŃSKA MAGDALENA

SUPERVISOR(S): ALEKSANDRA MARKIEWICZ, PHD

Despite recent years' development in diagnostics and treatment, breast cancer (BC) remains one of the most common cancer in women globally, with second-highest mortality of all cancers. The presence of circulating tumour cells (CTCs) in the blood is crucial for bloodborne cancer spread and the development of metastases. What is more, the occurrence of other types of cells in the blood, such as cancer-associated fibroblasts (CAFs), may enhance the metastatic potential of CTCs. Enumeration of CTCs is validated as a prognostic marker in metastatic breast cancer and higher numbers of CTCs correlate with poor prognosis. Additional information may be obtained by further transcriptomic analysis of CTCs and CAFs about tumour heterogeneity and the biology of metastasis.

The aim of the study was to evaluate the potential of filtration-based ScreenCell® method combined with immunofluorescent (IF) staining for rapid and non-invasive isolation of breast CTCs and circulating CAFs from blood. As a model system, MCF7 cancer cells (model of CTCs) and fibroblasts p2202 (model of circulating CAFs) cells were added to blood samples of healthy donors. Two isolation approaches were tested – Direct ScreenCell® approach, where whole blood samples were subjected to CTCs/CAFs isolation followed by IF staining for cancer surface marker EpCAM or fibroblast associated protein (FAP); in the second approach - PBMC- ScreenCell®, peripheral blood mononuclear cells were first isolated by density gradient centrifugation, followed by IF staining of EpCAM or FAP, and then ScreenCell® was performed. For testing specificity of the used markers, blood samples from healthy donors (N=5) processed with Direct ScreenCell® protocol were screened for presence of EpCAM-positive or FAP-positive cells.

The mean recovery rates in the Direct ScreenCell® protocol, were 28% (SD=8.9%) for MCF7 and 14% (SD=7.8%) for CAFs; the difference did not reach statistical significance ($p=0.16$). Similarly, for PBMC-ScreenCell® protocol recovery rates were 26% (SD=9.9%) and 13% (SD=3.5%) for MCF7 and CAFs, respectively ($p=0.10$). Analysis of the blood of healthy controls showed high specificity of the CTCs and CAFs markers used for the IF staining. Direct ScreenCell® protocol allowed for faster sample processing (~1.5 h) than PBMC- ScreenCell® protocol (~3 hrs), however the number of cells remaining for screening under fluorescent microscope were many times larger in case of the Direct ScreenCell®.

The results of recovery rates were not satisfactory, especially for CAFs, possibly due to losses of cells occurring during IF staining. Further improvement in the IF staining protocol could improve the CTCs' recovery rate and allow isolation of cells suitable for transcriptomic profiling of single CTCs.

LABORATORY OF TRANSLATIONAL ONCOLOGY

TITLE: ASSESSMENT OF PROGNOSTIC VALUE OF KERATIN PHENOTYPES IN PROSTATE CANCER

SPEAKER: WENTA ROBERT

SUPERVISOR(S): NATALIA BEDNARZ-KNOLL, PHD

Assessment of prognostic value of keratin phenotypes in prostate cancer.

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Background

Prostate cancer (PCa) is the most common malignancy in men worldwide. Approx. 10% of PCa is classified as high-risk disease, however, this subgroup of patients still includes the ones with a wide range of prognoses. Therefore, there is an urgent need for prognostic markers enabling precise PCa stratification. Keratins are common proteins in tumour cells, proved to be clinically useful in diagnosis of epithelial malignancies and detection of circulating tumour cells (CTCs), which are shown to be a non-invasive, prognostic biomarker of cancer dissemination (so called liquid biopsy). However, in PCa their composition is not fully understood yet. Therefore, in this study the clinical relevance of keratin phenotypes (KP) was assessed in PCa primary tumours (PT) and CTCs of d'Amico high risk PCa patients.

Methods

PT samples in the form of tissue microarrays from 55 high-risk d'Amico PCa patients were stained immunofluorescently for basal (K5/6/14) and luminal (K8/18/19) keratins and analysed using QuPath ver. 0.2.3. software (Bankhead et al., 2017) to assess their KP. Luminal, basal, transit-amplifying and double-negative phenotype was defined as K8/18/19(+)/K5/6/14(-), K8/18/19(-)/K5/6/14(+), K8/18/19(+)/K5/6/14(+) and K8/18/19(-)/K5/6/14(-), respectively. Results were compared to clinico-pathological parameters and patients' survival. In order to detect CTCs, matched peripheral blood (7.5 ml, EDTA tubes) samples from 20 PCa patients were collected, stained immunofluorescently and analysed using Amnis® ImageStream®X Mk II Imaging Flow Cytometer (Luminex) in order to assess their KP and compare it to PT phenotype.

Results

Luminal and double negative KP was detected in 36 (52.17%) and 33 (47.83%) of 69 PCa, respectively. Basal and transit-amplifying KP were observed only in adjacent fragments of benign tumour. Double-negative KP correlated with shorter time to biochemical recurrence (p=0.001), higher pT status (p=0.006) and higher Gleason score (p=0.001) as well as decreased expression of androgen receptor. In concordance, as proof of principle double-negative phenotype was found in CTCs and coincided with the lack of androgen receptor. In order to improve detection of double-negative CTCs vimentin was added as a marker what resulted in an increased detection of CTCs in blood samples. Luminal KP was found in 130 (66.67%), double-negative KP in 65 (33.33%) of 195 PCa CTCs. Double-negative KP correlated with higher pT status (p=0.006) and higher Gleason score (p=0.001).

Conclusions

Luminal and double-negative KP were found in PCa, both within primary tumours and in CTCs. Double-negative KP correlated with more advanced disease, which suggest potential clinical relevance of this phenotype as potential marker of PCa progression.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: SEARCH FOR THE OPTIMAL CELL MODEL FOR SARS-COV-2 INFECTION

SPEAKER: KRAWCZYK FILIP

SUPERVISOR(S): ANDREA LIPIŃSKA, PHD

Coronavirus SARS-CoV-2 requires no introduction, it is now one of the most extensively studied viruses globally. Currently, the most popular cell line for SARS-CoV-2 infection research is VeroE6. It can produce high virus yields, yet it causes adaptive mutations in the viral genome which interfere with the research.

This project aims to obtain a reliable platform to improve SARS-CoV-2 infection rate in commonly used laboratory cell lines (mostly of cancer origin) by overexpressing 2 receptor proteins involved in virus entry to the host cell. I have performed screening for ACE2 & TMPRSS2 expression using Western blotting for total protein level and flow cytometry to measure the protein level on the cell surface of 9 selected cell lines. The results confirm the high level of ACE2 and TMPRSS2 in VeroE6 cell line, known for the high efficiency of virus infection. I have constructed two retroviruses for overexpression of ACE2 and TMPRSS2, transduced VeroE6, and confirmed overexpression by Western blotting and flow cytometry.

My results suggest that some commonly used cell lines could benefit from overexpression of either ACE2 or TMPRSS2 (or both). This project will enable researchers to select and modify cell lines, in which viral replication is less prone to adaptive mutations, hence shortening the development of new vaccines, medications, and boosting the research on virus molecular structure.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: OPTIMIZATION OF SARS-COV-2-BASED VIRUS-LIKE PARTICLES SYSTEM

SPEAKER: RUIZ FERNÁNDEZ CARMEN

SUPERVISOR(S): MAŁGORZATA GRAUL, PHD

The COVID-19 pandemic caused by the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a leading cause of death globally. SARS-CoV-2 virions contain four structural proteins. Spike protein is responsible for the recognition of ACE2- the specific receptor on the host cell and the fusion of virus-host membranes. While Membrane and Envelope proteins are needed in virions assembly, Nucleocapsid protein interacts with virus single-stranded RNA+ genome and packs it into virions. The co-expression of four structural proteins generates virus-like particles (VLP), which resemble the virion structure and may activate the host immune system but do not contain the virus genome.

The aim of this bachelor thesis was to optimize the SARS-CoV-2 virus-like particle system (SC-VLP), first presented in Syed et al. Science, 2021 Dec 24;374(6575):1626-1632, where luciferase mRNA transporting SC-VLPs were used as a method to test mutations within SARS-CoV-2 structural proteins. In order to produce SC-VLP, HEK293T cells were transfected with plasmids carrying SARS-CoV-2 structural genes and Luc gene with a specific signal sequence recognized by N protein. Next, SC-VLP-containing medium was added to receiver cells expressing the receptor of SARS-CoV-2. Finally, the luciferase expression was measured.

I optimized the conditions for SC-VLP production such as choice of the transfection reagent, amount of DNA and plasmids ratio, as well as the time of incubation and media filtration necessity. What is more, SC-VLP's ability to enter uptaking cells was analyzed in two different cell lines (HEK and A549) with or without the expression of ACE2. Finally, the time of incubation of receiver cells with SC-VLPs, cell lysis conditions, and following luciferase assay were optimized.

