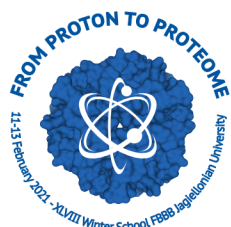
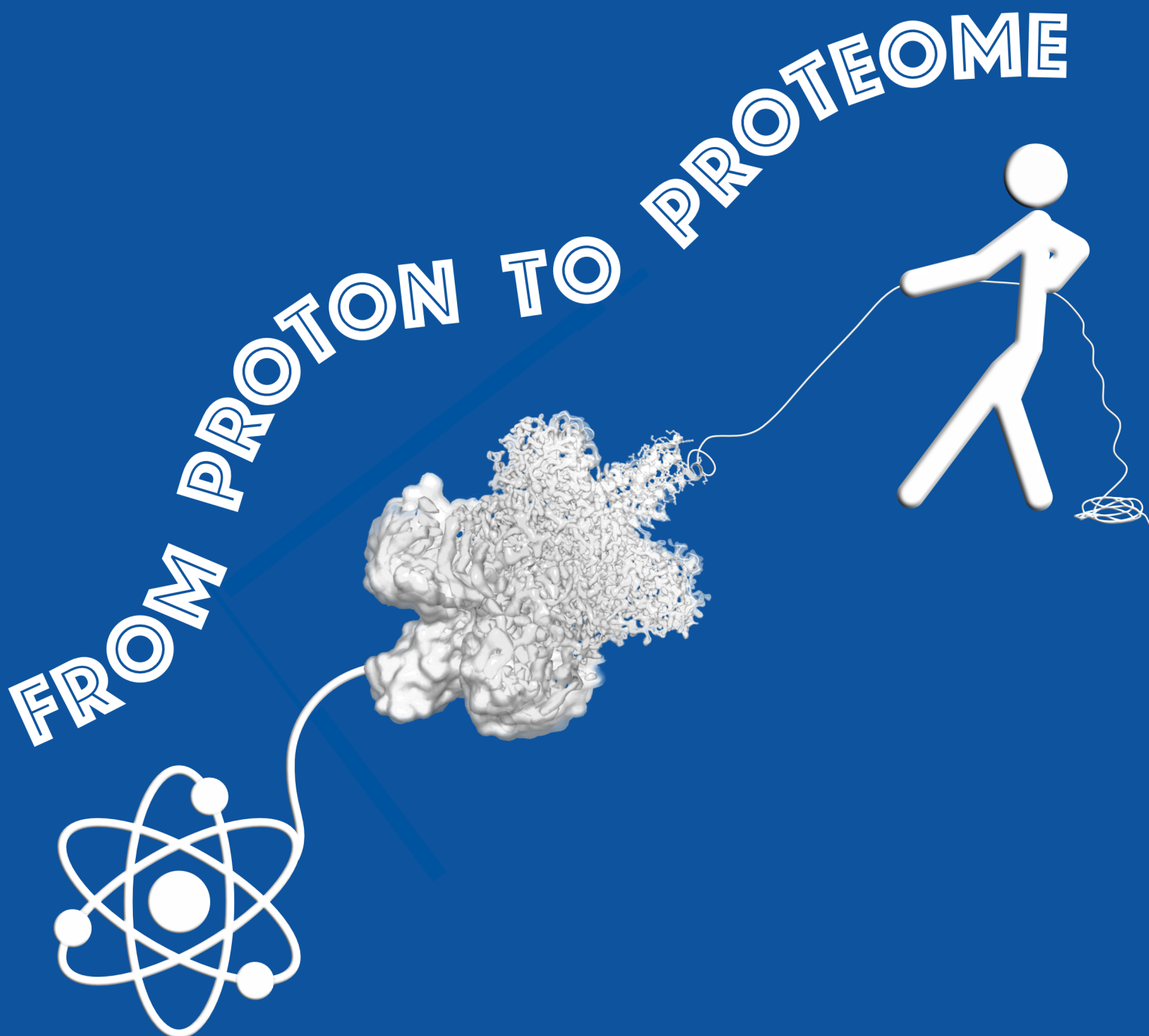


XLVIII WINTER SCHOOL

Faculty of Biochemistry, Biophysics and Biotechnology
Jagiellonian University

11-13 February 2021, on-line



50
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PROF. KRYSZYNA URBAŃSKA

“From proton to proteome”

Research interests of prof. Krystyna Urbańska focus on the biology of cancer, especially melanomas, the role of melanin, and methods of melanoma treatment, including radiotherapy and phototherapy.

In 1970-75 she studied biology at the Jagiellonian University. By defending her master's thesis entitled "Modifications to radio-resistance of human melanoma in extracorporeal culture", she began her fascination with melanomas, cancers, which she tried to understand for 45 years of her professional work (1975-2020). Her entire academic and professional career was connected with the Jagiellonian University in Krakow.



In 1976-81, still as Krystyna Cieszka, she studied animal melanomas in vivo and in vitro models after treatment with X-rays and neutrons. The defense of the doctoral dissertation entitled: "Studies on the mechanisms of radio resistance of pigmented cancer cells", took place at the Faculty of Biology and Earth Sciences of the Jagiellonian University in 1981. Her supervisor was Professor Stanisław Łukiewicz, founder and head of the Department of Biophysics at the Faculty of Biology at the Jagiellonian University. Ms. Krystyna Cieszka was at that time engaged in the implementation of various grants. In 1986 she became an assistant and then assistant professor at the Department of Biophysics.

After her doctorate, Krystyna Cieszka completed research internships at several American research centers: at EPR Center in Milwaukee Medical College (Prof. J. Hyde), State University of New York (C.S. Lange), and University of Medicine and Dentistry of New Jersey (Prof. H.Z. Hill). After returning to Poland (1994), she continued research on the radiosensitivity of melanoma cells, and began experiments related to cancer phototherapy. In 1998, after her marriage, she changed her name to Urbańska. She was awarded the D.Sc. degree in 2000, by the Faculty of Biotechnology of the Jagiellonian University. The title of her D.Sc. thesis was "Sensitization of pigmented cells and tumors of animal melanomas to ionizing radiation and light". In 2011 she became Professor.

In 2003, she became the head of the Cancer Radiospectroscopy and Radiobiology Laboratory at the Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, which she managed until 2018. Since 2013, she was also the head of the Department of Biophysics. She was a leader in several projects granted by KBN, NCN and one international (Euronanomed II). She cooperated with many domestic and foreign centers. For many years she was the Scientific Secretary of the Institute of Molecular Biology, and then FBBS of the Jagiellonian University. She was, among others co-organizer of the first conferences of the series "EPR Workshop in Biology and Medicine" organized in Krakow every 3 years since 1989 and enjoying great interest of scientists from around the world. Winner of several Rector's Awards,

as well as the Award of the President of the City of Krakow for the publication of the book "Nitric Oxide in Allograft Rejection and Anti-Tumor Response", Eds. S.J. Łukiewicz and J.L. Zweier, 1998.

She was the supervisor of 9 PhDs and over 30 MSc students, is the author of several monographic lectures: "Radiobiology", "Biology of cancer", "Hyperthermia, hypothermia and thermotherapy", "Radiation biophysics". Prof. Urbańska is a co-author of 90 original scientific publications, 4 book chapters and over 100 conference abstracts presented at national and international conferences.

Program of the Jubilee Session of Prof. Krystyna Urbańska shows the most important scientific themes in her research - from the therapy of melanoma cells and tumors with a proton beam, through research on the role of melanins in melanoma, the role of vitamin D in anti-cancer therapy, through gold and silver nanoparticles, to a wide range area of phototherapy and application various derivatives - chlorins, bacteriochlorins and chlorophyllides. New research areas and questions emerging from Her work and some of the new perspectives will be discussed during the session.