Although it was difficult to reproduce the protocol described in the above-mentioned publication, the system was optimized for our laboratory conditions. SC-VLP were produced and able to successfully transport luciferase mRNA to receiver cells expressing the SARS-CoV-2 receptor.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: COMPARISON OF ENTRY PROCESS OF HUMAN CORONAVIRUSES INTO DIFFERENT TYPES OF CELLS

SPEAKER: PIOTRKOWSKI MIKOŁAJ

SUPERVISOR(S): PROF. KRYSTYNA BIEŃKOWSKA-SZEWCZYK

The SARS-CoV-2 virus has spread around the world and had a big impact on human health, life quality and the global economy. Current knowledge shows that, apart from infection of respiratory tract, COVID-19 can affect other organs: brain, kidney, intestines, liver or circulatory system. During the pandemic, different variants of the virus revealed differences in infectivity and pathogenesis . Therefore, it is important to investigate the entry of different variants of the SARS-CoV-2 virus into cells connected with observed COVID-19 symptoms. Spike protein located on the virion's surface is responsible for attachment to ACE2 receptor, fusion with host membrane and entry into the cell. The restriction in the experimental work with SARS-Cov2 is the very high biosafety level, required for such studies. To overcome that problem, lentiviral pseudoparticles, harboring spike protein were used in this project as a research model for analyzing entry abilities of SARS-CoV-2. Three different types of pseudoparticles were made - containing spike protein from Wuhan-Hu-1, Delta and Omicron variants. Each of them was used for transduction of human cell lines derived from neuroblastoma (SH-SY5Y), colorectal cancer (SW620), lung adenocarcinoma (Calu3), dermal microvascular endothelium (HMEC-1) and human fibroblasts. The entry of Wuhan-Hu-1 and Delta pseudoparticles into colorectal cells and neuroblastoma cells was detected, with higher entry ability of Delta variant. These results, in accordance with some published reports, confirmed that SARS-CoV-2 virus can enter intestinal and neuronal cells with different efficiency depending on the variant. The separate experiments performed for Omicron (still inconclusive), suggest weaker infection efficiency of this variant.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: CHARACTERIZATION OF SHBSAG-BASED VIRUS-LIKE PARTICLES EXPOSING SARS-COV-2 SPIKE EPITOPES

SPEAKER: **KUNDA** MARCIN

SUPERVISOR(S): PROF. KRYSTYNA BIEŃKOWSKA-SZEWCZYK

Ongoing COVID-19 pandemic enforced rapid development of vaccines targeting SARS-CoV-2. Majority of them have shown high efficacy in clinical trials and become available for large-scale vaccination. However, concern about vaccine effectiveness has arisen with the emergence and spread of the SARS-CoV-2 variants, which carry multiple mutations in the spike (S) protein—the main target for the COVID-19 vaccines. Therefore, the design of effective, novel vaccines against SARS-CoV-2 targeting the conserved regions of the S protein could be beneficial in terms of newly arising variants. Furthermore, currently approved vaccines against SARS-CoV-2 still remain less available in low-income countries due to high costs, and storage requirements.

Virus-like particles (VLP) are a versatile, safe and cost-effective vaccine platform. Hepatitis B virus (HBV) small surface antigen (sHBsAg) has the ability to self-assemble into non-infectious, highly immunogenic VLPs which are currently used as a vaccine against HBV. sHBsAg contains highly immunogenic region where various foreign epitopes can be inserted. In this study, I characterized sHBsAg-based VLPs exposing the selected conserved epitopes of SARS-CoV-2 spike protein. Results shown a successful chimeric VLPs expression in unconventional, economically attractive Leishmania tarentolae expression system, which allows for protein production with similar to mammalian-type glycosylation. The antigens with confirmed VLPs assembly were used for mouse immunization studies. All chimeric VLPs elicited strong and specific humoral response against SARS-CoV-2 epitopes. The ELISA assays have shown that sera from mice immunized with chimeric VLPs were able to react with native full-length S and sHBsAg carrier protein. This study presents an alternative approach for vaccine design, which may be helpful in pan-SARS vaccine development.

LABORATORY OF VIRUS MOLECULAR BIOLOGY

TITLE: THE ROLE OF SARS-COV-2 MEMBRANE PROTEIN M IN THE REGULATION OF IFITM3

SPEAKER: ZIMNAWODA MAJKA

SUPERVISOR(S): ALICJA CHMIELEWSKA, PHD

The SARS-CoV virus belongs to the Coronaviride family and is responsible for causing the COVID-19 respiratory disease pandemic. Despite the intensive efforts of scientists from all over the world, knowledge about the interaction of the SARS-CoV-2 virus with the immune system is still limited. Expanding knowledge of the potential immunosuppression and pathogenicity of SARS-CoV-2 may be critical to explaining the differentiated course of COVID-19 observed in humans.

The first line of defense against viral infections is the innate immune response. One of its elements is the interferon-induced membrane protein (IFITM3), which is responsible for inhibiting the fusion of enveloped viruses with host membranes. This protein inhibits the entry of various groups of viruses, including influenza A virus, hepatitis virus type C (HCV) or human immunodeficiency virus (HIV). However, it has been proved that in the case of the SARS-CoV-2 virus, the IFITM3 protein may exhibit both pro- and anti-viral activity.

Recently published proteomic studies indicate that the structural M protein of SARS-CoV-2 could potentially interact with the cellular protein IFITM3. The aim of this project was to verify the hypothesis that the M protein of the SARS-CoV-2 virus interacts with the cellular protein IFITM3.

In the project, the A549 lines and Mel JuSo were used to study endogenous IFITM3, the level of which depends on IFN signaling, as well as the A549 line with exogenous IFITM3 overexpression. The total and surface levels of the tested protein were confirmed by Western blot analysis and flow cytometry. In order to investigate the role of the M protein in the regulation of IFITM3, lines with stable expression of the gene encoding the SARS-CoV-2 M protein were constructed by transduction with a retroviral vector. The obtained lines were sorted by a flow cytometer to isolate positive cells. The analysis of the protein levels in stable lines, by means of the Western blot technique, indicates the regulation of the IFITM3 protein level by the viral M protein. The interaction between the tested proteins was analyzed by co-immunoprecipitation. The obtained results indicate the interaction of the cellular protein IFITM3 with the M protein of the SARS-CoV-2.

This work provides an insight into the role of the SARS CoV-2 M protein in the regulation of the cellular protein IFITM3. Further understanding of SARS-CoV-2 virus immunomodulatory strategies and the analysis of critical virus-host interactions may contribute to the development of new therapeutic strategies in the future.

LABORATORY OF RECOMBINANT VACCINES

TITLE: REAL-TIME DNA SEQUENCING IN DIAGNOSTICS OF HUMAN RESPIRATORY VIRUSES

SPEAKER: NIZIŃSKI ERYK

SUPERVISOR(S): ŁUKASZ RĄBALSKI, PHD

Respiratory viruses are a burden to humanity, and viral infections might be life threatening. Early and accurate detection is the key to prevent the spread of viruses, and take the appropriate actions to limit the negative outcomes that they can have on our lives. Nanopore DNA sequencing gives a possibility to precisely detect simultaneous infections with multiple viruses, allowing us to control viruses with epidemic potential and prevent major outbreaks. In this presentation I will describe the steps of method optimization and show the results reflecting its gradual improvement. At the end I am going to prove the method's efficiency, by referring to the limit of detection (LOD) and the ability to detect many various respiratory viruses.

LABORATORY OF RECOMBINANT VACCINES

TITLE: PRODUCTION OF POTENTIAL VACCINE ANTIGENS BASED ON STRUCTURAL PROTEINS OF WEST NILE VIRUS IN THE LEISHMANIA TARENTOLAE EXPRESSION SYSTEM

SPEAKER: WIŚNIEWSKA EWA

SUPERVISOR(S): EWELINA KRÓL, PHD, DSC

West Nile virus (WNV) is a single-stranded RNA pathogen belonging to the Flavivirus genus that can cause a fever-like disease - West Nile fever. In some cases, infection can result in serious neurological complications, such as encephalitis, meningitis or paralysis. In the recent years the virus has been spreading in Europe due to climate change and simultaneous expansion of its vector – mosquitoes of Culex genus. Currently, there are no human vaccines against West Nile virus on the market, although virus-like particles (VLPs) of WNV are a possible vaccine candidate due to their non-infectivity and high structural similarity to viral particles.

The aim of my master's project was to produce prM/M and E structural proteins of West Nile virus using Leishmania tarentolae expression system. L. tarentolae is a single-cell, eukaryotic organism that parasitizes geckos. It was found to be a cheap and easy to handle expression system that allows for efficient production of proteins, including those that require post-translational modifications. I additionally characterized produced proteins to assess their ability to form virus-like particles and the potential for their application as vaccine antigens.

The results of my master's project demonstrate that gene constructs needed for expression of structural proteins of West Nile virus have been successfully obtained by PCR and then cloned into expression plasmids. Stable lines of L. tarentolae with gene constructs integrated into their genome have been constructed using transfection by the electroporation method. The expression of target proteins has been confirmed by Western blot. The results of ultracentrifugation in sucrose density gradient indicated that obtained proteins form virus-like particles. ELISA assay results confirmed that produced proteins efficiently bind neutralizing antibodies, which points to the fact that they could potentially be used as vaccine antigens.

In conclusion, in this project it has been shown for the first time that the Leishmania tarentolae expression system can be successfully used for the production of virus-like particles of West Nile virus. Moreover, initial results suggest that WNV VLPs could potentially serve as potential vaccine antigens.

LABORATORY OF RECOMBINANT VACCINES

TITLE: CONSTRUCTION OF A TEMPLATE FOR IN VITRO SYNTHESIS OF MRNA FOR VIRAL ANTIGENS

SPEAKER: GUPALO ANDRII

SUPERVISOR(S): PROF. BOGUSŁAW SZEWCZYK

Work on the creation and application of mRNA technology on an industrial scale has been going on for over 30 years. In the last 5 years, this technology has found widespread application in the field of vaccinology. Today, more than 10 pharmaceutical companies are successfully working on the creation of mRNA-based vaccines against the SARS-CoV-2 virus with an efficiency of more than 90%. The increase in the occurrence of outbreaks of infection in different parts of the world and a high tendency for mutations in RNA genomic viruses show that there is an urgent need for a quick response to the emergence of new pathogenic variants of viruses and prevention of their spread by prophylactic methods. The mRNA-based technology allows for the rapid creation of vaccines and rapid production of large volumes of thesedrugs. In my work, I tried to develop a universal, fast, and cheap method of RNA synthesis for any encoded sequence. To do this, I cloned genetic structures into a plasmid, which became the template for transcription. I tested the functionality of my construct. I obtained the functional mRNA encoding mCherry fluorescent protein, transfected mammalian cells HEK293, and observed intense fluorescence of the mCherry protein in the cells. I found the optimal dose of mRNA that does not induce cytotoxicity. Next, I easily exchanged the mCherry protein sequence for the N protein sequence of the SARS-CoV-2 virus. I transfected mammalian cells with the obtained mRNA and checked whether there is expression of N protein in mammalian cells using the Western-Blot method. Based on the obtained data, I determined if my proposed optimized method allows for the rapid synthesis of mRNA coding for the selectedproteins.