LOCAL ORGANIZING COMMITTEE

Conference Chair

dr hab. Martyna Elas, prof. UJ

Short Oral Presentations and Posters

dr Anna Kozińska

dr Martyna Krzykawska-Serda

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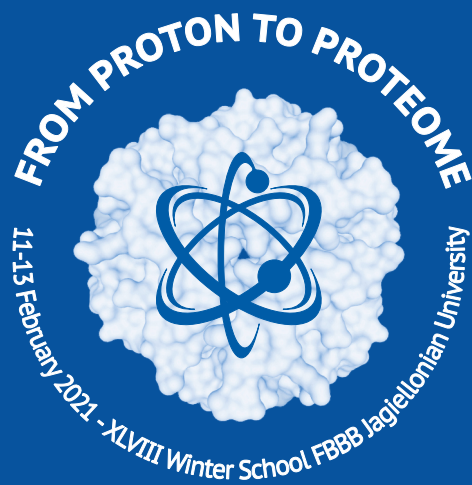
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Book of Abstracts and Graphics

dr Adam Kłóś

* * *

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PROGRAM

XLVIII WINTER SCHOOL

From proton to proteome

Conference Schedule, 11-13 February 2021, OnLine

11 Thursday

/ Session 1 - Chair: Martyna Elas /

- 12:00 - 12:05 Jolanta Jura**
Opening remarks
- 12:05 - 12:20 Martyna Elas**
From proton to proteome - scientific contribution of Prof. Krystyna Urbanska
- 12:20 - 12:40 Bożena Romanowska-Dixon**
Eye melanoma research – beyond the scientific collaboration
- 12:40 - 13:00 Przemysław M. Płonka**
Melanin as a natural, endogenous spin probe: old tracks and new perspectives
- 13:00 - 13:20 Maja Grabacka**
Fenofibrate – an old drug with new powers reaching far beyond the PPAR α activation
- 13:20 - 13:40 Marcin Kruszewski**
The influence of Ag and Au nanoparticles on tumor metastasis
- 13:40 - 14:00 Andrzej Słomiński**
Vitamin D derivatives and melanoma
- 14:00 - 14:10 break**
- / Session 2 - Chair: Leszek Fiedor /
- 14:10 - 14:30 Alicja Ratuszna**
Photodynamic therapy of cancers (PDT) – novel resolutions
- 14:30 - 14:50 Grażyna Stochel**
Bacteriochlorins in Photodynamic Therapy of cancer
- 14:50 - 15:10 Janusz Dąbrowski**
Photodynamic therapy of immunogenic cancers combined with PD-1/PD-L1 checkpoints blockade immunotherapy
- 15:10 - 15:30 Leszek Fiedor**
Towards Green Phototherapy
- 15:30 - 15:50 Zenon Matuszak**
From Lambert-Beer-Bourger law to proton therapy. A system approach. The long pathway

12 Friday

- 10:30 - 11:30 poster & short oral session**
- 11:30 - 12:00 break**
- / Session 3 - Chair: Maja Grabacka /
- 12:00 - 12:20 Jacek Stępniewski**
New tools for disease modeling and drug research
- 12:20 - 12:40 Anna Grochoń-Przęczek**
Beyond repression of Nrf2: the role of Keap1 in endothelial cells
- 12:40 - 13:00 Piotr Brzoza**
Role of protein chemerin in skin defense
- 13:00 - 13:20 Mikołaj Baranowski**
3D whole-body high temporal resolution electron paramagnetic resonance imaging
- 13:20 - 13:40 Paweł Ferdek**
"Walking a thin line": Bcl-2 family proteins, Ca²⁺ signalling and the pancreas
- 13:40 - 14:00 break**
- / Session 4 - Chair: Agnieszka Łoboda /
- 14:00 - 14:20 Martyna Krzykawska-Serda**
Calcitriol and hyperthermia treatment combined with chemotherapy against mouse and human pancreatic cancer in vitro
- 14:20 - 14:40 Małgorzata Durbas**
Swaying autophagy in neuroblastoma for therapeutic advantage
- 14:40 - 15:00 Mariusz Madej**
Dynamic oligopeptide acquisition by the RagAB transporter from Porphyromonas gingivalis
- 15:00 - 15:20 Justyna Karkowska-Kuleta**
The involvement of candidal moonlighting proteins in mixed biofilm formation and interactions with host
- 15:20 - 15:40 Aleksander Grabiec**
Epigenetic regulation of inflammation in periodontitis: targeting histone acetylation

13 Saturday

- 10:30 - 11:30 poster & short oral session**
- 11:30 - 12:00 break**
- / Session 5 - Chair: Grażyna Stochel /
- 12:00 - 12:20 Magdalena Olchawa**
The effect of quercetin on photic stress mediated by rhodopsin photobleaching products in retinal pigment epithelium (RPE)
- 12:20 - 12:40 Agnieszka Banaś**
That's what the Arabidopsis photolyases are about
- 12:40 - 13:00 Nada Tokodi**
Variable response of Raphidiopsis raciborskii strains to chill/light stress
- 13:00 - 13:20 Jan Łyczakowski**
Molecular architecture of wood: from sugars to trees
- 13:20 - 13:40 Adam Antosiak**
Cyanophages hijack and maintain the metabolism of cyanobacteria
- 13:40 - 14:00 break**
- / Session 6 - Chair: Sylwia Kędracka-Krok /
- 14:00 - 14:20 Mateusz Wilamowski**
Methylation of viral mRNA Cap by Nsp10/16 2' O MTase from SARS-CoV-2
- 14:20 - 14:40 Agnieszka Hoang-Bujnowicz**
Dynamics of 53BP1 and chromatin fibres in DNA repair foci
- 14:40 - 15:00 Dorota Mularczyk**
A Closer Look at Tanford Transition of β -lactoglobulin
- 15:00 - 15:20 Edyta Żyła**
Structural characteristics of macrocyclic peptide as a potent modulator of PD-1/PD-L1 immune checkpoint axis
- 15:20 - 15:40 Jerzy Kotlinowski**
Regulation of liver homeostasis by Mcip1 RNase



PLENARY LECTURES

Cyanophages hijack and maintain the metabolism of cyanobacteria

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There is a growing interest in how cyanophages maintain the vital functions of their hosts by introducing rearrangements in their metabolic processes. However, the existing hypotheses have been formulated mainly on the basis of genomic analyses while knowledge about the physiology of infections remains very limited. Therefore, our aim was to investigate how the vb-AphaS-CL131 cyanophages influence the metabolism of *Aphanizomenon flos-aquae* 2012/KM1/D3, a trichome-forming cyanobacterium. We documented stable expression of selected host genes involved in photosynthetic electron transport and modified expression of genes involved in carbon metabolism. Chlorophyll fluorescence *in vivo* measurements showed that a viral infection caused a partial reduction of the photosystem II efficiency and a subsequent decrease of electron transport around PS II. Phage infections rearranged the host metabolism resulting in increased ATP and NADPH concentrations which suggests their reduced consumption by the host or/and redirection of these products to viral assembly. The conducted experiments provided an initial insight into the mechanisms of *A. flos-aquae* infection by the CL131 phage, however, further studies are needed to obtain a more comprehensive understanding of these processes.

That's what the Arabidopsis photolyases are about

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Ultraviolet irradiation may cause damage of many molecules including nucleic acids. Among pyrimidine dimers formed between adjacent pyrimidines in a DNA strand the most frequent are cyclobutene pyrimidine dimers (CPDs), followed by (6-4) pyrimidine-pyrimidone photoproducts (6-4 PPs). In most organisms, except placental mammals, these photolesions are repaired by specialized enzymes, photolyases. Photolyases belong to one protein family with blue light photoreceptors, cryptochromes. Photolyases recognize and bind the dimers and use UVA/blue light energy to directly split them back to the undamaged pyrimidines.

Five genes with known or putative photolyase activity were identified in Arabidopsis genome. Our research have focused on three of them: *AtUVR3* (6-4 PP specific photolyase), *At4g25290* and *At2g47590*.

AtUVR3 was found in nuclei, chloroplasts and mitochondria [1]. This protein partially colocalized with chloroplast nucleoid even in absence of UV. Surprisingly, *AtUVR3* expression was down-regulated by light in a photosynthesis -dependent manner. The red light-controlled retrograde signalling involved in regulation of this expression was modulated by phyA, but only in absence of phyB. Photorepair of 6-4 PPs was very efficient in both Arabidopsis and Nicotiana transgenic plants overexpressing *AtUVR3*.

Two putative photolyases encoded by *At4g25290* and *At2g47590* were found in chloroplasts. The mRNA levels of both genes increased upon light. The phenotypes of these plants suggest the role of encoded proteins in regulation of photosynthesis and chloroplast biogenesis. Moreover, functioning of *At2g47590* as a blue light photoreceptor cannot be excluded.

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3D whole-body high temporal resolution electron paramagnetic resonance imaging

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Low-frequency electron paramagnetic resonance imaging (EPRI) is a powerful tool for non-invasive measurements of the physiological status of different organs [1-3]. Here, we report on the functionality of an ultrafast dynamic EPRI. For the EPR imaging, ERI TM600 tomograph was used. As a spin probes 3-CP nitroxide ($3\mu\text{mol/g b.w}$) for head imaging and OX063 ($0.6\mu\text{mol/g b.w}$) for body imaging were used. During 1h experiment, 3D images were collected with respectively 23 and 4.5s intervals for head and body. For each voxel, the empirical mathematical model [4] (EMM) was fitted. Imaging was performed on two BalbC mice aged 7 weeks and body weight $\sim 27\text{g}$. The blood-brain barrier (BBB)-permeable nitroxide combined with EPRI was used to obtain 3D images of a mouse's head. The temporal resolution of the imaging was 23 s. A high-resolution mouse body images were acquired with 4.5 s intervals. Eight different pharmacokinetic maps were created from EMM results. The present results suggest that a dynamic 3D in vivo EPR imaging system combined with different spin probes is a useful non-invasive tool for assessing changes from various parts of the body in rodent models. Also, curvature analysis allows to increase the spatial resolution of the obtained images. In conclusion, this imaging method can be used for monitoring the effects of therapeutic response and the changes between different tissues.

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The influence of Ag and Au nanoparticles on tumor metastasis

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Despite many current and foreseen application of engineered nanoparticles (ENPs), there are still gaps in our knowledge regarding ENPs effects on human. One of the biggest challenges is the interaction of ENPs with carcinogenesis and tumour metastasis. This study was focused on the influence of silver and gold nanoparticles on cancer progression and metastasis.

The results showed that silver but not gold nanoparticles attenuate growth of primary mouse tumour and the number of metastases when given orally to mice. The same nanoparticles administered intravenously had no effect. However, when human derived cancer cells were inoculated to immunocompromised mouse no effect was observed. Supplementary in vitro experiments showed that neither silver nor gold nanoparticle affected cell motility or invasiveness. Gene expression analysis revealed also lack of effect of nanoparticles on expression of genes responsible for cancer progression and metastasis. In addition, very slight changes were observed in regards of epigenetic gene regulation.

The results of this project put new light on the potential applications of silver nanoparticles that, so far, were used mostly as a strong antibacterial agent. The results of this project enable also better understanding of the effect of nanomaterials on model organisms on the molecular and cellular level, including especially the effect of nanomaterials on human health and determination of the potential risk or benefit related with their use. Apart from the scientific value, results of this project give a new hope to the cancer patients, as if confirmed in clinical tests might be a valuable supportive therapy.

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Role of protein chemerin in skin defense

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Skin forms the first layer of defence against external environment. The ability of this barrier organ to react to environmental threats such as microbial invasion is dependent on a presence of a variety of proteins – responsible for direct antimicrobial activity, cell signaling or chemotaxis of immune cells.

Among them chemerin may play an important role as it exhibits both chemotactic function for specific immune cells as well as antimicrobial functions against skin-associated microbes. Liver and adipose tissue are the main expression sites of blood-circulating chemerin. However, this protein is also produced locally in epidermis and dermis, where its expression was shown to be altered in response to pathological conditions like chronic inflammatory disease-psoriasis or bacterial colonization.

To gain its chemoattractant properties, chemerin must undergo limited proteolysis at the c-terminus. Whereas various enzymes were reported to act as chemerin activators, factors responsible for chemerin cleavage in the skin are largely unknown. Here we show potential proteolytic mechanisms underlying chemerin activation in epidermis. We also discuss antimicrobial function of chemerin in the context of skin infection with cutaneous pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

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Photodynamic therapy of immunogenic cancers combined with PD-1/PD-L1 checkpoints blockade immunotherapy

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Although immunotherapy has shown promising clinical results recently, a large number of patients still have a poor response this treatment, and therefore, more potent combinational therapies are required. Immunotherapy based on the modulation of immune checkpoints such as programmed death-1 (PD-1) and its ligand (PD-L1) in combination with photodynamic therapy (PDT) may be an innovative strategy for more resistant cancers.

We employed a set of NIR absorbing photosensitizers of different polarity to compare the PDT efficacy after various drug-to-light intervals: 15 min. (V-PDT), 3h (Ė-PDT) and 72h (C-PDT). We also performed the analysis of the molecular mechanisms of PDT crucial for the generation of the long-lasting antitumor immune response and indicated that PDT-induced cell death might affect the integrity of the host tissue and develop acute local inflammation, which in turn led to the infiltration of neutrophils and macrophages. It was also possible to evaluate whether modulation of inflammatory mediators induced by PDT determines the mode of cell death, tumour microenvironment and the susceptibility to systemic PD-1/PD-L1. We have demonstrated that PDT in vivo contributed to an immunogenic environment in the tumour, which may enhance PD-1/PD-L1 checkpoint blockade therapy by stimulating a strong and long-term antitumor immune response. Moreover, based on rechallenge experiments, we assessed the generation of immune memory able to prevent tumour relapse, similar to the functions of cancer vaccines. We suggest that the combination of PDT with PD-1/PD-L1 axis inhibition designed in the current study offers a new strategy for treating metastatic cancers with primary tumours accessible by PDT.

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Swaying autophagy in neuroblastoma for therapeutic advantage

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GD2 ganglioside is one of the most important molecular targets for immunotherapy in neuroblastoma [1]. Another important target in neuroblastoma, which has achieved wide popularity, is aurora A kinase [1]. We have recently shown that combination of an anti-GD2 ganglioside 14G2a mouse monoclonal antibody (14G2a mAb) and an aurora A kinase specific inhibitor, MK-5108, significantly potentiates cytotoxicity against IMR-32 and CHP-134 neuroblastoma cells *in vitro*, as compared to the inhibitor used alone [2]. Our recent studies focused on the process of autophagy and its role in survival of human neuroblastoma cell cultures upon treatment with these two agents [3]. We explored autophagy-related mechanisms in the observed cytotoxic effects, exerted by both agents using cytotoxicity assays, RT-qPCR, immunoblotting, and autophagy detection methods. By assessing autophagic flux, we reported enhancement of the autophagy process in the 14G2a mAb- and MK-5108-treated IMR-32 cells. Application of chloroquine (CQ), being a lysosomotropic agent, affected the 14G2a mAb- and MK-5108-stimulated autophagic flux indicating that the 14G2a mAb (40 µg/ml) and MK-5108 inhibitor (0.1 µM) induce autophagy in the IMR-32 cells. Furthermore, the combinatorial treatment of the IMR-32 cells with the 14G2a mAb and CQ significantly potentiates cytotoxic effect, as compared to CQ used alone. Most importantly, we showed that interfering with autophagy at its early and late step augments the 14G2a mAb-induced apoptosis. Therefore, inhibition of autophagy is the primary mechanism of the CQ-mediated sensitization to the 14G2a mAb-induced apoptosis. Defining optimal strategies to modulate autophagy for therapeutic advantage can broaden the treatment options of neuroblastoma.