LABORATORY OF RECOMBINANT VACCINES

TITLE: CONSTRUCTION AND PURIFICATION OF EUROPEAN BROWN HARE SYNDROME VIRUS (EBHSV) VIRUS-LIKE PARTICLES IN SF9 INSECT CELLS

SPEAKER: PEPLIŃSKA MARTA

SUPERVISOR(S): PROF. BOGUSŁAW SZEWCZYK

European brown hare syndrome virus is the causative agent of the haemorrhagic disease of hares which is highly contagious and has very high mortality rate. The aim of the project was to produce the main capsid protein VP60 of the EBHS virus with the use of baculovirus expression system in Sf9 insect cells and to purify virus-like particles (VLPs) made of this protein. Purified VLPs can be used in packing mRNA of varius human viruses and transporting it inside the cells and also in diagnostics and epidemiology of haemorrhagic disease of hares. Three mutated versions of the VP60 protein were constructed to promote the binding and packing of mRNA into VLPs. Wild-type VP60-EBHSV protein produced in Sf9 insect cells with the use of baculovirus expression system was efficiently secreted to the medium and self-assembled into VLPs, which could be purified from the medium by sucrose gradient ultracentrifugation. All mutations introduced in the sequence of the protein resulted in lower efficiency of protein production or in the lack of self-assembling into VLPs.

LABORATORY OF BIOLOGICALLY ACTIVE COMPOUNDS

TITLE: ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF SECONDARY METABOLITES FROM SELECTED CARNIVOROUS PLANTS

SPEAKER: HUMIĘCKA ANNA

SUPERVISOR(S): MARTA KRYCHOWIAK-MAŚNICKA, PHD

Introduction: Antimicrobial-resistant nosocomial pathogens such as Pseudomonas aeruginosa and Staphylococcus aureus pose an increasing health threat. We need new antimicrobial substances, preferably those against which bacteria will not be able to develop resistance mechanisms. Carnivorous plants are source of a wide range of biologically active secondary metabolites with antimicrobial potential, such as flavonoids and naphthoquinones (e.g. plumbagin). Although there are many reports on promising biological potential of plumbagin, it is inactive against P. aeruginosa and it tends to be highly cytotoxic. However, carnivorous plants contain variety of secondary metabolites with unknown antimicrobial potential.

Aims: The main aim of the study was to determine antibacterial properties of secondary metabolites from selected carnivorous plant species from Droseraceae family against P. aeruginosa and S. aureus. Specific aims of the study were as follows: i) to investigate influence of culture duration on the accumulation of antimicrobial secondary metabolites in plant tissues, ii) to assess toxicity of species-specific secondary metabolites and their anti-infective potential, iii) to determine significance of reference secondary metabolite, i.e. plumbagin, as main antimicrobial secondary metabolite in carnivorous plant tissues.

Methods: Propagation of four carnivorous plant species through tissue cultures was used to obtain plant material. After 3, 4 and 6 months of cultivation plant tissues were collected and extracted with three solvents (tetrahydrofuran, water and chloroform). HPLC analyses of extracts were performed to determine plumbagin concentration. Minimal Bactericidal Concentration (MBC) of extracts was established using microdilution broth method. Survival rate of C. elegans after 24 h exposure to selected extracts was determined to investigate their toxicity and anti-infective potential.

Results: Tetrahydrofuran (THF) extracts from all plants showed the highest bactericidal activity against P. aeruginosa and S. aureus. Extracts from plant tissues cultured for shorter time tended to have lower MBC. THF extracts from D. muscipula turned out to be highly toxic towards C. elegans in contrast to other plant species. Toxicity of D. muscipula extracts corresponded with high content of plumbagin. On the contrary, THF extracts of D. omisa and D. nitidula, i.e. extracts without plumbagin, increased survival rate of nematodes infected with P. aeruginosa.

Conclusions: Analyzed carnivorous plant tissues contain secondary metabolites with antibacterial activity against P. aeruginosa and S. aureus. THF extracts from tissues of D. omisa and D. nitidula show low toxicity and anti-infective potential towards P. aeruginosa. Future studies are needed for identification of non-toxic secondary metabolites with antibacterial potential from aforementioned carnivorous plants.

LABORATORY OF PLANT BIOCHEMISTRY

TITLE: EFFECT OF DIFFERENT PDAT1 ENCODING GENE EXPRESSION LEVEL ON THE GROWTH OF A. THALIANA PLANTS AND LIPID CONTENT IN THEIR LEAVES AND SEEDS

SPEAKER: **KĘDZIERSKA** SARA

SUPERVISOR(S): PROF. ANTONI BANAŚ

In the era of the growing global demand for food, and thus for plant products, ways are sought to increase the efficiency of agricultural production, both in terms of the size and quality of the crop. Research on the metabolism of plant lipids can provide knowledge about the most favourable area for genetic engineering interference as a way of achieving that efficiency.

Phospholipid:diacylglycerol acyltransferase (PDAT) is an enzyme involved in the biosynthesis of plant triacylglycerols (TAGs) - the main component of plant oils. The another type of enzymes involved in this process is DGAT - acyl-CoA:diacylglycerol acyltransferase. DGAT is using acyl-CoA and PDAT is using phospholipids, especially phosphatidylethanolamine – PE – and phosphatidylcholine – PC – as acyl donors for acylation of diacylglycerol in TAG biosynthesis. As a by-product of PDAT reaction lysophospholipids are created. Thus, additionally to TAG biosynthesis, reaction catalysed by PDAT can affected membrane composition and indirectly influences their properties.

In the presented study, the effect of different expression of PDAT1 encoding gene on the development of Arabidopsis thaliana (control plants, plants with a silenced PDAT1 gene and plants with overexpression of this gene) was tested. Additionally, the effect of cold stress (approx. 6° C) on the development of such mutants and control plants was investigated. In the experiments, two kind of plant cultivation were use: in vitro on agar plates and in vivo in pots with soil. After defined time of growth/ incubation in growth chamber with standard or low temperature (23oC and 6oC respectively) the morphology of the plants has been recorded and the plants were subjected for farther analysis. The fresh and dry weight of the plants was measured as well as lipid content and composition in leaves and seeds (in this case only mature seeds were analyzed).

Obtained results show a big differences in growth rates between the tested lines. Those differences magnified under the growth in cold conditions. Dry weight of mutants with PDAT overexpression was even 2-3 times higher than dry weight of wild type plants. In case of total lipid content no major differences were spotted nor lipid composition were significantly affected. Admittedly the TAG content doubled in the leaves of overexpressors, however, it still constituted for only about 2% of all lipids. Thus we concluded that the changes in lipid content and composition are probably not directly responsible for the increased growth rate of overexpressors. To explained this effect, other processes related to lipid metabolism must be considered in further studies.

LABORATORY OF PLANT BIOCHEMISTRY

TITLE: EFFECT OF KNOCK-OUT MUTATION IN LYSOPHOSPHATIDYLETANOLAMINE ACYLTRANSFERASE (LPEAT) CODING GENE ON GROWTH AND DEVELOPMENT OF CAMELINA SATIVA

SPEAKER: MANCEWICZ JOANNA

SUPERVISOR(S): KATARZYNA JASIENIECKA - GAZARKIEWICZ, PHD

Camelina sativa is an oil crop belongig to the Brassicaceae family, thus closely realated to popular model plant Arabidopsis thaliana. Characteristic features for camelina with regard to agriculture and are for example its low nutrient requirenments and drought resistance. It is also used in food industry for high level of mono- and plyunsaturated fatty acids, including ω-3, and high concentration of vitamin E. Camelina sativa oil has also anti-inflammatory and antioxydative properties. In science the plant is appreciated for being more suitable model organism for studies concerning both storage and structural lipids metabolism than A. thaliana is. In this work Camelina sativa cv. Suneson was used for analysing role of the acyl-CoA:lysophosphatidylethanolamine acyltransferase (LPEAT) enzyme in growth and development of plants. LPEAT is membrane protein occuring in two isoformes in cells (LPEAT1 and LPEAT2). They differ with their subcellular location and specificity towords particular acyl-CoA molecules. Both isoforms carrie out reaction of transfering acyl groups from acyl-CoA cytosolic pool to the sn-2 position of lysophosphatidilethanolamine molecule and by this reaction phosphatidylethanolamine (PE) is synthesized. PE is the second most abundant phospholipid in membranes structures, what makes it to some extent responsiple for changing fluidyty of mambrenes in various environmental conditions.

To assess how LPEAT influences growth and development of C. sativa, knock-out lines with mutation in gene coding for LPEAT1 or LPEAT2 isoform as well as wild type plants were cultivated in soil in standard conditions. Observations concerned processes related to flowering and scenescence. In order to characterise changes in fatty acids metabolism, lipids were extracted from leaves. Classes of lipids were separated by thin layer chromatography, fatty acids were derivated into methyl esters and analyzed by gas chromatography in terms of their content and fatty acids composition.

During observations it was noticed that knock-out mutants develop differences in morphological traits. Lack of LPEAT1 enzyme caused opening of the first flower to delay, in lines with lpeat2 mutation the effect was opposite. Also duration of flowering was shortened in lpeat1 lines, but prolongated in lpeat2 lines. Plants of every tested line were significantly shorter than wild type. Data obtained from lipids analysis indicate that mutation in LPEAT coding genes couses significant increase of fatty acid content both in leaves in every tested line. The biggest increas occured in trienoic acids: hexadecatrienoic and α-linolenic wherase content of saturated palmitic fatty acid did not change. Although biosynthesis of all lipids classes significantly intensified, fatty acid composition did not change, neither in general content nor in separated lipid classes. In terms of lipid classes, almost two-fold rise was denoted for digalactosyldiacylglicerol (DGDG).

LABORATORY OF PLANT MICROBIOLOGY

TITLE: MOLECULAR DETERMINANTS OF BACTERIAL ADHESION TO BIOTIC AND ABIOTIC SURFACES – CASE STUDY OF THE OCHROBACTRUM GENUS

SPEAKER: BOROWICZ MARCIN

SUPERVISOR(S): SYLWIA JAFRA, PHD, DSC

However, the Ochrobactrum genus is not a model organism example but is attractive to study. It includes both human opportunistic pathogens and plant growth-promoting organisms. Due to the close relation to the virulent Brucella genus Ochrobactrum spp., is considered a harmful organism despite its relatively low virulence. The adhesion process is associated with colonization of the biotic and abiotic surfaces. It might be related to the potential pathogenicity against humans and be considered the major virulence factor of Ochrobactrum spp. Yet it might be involved in beneficial colonization of plants.

The study aimed to identify and inactivate genes involved in adhesion to biotic and abiotic surfaces in Ochrobactrum quorumnocens A44 (plant isolate) and Ochrobactrum anthropi ATCC 49188 (clinical isolate).

Based on the literature study and bioinformatic tools, eight genes potentially associated with the adhesion, present in at least one of the studied organisms, were identified. By allele exchange mutagenesis with the suicide vector, two mutants were obtained and confirmed by sequencing. The flgE knock-out in O. quorumnocens, gene relevant to the production of flagellum hook protein essential in flagellum assembly and the wbaP knock-out in O. anthropi, the gene relevant to the production of undecaprenyl-phosphate glucose phosphotransferase, a protein associated with polysaccharide synthesis.