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“Walking a thin line”: Bcl-2 family proteins, Ca²⁺ signalling and the pancreas

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Acute pancreatitis (AP) is a severe disease of the pancreas, often caused by alcohol abuse or migrating gallstones [1]. It is triggered by dysregulated Ca²⁺ signalling in enzyme-storing pancreatic acinar cells (PACs), which leads to premature activation of trypsin *in situ* and autodigestion of the organ [1]. While extensive necrosis of PACs correlates with the severity of AP, apoptosis becomes predominant in mild cases of AP [2]. As a result, it has been proposed that modulation of cell death in the pancreas, that is increasing the ratio of apoptosis to necrosis, could be used as a therapeutic strategy to ameliorate the severity of AP. Small molecule Bcl-2 inhibitors were suggested as potential tools for this purpose. These compounds bind to anti-apoptotic Bcl-2 family proteins (such as Bcl-2 and Bcl-xL) and disrupt their interaction with pro-apoptotic proteins (such as Bax and Bak) sensitizing cells to apoptotic death [3]. However, Bcl-2 family proteins not only control the programmed cell death, but are also involved in the regulation of the intracellular Ca²⁺ homeostasis by interacting with and/or modulating the activity of Ca²⁺ channels and Ca²⁺ pumps [3]. Therefore inhibition of anti-apoptotic Bcl-2 proteins in PACs might pose a risk of throwing Ca²⁺ signalling off balance and, as opposed to protecting against severe AP, promote premature activation of digestive enzymes and necrosis. This talk aims to provide a historical overview of selected Bcl-2 inhibitors, present their effects on intracellular Ca²⁺ homeostasis and to highlight the concept of cell death modulation in AP.

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Towards Green Phototherapy

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The life on our planet is energetically entirely dependent on the activity of chlorophylls, the major photosynthetic chromophores of organisms which are able to convert light energy into chemical energy. Inevitably, as the components of plants these pigment directly interact with the animal and human organisms. Also, in practical terms, polar derivatives of chlorophylls find their applications in phototherapy. The best example of that is a palladium-substituted derivative of bacteriochlorophyll, Tookad®, very recently admitted in many countries, including the UE, for clinical use as a photosensitizer in cancer photodynamic therapy. The present summary, the outcome of our joint investigations focused on the chlorophyll-animal organism interactions and on the development of novel derivatives of chlorophyll of potential use in PDT is discussed. Our results show that chlorophyll derivatives are harmful phototoxins and as such they are selectively recognized and removed from the body by the xenobiotic transport system [1,2]. Applying chlorophyll derivatives as intrinsic probes allowed us to discover that growing solid tumor strongly affects the expression of breast cancer resistance protein and enhances its transport activity [3]. On the other hand, the metallosubstituted chlorophylls showed a very high cytotoxicity and efficacy as photosensitizers in phototherapy of A549 tumors in mouse model [4]. The results of our cooperative studies on the chemically modified chlorophylls revealed their great potential as photosensitizers and diagnostic agents in cancer therapy.

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Fenofibrate – an old drug with new powers reaching far beyond the PPAR α activation

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Fenofibrate was developed in 1970s and used to normalize plasma lipid profile. Later, it has been identified as a potent agonist of the peroxisome proliferator-activated receptor alpha (PPAR α). Our research, dated from the end of 1990s, helped to shed light on much more pleiotropic and often PPAR-independent activities of fenofibrate, including: anti-metastatic action in hamster melanoma model in vivo; modulation of several signal transduction pathways involved in oncogenesis and metabolism; mitochondrial respiration blockade and alteration of phospholipid model membrane fluidity. All these activities predestine fenofibrate as a supportive therapeutic agent against neoplasms of neuroectodermal origin.

Epigenetic regulation of inflammation in periodontitis: targeting histone acetylation

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Epigenetic mechanisms, namely DNA and histone modifications, are critical regulators of immunity and inflammation which have emerged as potential targets for immunomodulating therapies. The prevalence and morbidity of periodontitis, in combination with evidence that genetic, environmental and lifestyle factors cannot fully explain the susceptibility of individuals to disease development, have driven interest in epigenetic regulation as an important factor in periodontitis pathogenesis. The expression of enzymes that regulate histone acetylation, histone deacetylases (HDACs), is dysregulated in periodontitis-affected gingival tissue and in gingival fibroblasts (GFs) and epithelial cells infected with oral pathogens. These epigenetic changes are associated with increased expression of inflammatory cytokines, chemokines, and matrix-degrading enzymes. Our studies show that excessive production of these inflammatory mediators caused by *Porphyromonas gingivalis* or inflammatory tissue environment is suppressed by small molecule inhibitors of HDACs (HDACi) (Lagosz et al., 2020) or BET bromodomain proteins that interact with acetylated histones (Maksylewicz et al., 2019). Importantly, these compounds have no effect on GF viability and on susceptibility to bacterial invasion. Through the combination of pharmacological and gene silencing approaches we identified HDAC3 as a key epigenetic enzyme involved in GF inflammatory activation. Finally, we demonstrated that HDACi and BET inhibitors are equally effective in suppressing *P. gingivalis*-induced inflammatory activation of GFs isolated from healthy donors and periodontitis patients. Our results, in combination with evidence that HDACi and BET inhibitors ameliorate inflammation and alveolar bone resorption in animal models of periodontitis, identify HDACs and BET proteins as potential targets for host modulation therapy in periodontal disease.

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Dynamics of 53BP1 and chromatin fibres in DNA repair foci

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Induction of DNA double-strand break results in recruitment of repair factors to the site of damage. P53-binding protein 1 (53BP1) is a multifunctional repair factor that recognizes chromatin marks induced by DNA double-strand breaks and accumulates at the site of damage. The aim of this study is to elucidate the role of 53BP1 in stabilizing DNA damage repair foci. We used fluorescence correlation spectroscopy (FCS) and fluorescence loss in photobleaching (FLIP) methods to characterize the dynamic properties of 53BP1 upon DNA damage induced locally by visible light. The FLIP results suggest that 53BP1 dynamically exchanges between DNA damage foci and the surroundings, and that the rate of this exchange is lower than the rate of exchange between intact chromatin of the treated nucleus and mobile pool in the surrounding nucleoplasm. Moreover, FCS data show that following damage induction mobility of 53BP1 increases in the whole nucleus, but the fraction of 53BP1 bound to chromatin in the repair focus is less mobile than in the surrounding nucleoplasm. Concomitantly, a large decrease in the dynamics of chromatin fibres based on FCS of the core histones was not observed after inducing damage. We conclude that, although 53BP1 accumulates at the site of damage, it forms a focus of a dynamic structure that does not affect severely the movement of chromatin fibres.

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The involvement of candidal moonlighting proteins in mixed biofilm formation and interactions with host

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Multitasking proteins that perform different, unrelated functions in a distinct cellular localisation are collectively named as moonlighting proteins. This group includes cytoplasmic enzymes involved in basic metabolic processes, which repeatedly are identified on the cell surface of microbial pathogens, including *Candida* pathogenic fungi [1]. Transport inside the extracellular vesicles might be one of the potential mechanisms of their atypical secretion to the fungal cell surface. In this unusual location, independently of their primary function, fungal moonlighting proteins demonstrate involvement in the interactions of *Candida* cells with host ligands or with other microorganisms coexisting in the same infectious niche. One example of such proteins is the glycolytic enzyme enolase and its surface display may be provided by attachment to the typical fungal cell wall protein, agglutinin-like adhesin Als3 [2]. Fungal enolase plays an important role in the formation of mixed biofilms by *C. albicans* and key periodontal pathogen, anaerobic bacteria *Porphyromonas gingivalis*. Moreover, modification of enolase by bacterial enzymes alters fungal interactions with important host plasma proteins, including human plasminogen and kininogen [3].

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Beyond repression of Nrf2: the role of Keap1 in endothelial cells

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Keap1 is a redox-sensitive protein directly repressing the Nrf2 transcription factor. The significance of Nrf2 in the orchestration of stress response and maintenance of cellular homeostasis has been broadly described. However, the role of Keap1 has hardly been used to outreach the regulation of Nrf2 activity and redox sensing. Our study shows that Keap1, not restrained by Nrf2 in the cytoplasm, modulates endothelial cell (EC) angiogenic response to GDF-15 and SDF-1 by regulating actin polymerisation via RhoGAP1-dependent mechanism. Moreover, Keap1 forms an S-nitrosation (SNO) enzymatic complex together with GAPDH transnitrosating protein and nitric oxide synthase (NOS), hence controlling SNO-dependent NOX4 activity and senescent/apoptotic fate of ECs. Finally, Keap1 facilitates the accumulation of protein aggregates in ageing and prematurely senescent ECs, thus emerging as a critical modulator of cellular proteostasis. These data point up the significance of Keap1 and the impact of balance between Nrf2 and Keap1 in endothelial cell biology.