T he mutants' phenotypes were tested under different conditions. Motility assays showed that the mutation in flgE gene of O. quroumnocensc caused the loss of swimming motility, yet mutation in wbaP of O. anthropi did not affected its motility. The in vitro grown potato plant colonization showed that both mutations do not influence potato colonization by the tested strains. Interestingly O. anthropi, a clinical isolate, colonizes potatoes more efficiently than O. quorumnocensc, a plant isolate. The biofilm formation on plastic was tested to check the ability to colonize the abiotic surface. Results showed that the wbaP O. anthropi mutant completely lost the ability to form biofilm on plastic. Finally, the simple and newly developed assay assessed the mutation influence on medical tube colonization potential. Showing that the wbaP O. anthropi mutant lost completely the ability to colonize medical tubes and that the flgE O. quorumnocens mutant colonization ability is significantly lower compared to the wild type strain.

To conclude, flgE knock-out in O. quorumnocens does not influence potato colonization and biofilm formation but decrease the medical tube colonization ability under tested conditions, therefore FlgE is non-essential for potato colonization in vitro but plays a potential role in tubes colonization. The wbaP knockout in O. anthropi does not influence potato colonization but prevents biofilm formation on plastic under tested conditions, therefore WbaP is essential for adhesion to abiotic surfaces.

LABORATORY OF PLANT MICROBIOLOGY

TITLE: ANALYSIS OF ACTIVITY OF MUTATIONS IN GENES ASSOCIATED WITH CYCLIC DIGUANYLATE ON BIOFILM FORMATION IN PSEUDOMONAS DONGHUENSIS P482

SPEAKER: DARLIŃSKA ANIELA

SUPERVISOR(S): MAGDALENA RAJEWSKA, PHD

Cyclic diguanylate (c-di-GMP) is a ubiquitous bacterial second messenger which has been associated with the regulation of a range of cellular processes, including motility and biofilm formation. Biofilm formation is a cyclic process that initiates with the surface attachment of planktonic bacteria. After the microcolony is formed, it matures into macrocolony until the biofilm-associated cells disperse to resume a planktonic lifestyle. Low intracellular c-di-GMP levels are associated with a motile lifestyle, while elevated levels of c-di-GMP promote surface attachment and biofilm formation. Also, environmental conditions, such as the availability of the carbon source, may affect biofilm formation. Pseudomonas donghuensis P482 is a tomato plant rhizosphere isolate, with the ability to inhibit the growth of bacterial and fungal plant pathogens. P482 is also a plant root colonizer and a potential plant growth-promoting rhizobacterium (PGPR).

The aim of the study was the identification of genes associated with cyclic diguanylate in Pseudomonas donghuensis P482. Analysis of the impact of mutations on biofilm formation and plant rhizosphere colonization by P482 with the use of gene knockout.

Based on the results obtained from in silico analysis, site-directed mutagenesis was performed with pKONOCK-Km suicide vector to inactivate genes associated with c-di-GMP. Results of the mutation were confirmed through genome sequencing.

First, to determine if any of the introduced mutations affect motility, P482 mutants were tested on M8 plates supplemented with 0,3% agar were used. Overnight bacteria cultures were injected into the plate and their swimming motility was observed. Secondly, exopolysaccharide (EPS) production was assessed through Congo red assay on M63 agar plates. To quantitatively assess biofilm formation crystal violet assays on minimal M9-salts medium were performed. To allow for visualization of the biofilm on abiotic and biotic surfaces, GFP-tagged strains of P. donghuensis P482 were obtained.

The biofilm formation on glass-bottom plates was analysed using M9-salts minimal medium. The impact of mutations in P482 on plant rhizosphere colonization was tested with the use of tomato seedlings, inoculated with the GFP-tagged bacteria and grown in sterile conditions. The visualization was performed utilizing Leica SP8X scanning confocal microscope.

In this study, 10 genes associated with c-di-GMP were selected. After transformation, eight pKNOCK – amplicon constructs were obtained and after biparental mating six P482 mutants were obtained and confirmed through genome sequencing. Interestingly, the swimming motility of mutant KN0005 was increased, while KN0873 was decreased in comparison to P482 wild-type (wt). Decreased ability to bind Congo red-stain to EPS was observed in mutants KN2145 and KN2375. The quantitive assessment showed an increase in biofilm formation for KN0005 and a decrease for mutant KN0873. Colonization of plant rhizosphere was decreased for mutants KN4501 and KN1313.

In conclusion fleQ gene in P482 (KN0873) is associated with motility. This can further be associated with lower biofilm production in given conditions. Mutation in locus BV82_2145 causes lowered EPS production which can be associated with lowered biofilm production in given conditions. Based on in silico analysis and plant root colony visualization, gene mutations KN4501 and KN1313 can be associated with decreased rhizosphere colonization.

LABORATORY OF PHYSICAL BIOCHEMISTRY

TITLE: EFFECT OF THYMOQUINONE ON ENTEROTOXIN TYPE A LEVEL IN CULTURES OF STAPHYLOCOCCUS AUREUS

SPEAKER: SEROCKA SYLWIA

SUPERVISOR(S): ROBERT ŁYŻEŃ, PHD

Staphylococcus aureus is a commensal bacterium commonly found in the skin and mucous membranes of humans and animals. However, it is also an opportunistic pathogen that can cause many diseases that range from mild skin infections to more severe problems like bloodstream infections, endocarditis, pneumonia, and osteomyelitis. S. aureus infections are difficult to treat because this bacterium produces numerous virulence factors to evade the host's immune system and adapt to different environments. Particularly notable virulence factors associated with S. aureus are enterotoxins. These proteins are stable even at high temperatures and low pH levels and can lead to toxic shock syndrome and food poisoning. Staphylococcal enterotoxins exhibit superantigen activity and can stimulate non-specific activation and proliferation of T-cells. One of the most common and the first described enterotoxin is enterotoxin type A. Another fundamental problem associated with S. aureus infections is the rapid emergence of antibiotic resistance. Therefore, nowadays it is important to find new antibacterial substances that can help with treatment of multidrug resistant bacteria. Plants are the big source of such compounds. Thymoquinone is the main active constituent of Nigella sativa essential oil, which shows great therapeutic potential. It can effectively inhibit growth of many bacteria, including S. aureus. It was also able to downregulate the transcription of toxin genes in some bacteria. However, nobody checked the level of the actual enterotoxin proteins in the cultures after thymoquinone treatment. Therefore, the aim of this thesis was to investigate the effect of thymoquinone on growth and enterotoxin type A production in cultures of Staphylococcus aureus.

LABORATORY OF PHYSICAL BIOCHEMISTRY

TITLE: MODELLING AND OPTIMIZATION OF THE PROCESS OF DRYING OF SILICA COMPOSITES – IMPACT OF THIS PROCESS ON THE ACTIVITY OF THE ENZYME ENTRAPPED INSIDE OF THE COMPOSITE

SPEAKER: STALKE HANNA

SUPERVISOR(S): LESZEK KADZIŃSKI, PHD

One of the materials that can be used as carriers for medicines and reusable media for selective removal of a variety of compounds from food products and environment are xerogels. The immobilization of proteins in their structure can widen the spectrum of potential applications. Unfortunately, the most commonly used xerogel synthesis method – sol-gel method, requires conditions unfavorable to most proteins and may lead to their denaturation. For that purpose, a silica composite build of partially hydrated silica xerogel, polydimethylsiloxane and β-galactosidase A from Aspergillus oryzae has been synthesized and conditions of a drying step have been optimized to maximize the enzyme activity. The activity has been measured using a colorimetric test with ortho-nitrophenyl-β-galactoside and a model explaining the observed linear relationship between enzymatic activity and two factors – the temperature and pressure applied during the drying step has been created. Additionally, the differences between pH and temperature optima for free and immobilized β-galactosidase A and the reusability of synthesized composite have also been checked. It has been found that the optima of immobilized enzyme do not differ from free enzyme, but the activity spectra are more narrow, which can be useful when more precise reaction conditions are advantageous. Since it maintains enzymatic activity even after multiple incubations, this study can broaden the current knowledge about tailoring of xerogels for the purpose of using them for protein immobilization in industry.

LABORATORY OF PROTEIN BIOCHEMISTRY

TITLE: CHARACTERISATION OF ORTHOLOGS OF B-LACTAMASE INHIBITORY PROTEIN II (BLIP-II) AS POTENTIAL TOOLS TO TACKLE ANTIBIOTIC RESISTANCE

SPEAKER: KRUK KACPER

SUPERVISOR(S): AGNIESZKA KŁOSOWSKA, PHD

Every year, more than one million people die because of antibiotic resistant infections, out of which β-lactam resistant bacteria cause a large part. One of the mechanisms of resistance to β-lactam antibiotics is related to β-lactamases – enzymes that are able to hydrolyze β-lactam antibiotics. There are known small molecule β-lactamase inhibitors but mutations in β-lactamases might result in gaining resistance to those inhibitors. Usage of small molecule β-lactamase inhibitors might also result in severe side effects and cause allergies. Here I present β-Lactamase Inhibitor Protein-II (BLIP-II) and its orthologs as a potential new tool to tackle β-lactam resistance.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: FGF/FGFR-DEPENDENT REGULATION OF THE AP-1 COMPLEX IN LUMINAL BREAST CANCER

SPEAKER: LASAKOWSKA ALEKSANDRA

SUPERVISOR(S): RAFAŁ SĄDEJ, PHD, DSC

Despite the increasingly effective diagnostic and therapeutic methods, breast cancer (BC) is still the most common cancer among women worldwide and is the leading cause of cancer deaths. Breast cancer is a heterogeneous disease in which several subtypes can be distinguished. The most common is luminal A type characterized by the presence of steroid hormone receptor – estrogen receptor (ER) which is considered to be the main driver of disease progression. After activation with a specific ligand – estradiol, it is translocated to the nucleus and binds to estrogen response elements (ERE) within DNA. This action leads to expression of ER-dependent genes. However, there are genes that are under control of ER but do not contain an ERE sequences in their promoters. In these cases, ER binds to DNA indirectly by other transcription factors, such as activator protein-1 complex (AP-1). This complex consists of four protein families, the main of which is the Jun protein family (c-Jun, JunB and JunD). Jun proteins can form dimers which allows them to bind to DNA at specific AP-1 sequences. The ER then attaches to the complex, leading to expression of the genes.

It is known that tumor microenvironment affects the development and progression of the disease. Its main component – fibroblasts by secreting fibroblast growth factors (FGFs) activate signaling pathways that lead to ligand-independent activation of ER, as well as increase in Jun protein level.

The aim of the project was to determine the involvement of FGF/FGFR pathway in regulation of AP-1 complex in luminal breast cancer. The obtained results showed that the increase in Jun proteins level occurs after stimulation with several growth factors, however the highest increase is observed after stimulation with FGFs.

The mechanism of FGF/FGFR-dependent regulation of Jun proteins occur probably by protein stabilization as well as at the mRNA level, as the increase in both protein and mRNA levels were observed after about 2 hours after stimulation with FGF. Also, the localization of Jun proteins in the cell after stimulation with FGF was examined, and the preliminary data suggest that there is a shift from cytoplasm to nucleus. Considering data from other publications showing that upon activation, Jun protein dimers lead to expression of the Jun genes in the nucleus, there is a probability that FGF/FGFR signaling leads to activation of this positive feedback loop. To determine the biological effects of Jun proteins, cell lines with c-Jun, JunB and double overexpression were established. The derived cell lines were growing faster than the wild type in the control conditions and in the presence of anti-ER drug, which suggest that these proteins may promote the progression of the disease.

The obtained results indicate that FGF/FGFR pathway by regulation of the AP-1 complex may lead to the development of resistance to anti-ER drugs and faster tumor growth, however more research is needed.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: EFFECT OF THE FGF7/FGFR2 SIGNALING PATHWAY ON THE REGULATION OF P62 EXPRESSION AND ACTIVITY IN LUMINAL BREAST CANCER

SPEAKER: LEŚNIAK ALEKSANDRA

SUPERVISOR(S): RAFAŁ SĄDEJ, PHD, DSC

Breast cancer (BC) is the most common cancer type and the second cause of death among woman worldwide. Patients with luminal A breast cancer subtype (positive for both estrogen and progesterone receptor) can benefit from endocrine therapy, such as tamoxifen or fulvestrant. Unfortunately, most of patients have intrinsic resistance or will develop acquired resistance. Reduced efficacy of anti-ER drugs may be linked with process of autophagy and/or Nrf-2-Keap1 pathway. In physiological conditions, autophagy protects cells through degradation of cytotoxic compounds, whereas Nrf-2-Keap1 complex is a master regulator of oxidative and electrophilic stresses. Both of these processes are regulated by p62 protein. Phosphorylation of p62 in serine 349 leads to dissociation of Nrf-2 from its repressor Keap1 and translocation of Nrf-2 to the nucleus and activation of antioxidant genes. Moreover, p62 can induce a positive feedback loop to activate Nrf2, which in turn activates transcription of p62 gene (SQSTM-1). Multiple studies have shown that alterations in fibroblast growth factor receptors (FGFRs) signaling, which are involved in the transmission of signals from tumour microenvironment, contribute to development and progression of breast cancer. Especially, FGFR2 has been shown to protects breast cancer cells from tamoxifen. On the other hand, tumour microenvironment can also regulate autophagy and Nrf-2-Keap1 complex.

The aim of the project is to determine the involvement of FGF7/FGFR2 signaling in the regulation of p62 protein expression level and activity and its role in the response of breast cancer cells to anti-ER drugs.

The obtained results showed that FGF7/FGFR2 signaling increases the protein expression level of p62 and Nrf-2 and mRNA level of Nrf-2. Cells with overexpression of Nrf-2 showed increase level of SQSTM-1 (p62) mRNA, which suggest formation of positive feedback loop. Moreover, overexpression of p62 increases the protective effect of FGF7 against tamoxifen and fulvestrant treatment. The obtained results indicate that FGF7/FGFR2 signaling pathway is involved in the regulation of p62 expression and activity in breast cancer cells, which can be linked with limited efficacy of tamoxifen and fulvestrant treatment. However, additional research on this issue is needed.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: ROLE OF FGFR2/AP-1 IN LUMINAL BREAST CANCER PROGRESSION

SPEAKER: **CHODORSKA** MAGDALENA

SUPERVISOR(S): KAMILA KITOWSKA, PHD

Background: Hormone-dependent luminal breast cancer (BCa) is the most frequent subtype that represents about 70% of all cases. Estrogen receptor (ER) plays a key role in a regulation of genes that are crucial for progression of the disease. ER-dependent gene expression is regulated by binding of ER to DNA directly within estrogen response element (ERE) but also indirectly by interacting with other transcription factors, e.g. activator protein 1 (AP-1). Growing evidence from in vitro and in vivo studies proves that AP-1 is involved in BCa tumor progression and metastasis. It is also suggested that it might contribute to resistance to cancer therapies, e.g. treatment with tamoxifen. Components of tumor microenvironment like fibroblast growth factors (FGFs) are also an important factor in tumor progression. It has been proved that activation of fibroblast growth factor receptor 2 (FGFR2) with a fibroblast growth factor 7 (FGF7) mediates tamoxifen resistance and also leads to an elevated level of JunB protein- one of the major components of AP-1. Thus the aim of the project was to investigate molecular mechanism underlying FGF7-induced increase of JunB protein level in breast cancer cells.

Results: This project revealed that FGF7/FGFR2-induced increase in JunB protein level is ER-independent. Moreover, results from luciferase reporter assay suggest that FGF7 induces shift in ER DNA binding from estrogen response element (ERE) to AP-1 site, which may suggest modulation in ER-mediated gene regulation. Interestingly, knock-down of JunB in luminal BCa cell lines did not affect FGF7/FGFR2-mediated resistance to tamoxifen in T47D cells.

Conclusions: Taken together these results imply that ER presence in a cell is not crucial for FGF7-induced effect observed in a JunB protein level. However, shift in ER binding to DNA suggests that there might be activation of genes involved in JunB protein stabilization.

LABORATORY OF MOLECULAR ENZYMOLOGY AND ONCOLOGY

TITLE: THE FGFR2/IRS-1 INTERDEPENDENCE IN THE PROGRESSION OF LUMINAL BREAST CANCER

SPEAKER: POLAKIEWICZ ZUZANNA

SUPERVISOR(S): KAMILA KITOWSKA, PHD

BACKGROUND: Luminal subtype accounts for more than half of all breast cancers (BCa) and is characterised by expression of hormone receptors, such as estrogen receptor (ER) and progesterone receptor (PR). Endocrine therapy is a golden standard for treatment of luminal BCa by direct or indirect inhibition of ER activity. Signalling from fibroblast growth factors (FGFs) and their receptors (FGFRs) is known to be responsible for the emergence of resistance to hormone therapy. In BCa patients FGFR2 protein level correlates with mRNA level of ER-dependent gene - IRS1 gene, which encodes insulin receptor substrate 1 (IRS-1), protein involved in the progression of BCa. The aim of this study was to investigate whether FGF7/FGFR2 affects changes in IRS1 gene expression or IRS-1 protein activation influencing the luminal BCa progression.

RESULTS: It has been shown that neither IRS1 gene expression nor IRS-1 protein had been affected by FGF7/FGFR2 signalling. However, FGF7 stimulation induced changes in IRS-1 phosphorylation suggesting its impact on IRS-1 activity in luminal BCa cells: MCF7 and T47D. Interestingly, IRS-1 degradation by selective inhibitor (NT157) led to ER degradation and abolished FGF7/FGFR2-promoted resistance to tamoxifen/ fulvestrant in luminal BCa cells by inhibiting tumorspheres growth in matrigel. Moreover, clinical data analysis revealed corelation between low levels of IRS-1 protein and higher recurrence free survival (RFS) and overall survival (OS) in patients with luminal BCa.

CONCLUSIONS: Taken together, these results suggest that IRS-1 protein may be involved in the mechanism FGF7/FGFR2-dependent protective effect on tumorspheres growth and emergence of resistance to hormone therapy induced by FGF7/FGFR2 signalling in BCa cells. Furthermore, FGF7/FGFR2 signalling impact on IRS-1 phosphorylation indicates that it can influence changes in signalling downstream from IRS-1 protein, IRS-1 protein cellular localisation or ER-IRS-1 complex formation. In addition, effect of NT157 on simultaneous IRS-1 and ER degradation suggests that combination of endocrine therapy with IRS-1 degradation may lead to more successful outcome.

LABORATORY OF MOLECULAR BIOLOGY

TITLE: ANALYSIS OF ESCHERICHIA COLI LON PROTEASE INTERACTION WITH DNA

SPEAKER: CHMURA WERONIKA

SUPERVISOR(S): PROF. IGOR KONIECZNY

Lon, one out of five ATP-dependent proteases in Escherichia coli, selectively degrades abnormal and short-lived regulatory proteins, mediating protein quality control and multiple important processes necessary to maintain homeostasis. DNA, by interaction with Lon, regulates its activity and influence directed proteolysis. This nucleoprotein complex formation is essential for the bacterial cell. Research conducted until now indicate that Lon have high affinity to dsDNA and binding is nonseqence-specific. The aim of this study was to check whether the Lon protease can bind two dsDNA fragments simultaneously.

LABORATORY OF MOLECULAR BIOLOGY

TITLE: CONSTRUCTION AND ANALYSIS OF MODIFIED VARIANTS OF ESCHERICHIA COLI LON PROTEASE

SPEAKER: HIRSZ ZUZANNA

SUPERVISOR(S): PROF. IGOR KONIECZNY

In Escherichia coli Lon protease is responsible for the degradation of misfolded proteins under optimal conditions. It also proteolyzes ribosomal and regulatory proteins like replication initiators during amino acid starvation. Under non-stress conditions, Lon is localized in the nucleoid fraction, but there are limited data about Lon localization in the bacterial cells under stress conditions. Based on in vitro tests, that revealed that Lon interacts with molecule synthesized during stress, polyphosphate, it is very likely that Lon changes intracellular localization under stress conditions. In this project, I used CRISPR/Cas9-assisted recombineering to obtain an E. coli strain expressing gene encoding fusion protein Lon-mScarlet. This technique is the combination of homologous recombination with the CRISPR/Cas9 system that can significantly increase the effectiveness of recombination by applying negative selection. First, to verify the functionality of the Lon-mScarlet recombinant proteins I purified them and performed biochemical tests. The construction of this system has required the use of two plasmids. The first one encodes a gene for Cas9 protein and bacteriophage lambda proteins Beta, Gam, and Exo. Bacteriophage lambda proteins are required for homologous recombination. In the case of the second plasmid, I cloned a guide RNA sequence that targets Cas9 nuclease to the site on the chromosome that should be replaced during recombination. With the use of the Gibson assembly and PCR, I obtained a donor DNA sequence required for recombination. To verify positive recombinant events I used colony PCR. Construction of E. coli strain expressing Lon-mScarlet fluorescence protein will enable a microscopic analysis of Lon localization in bacterial cells under native and stress conditions.