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Regulation of liver homeostasis by Mcpip1 RNase

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Monocyte chemoattractant protein-induced protein 1 (MCP1) is an endonuclease that acts as a negative regulator of inflammation, through degradation of proinflammatory cytokines transcripts. Additionally, by regulating stability of CEBP/β transcript and cellular pool of miRNA, MCP1 inhibits adipogenesis. Knowing that liver is a key organ regulating lipid and glucose metabolism we investigated MCP1 involvement in its homeostasis.

We used control C57BL/6 mice that were fed with chow or high-fat diet for 12 weeks. Additionally, Mcpip1^{fl/fl} and Mcpip1^{fl/fl}Alb^{Cre} mice, with genetic deletion of *Zc3h12a* were used.

Mcpip1 level is reduced both in adipose tissue and primary hepatocytes from C57BL/6 mice subjected to NAFLD development, but deletion of Mcpip1 in Mcpip1^{fl/fl}Alb^{Cre} animals did not influence NAFLD progression. Although we detected a reduced hepatic expression of genes regulating glucose metabolism and β-oxidation in these mice, they did not develop any abnormalities related to metabolism in comparison to Mcpip1^{fl/fl} controls. On the other hand, we found that Mcpip1 deficiency in the liver recapitulates most of the features of human primary biliary cholangitis (PBC). In Mcpip1^{fl/fl}Alb^{Cre} livers, intrahepatic bile ducts displayed proliferative changes with inflammatory infiltration, bile duct destruction, and fibrosis leading to cholestasis. In plasma, increased concentrations of IgG, IgM, and AMA autoantibodies (anti-PDC-E2) were detected.

In conclusion, we demonstrated that Mcpip1 protein plays an important role in the liver homeostasis. Mcpip1^{fl/fl}Alb^{Cre} mice display early postnatal phenotype that recapitulates most of the features of human PBC.

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Molecular architecture of wood: from sugars to trees

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Wood is primarily composed of plant secondary cell walls which are made from polysaccharides, such as cellulose and hemicelluloses, and a polyphenolic compound known as lignin. The woody secondary cell walls of trees are the largest repository of renewable carbon biopolymers on the planet, as such they are a perfect feedstock for use in alternative industrial processes that tackle the climate emergency. The arrangement and interactions of plant cell wall components, also known as the molecular architecture of wood, has tremendous importance for tree growth and industrial application of timber. Despite that, the molecular architecture of wood remains poorly understood. This presentation will explore recent progress in the study of wood molecular architecture, highlighting advances obtained using low temperature scanning electron microscopy and biochemical analysis of cell wall polysaccharides. Approaches and techniques used to analyse polysaccharide and lignin structure recently adapted at the Faculty of Biochemistry, Biophysics and Biotechnology will be presented. These tools can be applied in the study of various polysaccharides in both plant and non-plant systems. Finally, an attempt will be made to link the observed molecular features of polysaccharide structure with characteristics of wood, highlighting the role of cell wall molecular architecture in the maintenance of timber properties.

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Dynamic oligopeptide acquisition by the RagAB transporter from *Porphyromonas gingivalis*

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Porphyromonas gingivalis is the main pathogen involved in the development of chronic periodontitis. Due to its asaccharolytic character, this Gram-negative bacterium is unable to metabolise carbohydrates, but instead acquires peptides provided by proteolytic enzymes called gingipains. Despite the fact that peptides are crucial for growth and survival of *P. gingivalis*, the mechanism of their uptake is completely unknown. This study presents the structural and functional characterisation of a RagAB complex transporting peptides through the outer membrane of *P. gingivalis*. Therefore, it sheds new light on the uptake of peptides by Gram-negative bacteria.

The crystal structure of RagAB was solved by molecular replacement. RagAB forms the hetero-tetrameric complex composed of two subunits of RagA and two subunits of RagB (RagA2B2). Examination of the RagAB dimer interface showed the electron density which can be modelled as a peptide of ~13 residues in length suggesting that both RagA and RagB form a peptide binding site. Single-particle cryo-electron microscopy revealed three dynamic states of RagAB: double closed, single closed and double open and show conformational changes of the plug domain upon substrate binding. Specificity of both complexes towards peptide length and amino acid composition was determined using mass spectrometry. The structure-function studies were carried out by comparing the growth of RagAB mutants in minimal medium with bovine serum albumin as a sole peptide source.

The results obtained in this study will contribute to better understanding of peptide transport by Gram-negative bacteria and may allow development of specific drugs attenuating virulence of *P. gingivalis*.

From Lambert-Beer-Bourger law to proton therapy. A system approach. The long pathway

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To truly understand the mechanisms of phenomena (including biological), however, we must be able to describe the phenomena mathematically. This allows the experimenter to identify key factors and predict future behavior of the system under study. In this short summary, I would like to show some common elements, some mathematics, which is present in many of the undertaken issues and often at first glance, not obvious. This common denominator is counting of objects, from molecules to cells and probability. It is apparent in the research methods used, in absorption, fluorescence and ESR spectroscopy, and in investigated therapeutic strategies such as photodynamic therapy, ionizing radiation therapy, including proton therapy. It is present in all topics related to the study of melanins, melanoma and oxidative stress. In all these cases, counting of molecules and cell plays a fundamental role illustrating power of systemic approach. The presentation is illustrated with the results of some of studies done over the years in the Laboratory of Cancer Radiospectroscopy and Radiobiology (Department of Biophysics).

A Closer Look at Tanford Transition of β -lactoglobulin

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β -lactoglobulin is a milk protein which binds hydrophobic molecules inside a β -barrel. The binding is affected by a conformational change, so-called Tanford transition, triggered by a change of an environment's pH. When the pH falls below 7, one of the loops flanking the entrance to the barrel flips in a way that obstructs the entrance, thus impeding the entry of ligands and lowering the binding constant. The crucial role in Tanford Transition has been attributed to a change of protonation state of glutamic acid residue at position 89 (Qin *et al.*, 1998). However, the results obtained during our study indicate that leucin residues at positions 39 and 87 are also significant for the transition. In order to deepen the understanding of the role of the leucin residues, variants of β -lactoglobulin with amino acid substitutions at positions 39, 87 and 89 were examined. Circular dichroism spectra confirmed that the substitutions did not alter the overall structure of the protein while isothermal titration calorimetry allowed to compare binding of sodium dodecyl sulphate to different variants of the protein at pH 6.5 and 7.5. The results show that the binding constants are similar at both pH values for almost all variants, which suggests a cancelation of Tanford transition. More details on how the substitutions affect the binding are available through comparison of enthalpic and entropic contributions to the free energy of binding.

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The effect of quercetin on photic stress mediated by rhodopsin photobleaching products in retinal pigment epithelium (RPE)

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A growing body of experimental evidence suggests that rhodopsin (Rh) photobleaching products (RhPBP), such as all-*trans*-retinal (ATR), may be involved in light-induced damage to the retina. Here we asked if ATR formed in photoreceptor outer segments (POS) may contribute to dysfunction of the retinal pigment epithelium (RPE) under *in vitro* photic-stress conditions and whether quercetin can modify phototoxic potential of Rh photobleaching products.

Control ARPE-19 cells or quercetin enriched cultures were pre-loaded with Rh-rich POS isolated from bovine retinas and irradiated with green light (520-580 nm, 7 mW/cm²) to photobleach Rh, and subsequently irradiated with blue light (425 nm, 10 mW/cm²). Phototoxicity mediated by RhPBP was analyzed in control ARPE-19 cells and quercetin supplemented cultures by determining cell survival (MTT), mitochondrial membrane potential (MMP) (JC-1), oxidation of cellular proteins (CBA), and the cell specific phagocytic activity (flow cytometry). Aerobic photoreactivity of RhPBP in POS was analyzed by electron paramagnetic resonance (EPR) oximetry, EPR-spin trapping and time-resolved detection of singlet oxygen phosphorescence.