LABORATORY OF MOLECULAR BIOLOGY

TITLE: BACTERIAL INORGANIC POLYPHOSPHATE - INTERACTIONS WITH STRESSED E. COLI PROTEINS AND INFLUENCE ON EUKARYOTIC CELL METABOLISM

SPEAKER: **KUŚ** FILIP

SUPERVISOR(S): KATARZYNA WĘGRZYN, PHD

Inorganic polyphosphate (polyP) is a polymer of orthophosphate units, linked by high energy bonds, similarly as in ATP. PolyP is synthesized by all studied organisms, from bacteria to humans. PolyP regulates a range of cellular processes. Bacteria produce long-chain polyP that consists of often more than 1000 phosphate units. In bacteria, polyP production is enhanced during stress, which serves as a mechanism to adapt to unfavorable conditions. PolyP can act as an energy storage, and a chaperone to help proteins fold properly, but it can also bind several proteins: e.g., Lon protease, DnaA replication initiator or ribosomal proteins. These protein-polyP interactions result in Lon activation and targeted degradation of DnaA and ribosomal proteins, leading to cell cycle arrest (Ropelewska et al., 2020). Conversely, higher eukaryotes synthesize shorter polyP with a length of approx. 100 phosphate units. Eukaryotic polyP can be found in numerous cell types, including fibroblasts or astrocytes, but the highest concentration is found in platelets- polyP released from activated platelets initiate blood clotting and acts proinflammatory.

Recent data show that during infection, bacterial polyP acts anti-inflammatory on the host's immune system (Roewe et al., 2020). The anti-inflammatory actions of long-chain polyP are surprising considering that both prokaryotic and eukaryotic polyP is chemically identical, except for the difference in

length.

In our work, we investigated what proteins, in addition to Lon, DnaA, and ribosomal proteins interact with polyP in stressed bacteria. We utilized PolyP Binding Domain (PPBD), a protein with high affinity to polyP, and a microfluidics system Surface Plasmon Resonance to capture polyP-protein complexes from bacterial lysates. We identified several proteins that potentially interact with polyP. Defining the so-called polyP-ome may provide new insights into the yet unrevealed functions of bacterial polyP. We also studied how bacterial polyP influences eukaryotic cells metabolism in the context of bacterial infection, by treating endothelial cells with polyP of different lengths and measuring the aberrations in the intracellular ATP levels. Despite not observing significant changes in the measured parameter, more research is needed to clarify the potential influence of polyP on the endothelium.

Our work may serve as a preliminary research to better understand the role of polyP in prokaryotes under stress, and how polyP released from bacteria may influence host's organism during infection. With more bacteria becoming resistant to antibiotics, targeting polyP metabolism is considered as a potential approach to fight antimicrobial resistance (Bowlin and Gray,2021). Bowlin, M.Q., Gray, M.J., 2021. Inorganic polyphosphate in host and microbe biology. Trends Microbiol. 29, 1013–1023.

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LABORATORY OF BIOMOLECULAR SYSTEMS SIMULATIONS

TITLE: MARKOV STATE MODEL OF PROTEIN FOLDING

SPEAKER: BASIŃSKA KALINA

SUPERVISOR(S): RAJMUND KAŹMIERKIEWICZ, PHD, DSC

Protein folding is the physical process by which a polypeptide chain is translated to its native three-dimensional structure. That process is crucial for the biological function of the protein. Various methods to study protein folding are available. There are experimental ones, e.g. X-ray crystallography, Fluorescence spectroscopy, or Protein NMR, but especially in recent years, there has been a rapid development of faster and more efficient computational methods for studying protein folding processes. State-of-the-art computational methods use artificial intelligence or stochastic modeling such as Markov chains.

The project attempts to construct a Markov state chain for a small model protein. In the Markov state model, the dynamics of the protein are modeled as a stochastic network of transitions between metastable conformational states. The results of molecular dynamics simulations were used for the construction. The simulations were performed under various conditions, including using different force fields. The Markov chain constructing program used in the project was previously used only for proteins with a lower molecular weight and less diversified structure.

LABORATORY OF BIOMOLECULAR SYSTEMS SIMULATIONS

TITLE: DE NOVO DESIGN AND TOXICITY EVALUATION OF POTENTIAL SARS-COV-2 MAIN PROTEASE INHIBITORS

SPEAKER: KALINOWSKI BARTŁOMIEJ

SUPERVISOR(S): RAJMUND KAŹMIERKIEWICZ, PHD, DSC

Abstract not submitted in time.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: EFFECT OF PHOTODYNAMIC METHOD WITH GALLIUM-COMPLEXED PORPHYRIN DERIVATIVE ON PRODUCTION AND ACTIVITY OF PYOCYANIN IN *PSEUDOMONAS AERUGINOSA*

SPEAKER: BURZYŃSKA NATALIA

SUPERVISOR(S): MARIUSZ GRINHOLC, PHD, DSC

Pseudomonas aeruginosa is an opportunistic pathogen belonging to the ESKAPE group, responsible for both acute and chronic infections in humans. Bacterium produces a wide range of virulence factors, including dyes like pyocyanin. It is a blue redox-active secondary metabolite that interferes with multiple cellular functions and plays an important role in invasive pulmonary infections. Because of raising pathogen resistance to conventional antimicrobials, there is an urgent need to develop new terapeutic options One of the promising method is antimicrobial photodynamic inactivation (aPDI). It uses non-toxic photosensitizers, which when irradiated with an appropriate wavelength are excited and generate large amounts of reactive oxygen species (ROS) which leads eventually to bacterial cell death.

The aim of this study is to determine the effect of the photodynamic method with gallium-complexed porphyrin derivative as photosensitizer and blue light on the production and activity of pyocyanin in P. aeruginosa.

Three isolates of P. aeruginosa were used in the study, including two reference strains and one clinical. Sub-lethal and lethal doses were determined for all strains. GaCMP was used as photosensitizing compound while illumination was provided with a single-emitter diode lamp emitting blue light (λmax = 409 nm). In order to determine the effect of aPDI on extracellular produced pyocyanin, bacterial supernatants were obtained, which were then treated with previously established sub-lethal and lethal doses. The ability of P. aeruginosa strains to accumulate photosensitizer molecules was also analyzed. Moreover, the RT-qPCR method was performed to determine whether the expression of the phzB2, phzM and phzS genes responsible for pyocyanin production was altered after treatment with sub-lethal doses of aPDI.

P. aeruginosa strains respond similarly to photoinactivation with GaCMP in tested conditions. The conducted experiments confirm that lethal and sub-lethal doses of aPDI significantly reduce the activity of pyocyanin in bacterial supernatants. Moreover, P. aeruginosa isolates possess the ability to accumulate photosensitizer molecules, which influences the effectiveness of the photodynamic method. The analysis of gene expression with RT-qPCR method confirm that sub-lethal doses of aPDI do not affect the expression profile of genes involved in pyocyanin production.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: EFFECT OF PHOTODYNAMIC METHOD WITH WATER-SOLUBLE CATIONIC GALLIUM PORPHYRIN ON EXOTOXIN PRODUCTION AND ACTIVITY IN *STAPHYLOCOCCUS AUREUS*

SPEAKER: GAWROŃSKA AGNIESZKA

SUPERVISOR(S): JOANNA NAKONIECZNA, PHD, DSC

Atopic dermatitis patients are massively colonized with Staphylococcus aureus which is associated with the severity, pathogenesis and exacerbation of that disease. Staphylococcal exotoxins are important virulence factors in the epidemiology of atopic dermatitis-derived S. aureus. Antimicrobial photodynamic inactivation - aPDI could be a promising alternative therapy for the treatment of skin inflammation as well as skin colonization in atopic dermatitis patients, where S. aureus is a predominant species.

The aim of the study was evaluation of the efficacy of photodynamic method using water-soluble cationic gallium porphyrin on the production and activity of two exotoxins (SEC, TSST-1) in S. aureus.

It has been demonstrated that photodynamic method with Ga3+CMP and green light is effective against all tested clinical isolates of S. aureus. Expression of sec gene and the pool of SEC decreases after aPDI. Expression of tsst-1 gene increases after aPDI but it is not correlated with the pool of the protein and superantigenic activity of SEC and TSST-1 decreases after aPDI.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: DETERMINATION OF THE RISK OF TOLERANCE DEVELOPMENT TO ANTIMICROBIAL PHOTODYNAMIC INACTIVATION IN CANDIDA ALBICANS AS A REPRESENTATIVE OF FUNGAL PATHOGENS

SPEAKER: GOŁAWSKA JOANNA

SUPERVISOR(S): ALEKSANDRA RAPACKA-ZDOŃCZYK, PHD

Candida albicans, is a yest-type, dimorphic fungus, common element of human gut microbiota. However, besides being a commensal, due to its opportunistic nature, it is frequently associated with severe infections occurring in high-risk individuals (e.g. with altered microbiome or immunocompromised patients). Hospital acquired infections of C. albicans are still one of major health concerns causing many deaths all around the world. It is challenging to find antimycotic drugs that would specifically target fungi cells without affecting human cells. Moreover, long-term antifungal use may contribute to the development of resistance to antibiotics. For this reason, there is an urgent need to search for novel methods to combat fungi infections. One of such alternative method could be antimicrobial blue light (aBL) and antimicrobial photodynamic inactivation (aPDI). aBL (spectrum of 400–470 nm) is an approach based on naturally occurring endogenous photosensitizing compounds such as iron-free porphyrins which, after irradiation, lead to production of reactive oxygen species (ROS) that trigger damage in microbial cell inducing its death. In contrast to aBL, aPDI requires the supply of an exogenous photosensitizing compound.