Cytotoxicity measures and MMP analyses confirmed that supplementation with quercetin protected ARPE-19 cells against photic stress mediated by RhPBP. Furthermore, enrichment of ARPE-19 cells with quercetin significantly reduced the inhibitory effect of RhPBP-mediated photic stress on POS phagocytosis and the RBPB ability to photooxidize cellular proteins or BSA in model systems. Quercetin lowered also the detectable photoreactivity of RhPBP.

This data supports the hypothesis that quercetin may efficiently diminish the phototoxic action of RhPBP, necessary for restoring the phagocytic function of ARPE-19 cells.

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Melanin as a natural, endogenous spin probe: old tracks and new perspectives

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The most important lesson I was taught by Prof. Urbańska in the very beginning of my scientific work concerned cell cultures in vitro. Initially she herself prepared for me primary cultures of melanoma cells for to EPR measurements, and gradually I learned from her, how to implant and harvest a tumor, trypsinize and purify the cells, count them, how to prepare a primary and secondary culture. The present report concerns the still unpublished data from that time. They were focused on melanins as endogenous spin probes to investigate intracellular paramagnetomes [1] and interactomes of S-91 melanoma cells in vitro. It also contains preliminary in vivo data, which have turned out encouraging. Since then, this topic has been rarely analysed in literature but looking at the present state of melanometry [1,2], only recently have I found them satisfactory enough to be shown in public.

They concern saturability of EPR melanin signal with power of continually applied microwaves in cells of Cloudman S91 melanoma obtained from in-situ growing tumors and re-implanted or maintained in the primary in vitro culture, at X-band. These data may inform on the paramagnetic surrounding of melanin, and on melanin itself, and in general they encourage one to continue these investigations.

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Photodynamic therapy of cancers (PDT) – novel resolutions

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Scientific research conducted by physicists and chemists have become increasingly more important in developing new and effective cancer therapies, the disease that continues to take a heavy toll. It is worth noticing that radio - and chemotherapies would not have existed without the contribution of these sciences. Although applications of these therapies destroy cancer cells, unfortunately, they also destroy healthy cells.

Therefore, increasing expectations are associated with photodynamic therapy. The main advantage of that treatment is the application of photosensitizing materials which are completely safe for humans. Their destructive actions can be initiated by a beam of visible or infrared light.

The research presented will focus on the search of effective photosensitizing compounds producing large quantities of singlet oxygen, associated with high probability of intersystem transitions. Our research team has focused on modified porphyrins and chlorines.

We have studied their spectroscopic properties as absorption and emission, lifetime of excited singlet and triplet states, efficiencies of these processes, stability. We have also conducted biological studies, such as transfection of these substances into tumor cells, their localisation and efficiency of destruction. We have shown that the application of liposomes as carriers of therapeutic substances has improved their penetration into tissues.

One example of the obtained results will be presented. We will discuss the survival rate of adenocarcinoma tumor cells after administration of the chlorine c, encapsulated in the different types of liposomes. The observed effect is surprising.

Eye melanoma research – beyond the scientific collaboration

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Interdisciplinary cooperation is a prerequisite for achieving new, interesting results.

Connecting with researchers from a different specialty brings additional value to our work - extends the methodology, allows introducing novel views and a different perspective on difficult issues. Oncology is a very extensive field requiring the cooperation of doctors with biologists, chemists and physicists, especially in the case of problems of tumor metastasis and the search for methods of their treatment.

Such a fruitful cooperation has existed for many years between the Clinic of Ophthalmology and Ophthalmic Oncology of the University Hospital in Krakow and the Department of Biophysics, Faculty of Biotechnology of the Jagiellonian University. [1-4]

Uveal melanoma is the most common primary intraocular tumor in adults. It typically spreads metastasis to liver causing a high mortality rate. Animal models are crucial for evaluating new diagnostic and therapeutic modalities in cancer research. Developing a new animal model of any cancer disease is a difficult and multi-faceted process.

Newly established models of uveal melanoma (UM) allow studying their biological features and response to the therapy. In our research we focus on radiosensitivity and biological effects in UM cells and tumors.

I present briefly the history of our cooperation and some of its successes.

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Vitamin D derivatives and melanoma

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Novel pathways of vitamin D₃ activation by CYP11A1 to produce a variety of hydroxymetabolites were discovered. They are produced in vivo and are biologically active displaying anti-proliferative, anti-inflammatory, pro-differentiation and anti-cancer properties. They have low or non-calcemic activity. The mechanism of action includes activation of vitamin D (VDR) and arylhydrocarbon (AhR) receptors as well as inverse agonism on retinoic acid orphan receptors (RORs). Molecular and clinicopathological studies demonstrated correlation between defects in vitamin D signaling and progression of melanoma and disease outcome, with very poor outcome in VDR negative melanomas. Vitamin D hydroxyderivatives have been shown to have anti-melanoma activity in vitro in several human and rodent melanoma lines. Also 20(OH)D₃ inhibited melanoma tumor in vivo. To better define a mechanism of action of vitamin D hydroxyderivatives, we have knocked out (KO) the VDR in WM164 melanoma cells using CRISPR technology. Melanoma cells were tested for their proliferation, and ability to form spheroids or to form colonies in monolayer. The VDR-KO cells grew faster, and form more spheroids or colonies than scrambled cells and show different morphology. 20(OH)D₃, 1,20(OH)₂D₃ and 1,25(OH)₂D₃ showed lack of anti-proliferative effect in VDR KO cells at concentrations 10⁻¹⁰ and 10⁻⁹M, which was seen in scrambled cells. However, higher concentration of derivatives inhibited proliferation of both VDR-KO and scrambled cells. They also inhibited colonies formation with markedly higher potency in scrambled vs VDR-KO cells. In conclusion, VDR acts as tumor suppressor in melanoma and its presence is necessary for full anti-melanoma effects of vitamin D derivatives.

New tools for disease modeling and drug research

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Successful drug development is hindered by lack of sufficient human models of many diseases. Accordingly, numerous putative therapeutic compounds, although effective in pre-clinical studies, do not demonstrate beneficial effects in clinical trials. Organ toxicity, particularly cardiotoxicity adds another limitation which may additionally result in withdrawal of already approved drugs. As animal models fail to fully recapitulate pathophysiological changes observed in patients suffering from many diseases, novel human cell-based approach is needed to facilitate drugs transition from bench to bedside. Particularly, limited possibilities exist for studying molecular background of conditions affecting cells difficult to isolate, including cardiomyocytes and neurons. Human pluripotent stem cells (hiPSC), generated from easily accessible somatic cells by overexpression of defined transcription factors provide a novel platform to address these issues. hiPSC can be efficiently differentiated into many cell types of human body and thus have been extensively applied to: i) obtain patient-specific stem cells and differentiate them into cells affected by the patient's disease, ii) utilize such differentiated cells in investigating disease mechanisms and drug testing and iii) generate cells applicable in regenerative medicine. Importantly, our and others studies confirmed that patient-specific hiPSC recapitulate pathological phenotype and thus can be used to model various conditions including Duchenne muscular dystrophy, amyotrophic lateral sclerosis and diabetes. hiPSC-derived cardiomyocytes, on the other hand, are recognized as a valid model to evaluate cardiotoxicity of new drugs, and demonstrate substantial therapeutic effect in animal models of myocardial infarction. Application of hiPSC-based technology, therefore, provides an important milestone for facilitating drug development.

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Bacteriochlorins in Photodynamic Therapy of cancer

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Photodynamic therapy (PDT) is considered as the credible alternative to surgery, chemotherapy and radiotherapy in the management of cancer diseases [1]. The key factors in this method are spectroscopic, photophysical and photochemical properties of the photosensitizer used, light dose and dioxygen concentration. Among many groups of photosensitizers studied up to now, synthetic bacteriochlorins (Fig.1) are unique in their photodynamic efficacy and possibilities of tuning the required properties. Engineering of their electronic and geometrical structures *via* rings substituents (R, X in Fig.1) allowed for obtaining compounds interesting for various applications, from photocatalysis and photodiagnosis to photodestruction. From the library of synthetic bacteriochlorins, after many biological (*in vitro* and *in vivo*) studies [e.g 2-4], the most promising photosensitizer for head and neck cancer PDT treatment entered the second clinical phase [5-6].