The first aim of the study was to compare the responses of two Candida albicans strains (one reference strain and one clinical strain) to aBL (415 nm) and a novel, water soluble, cationic gallium porphyrin derivative (GaCMP)-mediated aPDI. GaCMP is a porphyrin derivative with additional gallium ion. Thanks to its dual nature, GaCMP mimics naturally occurring hem and inhibits internal iron metabolism (so it acts a trojan horse) and additionally acts as a photosensitizer (PS).

Due to its multi-target mode of action, aPDI and aBL treatments are considered low risk for the development of microbial resistance and tolerance. To date, tolerance development has only been observed for gram-positive and gram-negative bacteria, but not for fungi. This is the reason why the second aim of the study was to demonstrate whether tolerance development occurs in Candida species, as a representative of fungi pathogen. For this purpose, the reference C. albicans strain was repeatedly treated with subsequent sub-lethal irradiations with (GaCMP)-mediated aPDI. The cycle of exposure - regrowth - exposure was repeated 15 times. Potential reductions in the sensitivity to GaCMP-aPDI were examined after the 5th, 10th and 15th consecutive cycles.

Examination of aBL effect alone did not lead to a significant reduction in the survival rate of C. albicans strains, however, the antimycotic effect was enhanced with additional usage of non-toxic inorganic salt potassium iodide (KI) and after overnight pre-incubation with aminolevulinic acid (ALA), which is a precursor of heme. Although, the most promising treatment was GaCMP-mediated aPDI. Studies concerning potential tolerance development did not indicate adaptation to GaCMP-mediated aPDI, which may demonstrate the superiority of antimicrobial photodynamic inactivation over antibiotics.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: DETERMINATION OF THE RISK OF TOLERANCE DEVELOPMENT TO ANTIMICROBIAL PHOTODYNAMIC INACTIVATION IN CANDIDA ALBICANS AS A REPRESENTATIVE OF FUNGAL PATHOGENS

SPEAKER: SZAFRANEK ZUZANNA

SUPERVISOR(S): MAGDA RYBYCKA-MISIEJKO, PHD

Colorectal cancer is one of the most common cancers in both men and women. The incidence in Poland increased almost 4-fold between 1980 and 2013. In patients with stage II colorectal cancer (~25% of all colorectal cancers), management after surgical resection remains a clinical dilemma. Better risk markers for recurrence would identify a high-risk group and provide a basis for administration of additional chemotherapy. To date, the literature has demonstrated a relationship between the presence of circulating tumor DNA (ctDNA) in the postoperative period with the incidence of colorectal cancer recurrence in patients. However, the data obtained is not sufficient to be considered as a diagnostic method. Therefore, this study focuses on the determination of ctDNA levels in perioperative liquid biopsy in patients diagnosed with colorectal cancer. The analysis was performed on 250 plasma samples from 25 patients. Blood samples were collected in pre-operative period (1 day and 1 hour before the surgery), during the surgery (3 timepoints: after ligating vessels, after extracting specimen, before skin closure) and directly after the surgery (3h, 6h, 12h and 24h after). In the first step, circulating cell free DNA (ccfDNA) was isolated from serum using the QIAamp MinElute ccfDNA Mini Kit. The concentration of the resulting ccfDNA was measured in duplicate using NanoDrop. The obtained values ranged between 4.95 and 18.0 ng/µL. The next step will be the verification of the obtained values through use of real-time PCR (RT-PCR). At the same time, DNA isolated from FFPE tissues was subjected to the KRAS mutation detection by qPCR. This mutation is correlated with worse prognosis. The mutation was detected in 8 out of 24 screened patients, samples from those patients will undergo additional analysis using digital droplet PCR (ddPCR). Data from RT-PCR will be compared against ddPCR output.

LABORATORY OF PHOTOBIOLOGY AND MOLECULAR DIAGNOSTICS

TITLE: THE INFLUENCE OF BLUE LIGHT ON DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO EPIDERMAL CELLS

SPEAKER: WOJCIECHOWSKA MAGDALENA

SUPERVISOR(S): AGNIESZKA BERNAT-WÓJTOWSKA, PHD

Current protocols for differentiation of embryonic stem cells into keratinocyte lineage enable obtaining merely 5-25% of keratinocytes in the population. Thus, the search for other factors, including physical ones, is necessary to increase the efficiency of stem cell differentiation into the epidermal lineage. Several publications have shown that blue light irradiation reduces proliferation in favor of increased differentiation in primary skin cultures, it is also known that photobiomodulation can alter proliferation and differentiation rates in various stem cell types.

Here we present the influence of blue light on the differentiation of mouse embryonic stem cells into epidermal cells. For this, we developed and optimized a differentiation protocol of mouse embryonic stem cells (mESC) based on embryoid bodies' production and induction toward epidermal lineage with retinoic acid, bone morphogenetic protein 4, and ascorbic acid in a serum-based medium for 6 days. The cells were exposed to blue light at 411 nm and a dose of 10 J/cm2 at various time points: d0 (mESC culture), d2 (the hanging drop stage), d5 (induction), and d7 (specification).

We observed that the blue light exposure affected the integrity and size of generated embryoid bodies and changed the expression of pluripotency, ectodermal and keratinocyte specific markers: Nanog, Pax6, Nestin, keratins: Krt8, Krt14, and Krt18, as was verified by RT-PCR and immunofluorescent staining for pan-keratins.

LABORATORY OF EXPERIMENTAL AND TRANSLATIONAL IMMUNOLOGY

TITLE: INDUCTION OF A TOLEROGENIC PHENOTYPE AS A CONSEQUENCE OF DENDRITIC CELL STIMULATION IN TIME

SPEAKER: KLIMCZUK MIKOŁAJ

SUPERVISOR(S): DANUTA GUTOWSKA-OWSIAK, PHD, DSC

The term dendritic cell was first coined in 1973 when Ralph Steinman and Zan Cohn were observing murine splenocytes. During this they discovered a small population of cells with unique stellate morphology, these cells did not fit the definition of any other that scientists have encountered previously. At that time, they did not know what the impact of their finding would have on the field of immunology. Now we know, that DCs were the missing piece in the puzzle that would finally connect the innate and adaptive immune system into a coherent whole. For this Ralph Steinman was awarded the Nobel prize in physiology and medicine in 2011. Years later we are still learning about this type of cells.

DCs are a type of professional antigen presenting cells (APCs). Their main function is to uptake and process antigens to present them to the adaptive immune system, specifically T cells. What's more, these cells can decide whether to elicit an immune response or rather induce a state of tolerance. In our body we can generally find DCs in two distinct states, the immature and mature state. The most important difference between these two is if they have encountered an antigen or not. Immature DCs have yet to encounter an antigen and are more prone to induce tolerance on the other hand mature DCs have already processed their captured antigen and are presenting them on their cell membrane hoping to induce an immune response. These two phenotypes have significant differences in cell morphology, function, and uptake capabilities. There is also a third state that DCs can enter – the tolerogenic state. Using specific tolerogenic agents such as interleukin-10 (IL-10) we can induce a state of tolerance of DCs resulting in them being more comparable to the immature phenotype despite their encounter with an antigen. These cells are called tolerogenic dendritic cells and throughout the years they have found an application in allergy therapy as well as transplantation medicine. The key factor when rendering DCs tolerogenic is the time when the tolerogenic agent is provided, based on this we can observe many different outcomes. The results in the presentation were obtained with novel techniques as well as the ones that are widely used in immunology studies. They will cover the differences between several generated tolerogenic DCs models, differing in the time of IL-10 provision, furthermore their comparison to immature and mature phenotypes.

LABORATORY OF BIOPHYSICS

TITLE: INTERACTION BETWEEN CHEMOTHERAPEUTIC DAUNOMYCIN AND PLATINUM NANOPARTICLES

SPEAKER: ŻAKOWSKA NATALIA

SUPERVISOR(S): JACEK PIOSIK, PHD, DSC

Cancer is the leading cause of death worldwide. Modern therapies and existing methods of cancer treatment do not bring the expected results. Therefore, there has been an increased need to introduce an effective treatment and a method to reduce the toxicity of currently used chemotherapy.

Daunomycin (DAU) is an anthacycline antibiotic used in chemotherapy, mainly in acute lymphocytic and myeloid leukemia. Due to the high toxicity of daunomycin and the phenomenon of acquiring resistance to cancer treatment, the need to reduce the toxicity of a given chemotherapeutic agent has increased.

Platinum nanoparticles (PtNPs) are a promising solution to the modern problem of cancer treatment. Due to their small size, they penetrate cell membranes, delivering the drug faster. Moreover, due to their selectivity towards the tumor microenvironment, they are an ideal nanoplatform for DAU. The creation of the PtNPs-DAU complex will contribute to more effective treatment of cancer patients in the future. The hypothesis of the work is that the combination of PtNPs with DAU, a potent chemotherapeutic agent, would be more effective than a single drug. The present study was designed to assess the occurrence of bonds between PtNPs and DAU and the effect of the complex on the mutagenicity of DAU.

The research methods include the use of biophysical dynamic light scattering, titration microcalorimetry and the Ames biological mutagenicity test. Biophysical techniques will enable the study of changes in the biophysical properties of DAUs after the administration of PtNPs and will enable the confirmation of the formation of potential heteroaggregates between DAU and PtNPs. On the other hand, using the biological method, the mutagenicity of DAU and the change in biological activity of DAU after administration of PtNPs will be tested.

The obtained results will enable a wider understanding of the mechanism of action of platinum nanoparticles as a nanotransmitter for DAU and many other drugs. An important aspect of the conducted research is the introduction of a therapy that enables effective treatment of patients and the simultaneous prevention of the development of resistance to therapy, which in turn will contribute to reducing the number of cancer patients' deaths. An important aspect is to conduct further experiments with the use of PtNPs with other drugs and to expand the knowledge in the field of creating interactions between a drug and metal nanoparticles.

However, futher research is needed and a more detailed analysis, involving a large group of drugs, is necessary as only a small number of results has been presented. Overall, the emerging field of PtNP applications in nanomedicine is growing fast, and it is possible to expected numerous and promising outcomes for these nanomaterials in the next future.

LABORATORY OF BIOPHYSICS

TITLE: MODULATION OF DAUNOMYCIN WITH PLATINUM NANOPARTICLES

SPEAKER: ZAKRZEWSKI MARCIN

SUPERVISOR(S): GRZEGORZ GOŁUŃSKI, PHD

Nanoparticles have been extensively studied over the last few decades. Among many applications, they are known for their anticancer activity. What is more, they have high affinity towards cancer cells, hence they can be used as nanocarriers, enhancing the anticancer activity of commonly used drugs. Herein, we tested the effects of 50 nm and 70 nm platinum nanoparticles (PtNPs) on daunomycin (DAU), an antibiotic used in treatment of different leukemia types.