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Variable response of *Raphidiopsis raciborskii* strains to chill/light stress

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Invasive cyanobacteria *Raphidiopsis raciborskii*, originally thought to proliferate only in more tropical regions of the world, is being detected in temperate climates, suggesting that it has the ability to rapidly adapt to changing environmental conditions (e.g. temperature, solar radiation) due to high phenotypic plasticity. Therefore, the adaptation mechanisms of *R. raciborskii* strains (differing in their climatic origin and toxicity) exposed to the chill/light stress (low temperature and high light), were examined. Previous results showed that non CYN (cylindrospermopsin) producing strain isolated from the temperate region (Poland), displayed fast initiation of different mechanisms required to maintain the main metabolic functions, particularly ones sensitive to chill/light stress. In contrast, a CYN producing Australian strain was not able to adapt quickly, with *cyrB* gene (involved in CYN synthesis) significantly upregulated, suggesting other mechanisms involved in the response to chill/light [1]. Further research, with more strains (three non CYN producing strains from temperate regions in Poland, and one toxic Australian strain), were conducted to verify these results. Preliminary experiments indicated limited transcriptomic upregulation of genes in strains from Poland, while upregulation was noted in genes sensitive to chill/light stress as well as one associated with CYN synthesis of another Australian strain. These results suggest that adaptive evolution to environmental stress is not reserved only for strains from temperate regions and that mechanisms of adaptations could be specific for each ecotype. Elucidation of reasons for such distinctive responses of *R. raciborskii* strains present a challenge and should be investigated more thoroughly in the future.

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Calcitriol and hyperthermia treatment combined with chemotherapy against mouse and human pancreatic cancer in vitro

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Gemcitabine chemotherapy is a well-known treatment against human pancreatic cancer. Because of insufficient pancreatic cancer drug accumulation and side effects during treatment, it is believed that gemcitabine effectiveness can be improved by combinatory therapy with calcitriol and local hyperthermia. Gemcitabine is a specific and robust analog of deoxycytidine affecting the DNA synthesis process [1]. Mild hyperthermia (<42°C) can modify the cell membrane and support drug delivery or lead to cell death [2]. Calcitriol, an active form of vitamin D₃, has a wide range of effects related to cell proliferation, differentiation and apoptosis, oncogenesis, and angiogenesis regulation [3].

The study's goal was to verify synergetic effects of hyperthermia and the active form of vitamin D with chemotherapy against mouse and human pancreatic cancer cells.

Human (PANC-1) and murine (PAN_O2) pancreatic cancer cells were heated to 41°C for 20 minutes and treated with gemcitabine (0.001 - 1000 µM)

and calcitriol dose of 100 nM. Cells condition was evaluated with hemocytometer cells counting, MTT, cytometer, and long-time microscopic observation test. Western Blott technique was used to assessed selected proteins.

In conclusion, the combination of hyperthermia and calcitriol with gemcitabine can lead to better anti-cancer effects than using the drug alone.

Acknowledgements

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Methylation of viral mRNA Cap by Nsp10/16 2'O MTase from SARS-CoV-2

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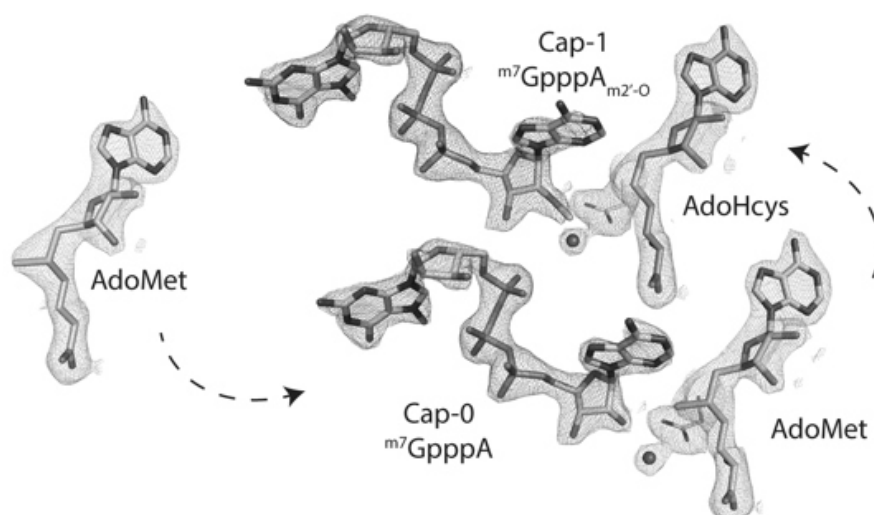
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Three coronaviruses induce clusters of severe respiratory diseases in humans: SARS, MERS and SARS-CoV-2. Promising vaccines against SARS-CoV-2 are being introduced but there is no effective drug to control infections and stop virus proliferation. The genome of the SARS-CoV-2 comprises 15 nonstructural proteins (Nsp). One of the SARS-CoV-2 protein complexes is a Nsp10/16 heterodimer that function as a 2'-O methyltransferase. Activity of Nsp10/16 is required for the methylation of viral RNA Cap at the 5' terminus. The methyl transfer is dependent on the S-adenosyl-L-methionine (AdoMet), which serves as the donor of the methyl group that is moved to 2'-O position of ribose at first nucleotide of the Cap0 to produce Cap-1 and AdoHcys. The occurrence of methylated Cap-1 allows SARS-CoV-2 RNAs mimic host transcripts, and therefore trick innate immunity mechanism that recognizes exogenous RNA. We used fixed-target serial synchrotron crystallography (SSX) to determine crystal structures of Nsp10/16 with substrates and products of catalyzed reaction. SSX allows to collect data from thousands of independent crystals, and significantly reduce radiation damage of crystals. Using fixed-target SSX system that utilize ALEX chip developed at Structural Biology Center 19-ID at APS we determined room-temperature structures of Nsp10/16 using low X-ray dose. We solved the first structure of Nsp10/16 in a complex with Cap-1. We hypothesize that molecules which affect activity of Nsp10/16, and inhibits viral RNA maturation are potential drug compounds against COVID-19, making SARS-CoV-2 sensitive to human innate immune response.



Structural characteristics of macrocyclic peptide as a potent modulator of PD-1/PD-L1 immune checkpoint axis

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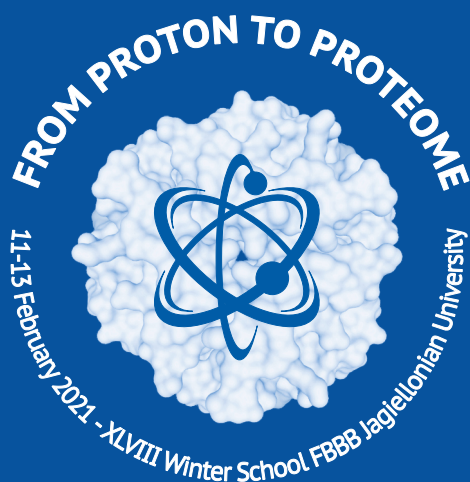
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Blocking therapy targeted towards the immune checkpoint PD-1/PD-L1 pathway has proven to be a very effective approach for treatment of a number of tumor types and has become an important tool in immunotherapy harnessing therapeutic antibodies [1]. Although successful, these applications face several constraints such as potential immunogenicity, poor solid tumor penetration or monoclonal antibody-related toxicity for example immune-related adverse events [2]. Potential therapies involving several groups of small-molecule inhibitors targeting immune checkpoints could completely avoid the aforementioned problems [3]. Small-molecules such as macrocyclic peptides act as PD-1 agonists and not only exhibit high affinity and specificity of binding but also and even more importantly lack immunogenicity. Here, we report characterization of 3rd class of macrocyclic peptide inhibitors capable of binding the distal extracellular domain of PD-L1. Based on the crystal structure we determined the details of the macrocyclic peptide ligand-PD-L1 interactions revealing their hydrophobic and electrostatic nature. We also report unusual sulfur binding mode between PD-L1 and the ligand involving interaction between sulfur atom of methionine from PD-L1 and phenyl aromatic ring of phenylalanine from the ligand which to date has been only scarcely described in the case of one of the macrocycles binding modes. Our results clearly indicate wide diversity among 3rd class of macrocycle peptides.