To assess interactions between PtNPs and DAU we conducted fluorescence spectroscopy of the drug titrated with increasing concentration of nanoparticles. We also investigated the changes of PtNPs size when combined with different concentrations of DAU using Dynamic Light Scattering (DLS) measurements. Furthermore, we performed dialysis experiment to check the rate of drug release at three different pHs. In order to investigate the impact of PtNPs on biological effects of DAU, we performed the Ames mutagenicity test using Salmonella typhimurium TA98 strain. We analyzed different concentrations of PtNPs with the previously chosen concentration of DAU, either with 24h preincubation or without peincubation, and assessed changes in mutagenic activity of DAU induced by the nanoparticles.

The results of spectroscopic experiments indicate interactions of both tested sizes of PtNPs and DAU evidenced by changes in the fluorescence spectra of daunomycin upon titration with PtNPs. The DLS analysis shows changes in hydrodynamic diameter indicating possible aggregation of nanoparticles with the tested drug. The dialysis experiments reveals a faster and overall higher level of drug release within 48h at pH 5.4 than at pH 6.4 and 7.4 both in case of 50 nm and 70 nm PtNPs-DAU aggregates. The results of the Ames test show a change in the number of revertant colonies with increasing concentration of PtNPs. However, the 24h preincubation of PtNPs with DAU seems to reduce DAU mutagenic activity significantly more than lack of preincubation. This phenomenon is most prominent at PtNPs concentrations 500 ng and 1000 ng per plate.

All the obtained results indicate direct interactions between DAU and both tested sizes of PtNPs and their influence on DAU biological activity. Therefore there is the need to further investigate these interactions and effects of platinum nanoparticles on biological activity of daunomycin.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: FUNCTIONAL ANALYSIS AND BINDING OF ANTI-C4D IMMUNOGLOBULINS TO HUMAN LYMPHOMA CELLS TREATED WITH RITUXIMAB

SPEAKER: KAWECKA ADA

SUPERVISOR(S): MARCIN OKRÓJ, PHD, DSC

Introduction: Rituximab is a monoclonal antibody, which targets CD20 transmembrane antigen present on the surface of B lymphocytes and induces their lysis by e. g antigen and complement-dependent cellular cytotoxicity and apoptosis. It was approved as a drug for non-Hodgkin's lymphoma, but it is currently used in patients with e. g chronic lymphocytic leukemia, rheumatoid arthritis, lupus, and vasculitis. One of the main challenges during rituximab treatment is the resistance of some cancer types, presumably connected to mechanisms including excessive complement inhibitors expression, loss of CD20 epitope due to mutations, internalization, removal by monocytes, or selection of CD20low population of tumor cells due to incorrectly administered dosage of the antibody. C4d is a by-product generated after activation of classical and lectin pathway of complement system due to activity of Factor I on C4b protein. It is deposited on the membrane of B-lymphocytes with overly expressed complement inhibitors, which makes it a good potential target for secondary activation of complement in cells resistant to rituximab treatment.

Aims: The aim of this study was to analyze polyclonal anti-C4d antibodies obtained from rabbits immunized with C4d epitopes for their specificity, functionality, and binding to C4d deposited on the cell membrane of lymphoma cells and to evaluate their ability to cause lysis of human lymphoma cells resistant to rituximab treatment.

Materials and methods: Antibodies were purified from serum of rabbits immunized with epitopes derived from the C-terminal domain of C4d by two-step affinity chromatography, which ensured antibodies binding not only to C4d but also to C4 and C4b were excluded. The specificity and functionality of obtained antibodies to C4d were confirmed and compared by ELISA assay. Antibody characterized by the best functionality was tested for binding to human lymphoma cell line Ramos treated with rituximab by flow cytometry and subsequently induction of lysis of cells resistant to rituximab treatment was evaluated with calcein cytotoxicity test in Ramos and Raji cell lines.

Results: Although in serum from one of the rabbits immunised with 13-amino acid (aa) peptide relatively high amount of antibodies specific to C4b was present, after two-step purification antibodies specific to C4d were obtained. Those antibodies, however, were less functional than antibodies obtained after immunization with shorter epitopes of C4d. Antibodies recognizing 9aa epitope of C4d bound to C4d deposited on lymphoma cells stimulated with rituximab but had no effect on the percentage of cell lysis.

Conclusions: Functional polyclonal anti-C4d rabbit antibodies specific exclusively to C4d were obtained due to two-step affinity chromatography purification. Although those antibodies were able to bind to C4d deposited on human lymphoma cells, they failed to induce cytotoxicity in the examined cell lines.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: ANALYSIS OF THE SPECIFICITY OF ANTIBODIES RECOGNIZING THE C-TERMINAL LINEAR NEOEPITOPE OF THE COMPLEMENT C4D FRAGMENT

SPEAKER: JURKOWSKA PAULINA

SUPERVISOR(S): MARCIN OKRÓJ, PHD, DSC

During the activation of the classical complement pathway on the surface of cancer cells by therapeutic anti-CD20 antibodies, the C4 protein is proteolyzed to the C4b and C4a fragments. At this stage, it is possible to inhibit the cascade by complement inhibitors whose increased expression level is a feature of cancer cells. Factor I, together with the appropriate cofactors (e.g. CD46, C4BP), further proteolyzes the C4b fragment into iC4b and finally from the C4d fragment. The C4d fragment covalently bound to the membrane of the originally attacked cell is a distinguishing feature of complement-resistant populations. Generating an anti-C4d antibody and using it as a second-line targeted immunotherapy would channel a second wave of complement activation to cancer cells that are primarily more resistant.

The aim of the research was to analyze the specificity of the antibodies obtained by immunizing rabbits with peptides 5, 7 and 9 amino acids long, corresponding to the C-terminal sequence of the C4d fragment. The rabbit antiserum was purified on affinity chromatography media to which the peptides used for immunization had been immobilized. The purified antibodies were then analyzed by enzyme immunoassay (ELISA). It has been shown that thanks to the application of the above-mentioned peptides, it is possible to obtain rabbit anti-C4d antibodies devoid of cross-recognition properties of the native C4 protein and the C4b fragment. The obtained results open the way to designing an effective immunotherapeutic agent to overcome the resistance of cancer cells to complement activation. At the same time, the usefulness of the obtained antibodies for applications in immunihistochemistry as a diagnostic tool wasdemonstrated.

LABORATORY OF CELL BIOLOGY AND IMMUNOLOGY

TITLE: EXTRACELLULAR METABOLISM OF ADENINE NUCLEOTIDES REGULATES DIFFERENTIATION OF THE BREAST CANCER AND MELANOMA CELLS

SPEAKER: MUCHA PATRYK

SUPERVISOR(S): PATRYCJA KOSZAŁKA, PHD, DSC

Introduction: Breast cancer (BC) is the most common neoplastic lesion among women, while melanoma accounts for about 1% of all diagnosed skin cancers. For both cancers, distant metastases are the most frequent cause of death and complications during therapy. Hypoxia-induced accumulation of extracellular adenosine (eAdo) generated by ecto-5'-nucleotidase (CD73) is a hallmark of solid tumors. Adenosine signaling regulates the metastatic phenotype of cancer cells but it might also play a crucial role in processes of cancer cell differentiation, including the formation of cancer stem cells (CSCs), epithelial to mesenchymal transition (EMT), and its reverse phenotype change – MET.

Aim: Analysis of the role of extracellular adenine nucleotides metabolism in the regulation of cancer cell differentiation: (i) in epithelial to mesenchymal transition; (ii) in cancer stem cell functionality; (iii) and the universality of these changes through both in vitro and in vivo analysis in breast cancer and melanoma model.

Materials and Methods: For in vitro study cell lines MDA-MB-231 (triple-negative BC), E0771 LA (Luminal A-like BC), and B16F10 (metastatic melanoma) were used. For in vivo analysis model of CD73 knockout mice was used. The activity of CD73, adenosine deaminase (eADA), and adenosine receptors (A1, A2A, A2B, and A3) was modulated by inhibitors or agonists. The level of EMT/MET and CSCs markers was analyzed by the Western Blotting technique (WB) and confirmed with immunohistochemistry staining.

Results: Inhibition of CD73 increases the level of mesenchymal ALDH1A1+ CSCs in MDA-MB-231 and in B16F10 cells. Furthermore, in vivo studies show the crucial role of A2A and A3 receptors in CSCs formation. In MDA-MB-231, stimulation of A2A, A2B, and A3 receptors increases the level of EMT markers. In E0771 LA stimulation of A2B receptor increases EMT, while inhibition of CD73 and eADA has an opposite effect.

Summary: CD73 modifies EMT/MET and CSCs differentiation, while the specific adenosine receptor involved in the regulation is dependent on the type/subtype of the tumor.

LABORATORY OF BIOPOLYMERS STRUCTURE

TITLE: PEPTIDOME COMPOSITION OF SALIVA FROM PATIENTS WITH SALIVARY GLAND TUMOURS

SPEAKER: **ODRONIEC** AMADEUSZ

SUPERVISOR(S): STANISŁAW OŁDZIEJ, PHD, DSC

Salivary gland tumours belong to the head and neck tumours group and constitute 3-10% of all head and neck tumours. According to the World Health Organisation (WHO), salivary gland tumours are a highly diverse group with 30 different histological types. Most of them are benign, but the most common benign salivary tumours have a high risk of becoming malignant, with an additional risk of developing metastasis mainly towards lymph nodes. Currently, salivary gland tumours are diagnosed either by using fine needle biopsy under the USG control or other imaging techniques like X-ray, CT scan and MRI. In recent years, saliva emerged as a potential biological fluid which contains many components that reflect the health status of the whole body. Additionally, the fact that saliva is produced in salivary glands might be an additional incentive to search for molecular markers of salivary gland tumours in the body fluid.

In this project, we were able to develop and optimize the protocol of saliva sample preparation for the peptidome mass spectrometer analysis. We created the saliva peptide library of 5 patients with salivary gland tumours and 8 healthy individuals. We found 25 frequently repeating peptides and 56 frequently repeating de novo peptides between tested patient samples. Due to the low amount of patient samples, further tests should be performed to create a comprehensive list of potential salivary gland tumour markers.