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SHORT ORAL PRESENTATIONS

The novel gold nanorod-based nanotherapeutic for multi-module pancreatic cancer therapy

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Pancreatic cancer is one of the worst human neoplasms based on survival prognosis and poor treatment effectiveness [1]. One of the proposed strategies to solve this problem is multi-modality therapy and modern imaging techniques. Gemcitabine is a commonly used chemotherapeutic agent in the fight against pancreatic cancer [2]. That is why a novel nanotherapeutic was designed to perform gemcitabine-based chemotherapy combined with mild hyperthermia treatment [3,4]. Additionally, computed tomography (CT) imaging could optimize treatment procedures and introduce radiotherapy as a complementary treatment technique.

The study's major goal was to design and characterize a new theranostic compound and verify the mechanism of its action in *in vitro* model. The successful synthesis of gemcitabine conjugate with carboxylated gold nanorods was performed using a water-soluble carbodiimide methodology (EDCI/NHS), attaching gemcitabine amino groups with carboxyl coating of the gold nanorods. New nanotherapeutic was further characterized using transmission electron microscopy, DLS, IR, HPLC, and XRF techniques. The intense absorbance spectrum for the construct was observed at 828 nm. The hyperthermia effect strongly depends on the power density and wavelength of the light source. The solubility, stability, and toxicity were studied *in vitro*. MTT and long-term microscopic (JuliStage®) cell survival tests were performed in Panc-1, AsPc-1, Pan_02, Panc 03.27 and Panc 08.13.

In conclusion, the gemcitabine chemotherapy can be successfully performed with hyperthermia treatment induced by gold nanorods and infra-red light. The next step will involve complex *in vitro* and *in vivo* toxicity studies and evaluating CT imaging contrast capability.

Acknowledgements

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Identification of SAGs virulence factors

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Streptococcus anginosus group, formerly known as *Streptococcus milleri*, consists of three distinct streptococcal species: *Streptococcus anginosus*, *Streptococcus constellatus*, and *Streptococcus intermedius*. Those microorganisms were subsequently classified as commensals colonizing the oral cavity, colon or vagina but recent data announced them as potent pathogens found in brain or liver abscess. Despite the strong set of clinical data there is still a gap in our knowledge about the mechanisms of SAGs pathogenesis. Therefore we aimed in our study to characterize the unique collection of SAGs isolates (n=41) in the respect of their adhesion properties, as well as hemolysins, DNases and proteases activity. Then, we optimized and applied the infection model using *Dictyostelium discoideum* for screening the virulence of all tested SAGs strains. Finally, we established and characterized the infection process of selected SAGs strains by wax worm *Galleria mellonella*. Obtained results indicate *D. discoideum* as a perfect model for a large scale screening of SAGs virulence. Moreover, we showed that *G. mellonella* could be applied to evaluate the molecular mechanism of SAGs pathogenesis to corrupt the innate immunity.

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Influence of PemK toxins on *Staphylococcus aureus* transcriptome and extracellular proteome

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Staphylococcus aureus is an opportunistic pathogen responsible for a variety of infections. Toxin-antitoxin (TA) systems are widespread in bacteria. TA systems, here exemplified by PemIK, compose of toxin (PemK) and antitoxin (PemI). The system has been shown to be implicated in maintenance of genetic material and regulation of staphylococcal virulence, however molecular mechanisms of its activity remain obscure.

Two homolog toxins, PemK_{Sa} from *S. aureus* CH91 and PemK_{Sd} from *S. delphini*, significantly differ in toxicity, although both have RNase activity. Here we researched the influence of the toxins on *S. aureus* transcriptome and extracellular proteome with RNA-seq and two-dimensional differential gel electrophoresis, respectively. Recombinant strains *S. aureus* SH1000, transformed with PemK expressing plasmids, were grown. Then total RNA and proteins excreted to the medium were collected for analysis.

Overexpression of toxic PemK_{Sa} resulted in severe changes in transcriptome, which deepened over the time (387 and 1061 genes, 20 and 90 minutes after induction of toxin expression, respectively). In contrast, non-toxic PemK_{Sd} affected only a few dozens of genes (70 and 20). Similar trend was observed in extracellular proteomes, however the numbers of differentiating proteins were lower (17 and 2, for PemK_{Sa} and PemK_{Sd}, respectively).

Obtained results show that different toxicity of PemK toxins has the reflection in transcriptome and secretome. Further analysis of the omics data will provide information about molecular mechanisms of the toxicity, with implications on the role of TA systems in bacteria.

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MCPIP1 regulates the expression of inhibitors of the WNT pathway through its RNase activity

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The MCPIP1 (Monocyte Chemoattractant Protein-1 Induced Protein) protein is important regulator of inflammation and homeostasis of the immune system. MCPIP1 thanks to its RNase activity specifically digests of pre-miRNAs and regulates gene expression. MCPIP1, acting antagonistically to the DICER enzyme complex, is responsible for the formation of mature miRNA. Although some pre-miRNAs are known to be degraded by MCPIP1, the long-term biological effect remains unknown. Changes in the miRNA levels may be associated with the increased resistance of cancer cells to treatment and the acquisition of migratory and invasive properties leading to metastasis.

Our results showed that in patients with ccRCC, the level of β -catenin increases together with a decrease of MCPIP1. Furthermore, in a xenotransplant model of ccRCC established in NOD-SCID mice, the transcript level of β -catenin was increased in tumors with the mutant form of MCPIP1 lacking RNase activity. Regulation of β -catenin activity occurs via its phosphorylation, which is crucial for the degradation process. We observed that MCPIP1 regulates the activity and phosphorylation status of β -catenin in ccRCC cells. Moreover, MCPIP1 regulates the expression of inhibitors of the WNT pathway through its RNase activity. We identified several miRNAs dependent on the RNase activity of MCPIP1. Based on a database, we identified several target genes, which act as negative regulators of WNT signaling pathways and reduce the level of active β -catenin.

Our results may contribute to a better understanding of ccRCC progression, which may help in the development of more effective therapy in the future.

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Promising starting models for simulating spontaneous creation of an inverse hexagonal phase of poly-unsaturated monogalactolipids at atomic resolution

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Monogalactosyldiacylglycerol (MGDG) is the most abundant lipid component in the thylakoid membrane of chloroplasts. It plays an important role in the violaxanthin cycle and photosynthesis. MGDG consists of a β -galactose moiety and two α -linolenoyl (di-18:3, *cis*) acyl chains bound at the *sn*-3, *sn*-1 and *sn*-2 positions, respectively, of the glycerol backbone. There is a close relationship between the type of the galactolipid acyl chains and the lyotropic phase formed spontaneously by this molecule in water, and MGDG with both α -linolenoyl chains forms an inverse hexagonal phase (H_{II}).

Four di-18:3 MGDG systems were constructed and molecular dynamics (MD) simulated in the OPLS-AA force field by using the GROMACS software package. Each of them consisted of two 450-lipid bilayers separated by two water layers. The number of water molecules in the inner water layer varied and was 8, 12, 15 and 30 H₂O/MGDG (W8, W12, W15, and W30, respectively); the outer layer of each system contained 30 H₂O/MGDG. The W8, W12, W30 systems were MD simulated for 500 ns and the W15 system for 1425 ns at temperatures of 333 K (W12, W15) and 353 K (W8, W30). Among the tested systems, the most promising starting model for spontaneous inverse hexagonal phase formations is so far W15. The number of 8 and 12 H₂O/MGDG in the inner layer is probably too low for the stalk formation. On the other hand, 30 H₂O/MGDG seems too high.

Unravelling the mechanisms of abdominal aortic aneurysm: focus on the endothelial cells

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Abdominal aortic aneurysm (AAA) is a permanent dilatation of the abdominal aorta, which bears a high risk of rupture and sudden death of the patient. The pathogenic mechanisms of AAA remain elusive, and surgical intervention represents the only treatment option. Our research shows that a predisposing factor to AAA may be the altered intimal ultrastructure with fenestration in the aortic endothelial layer. Therefore, in this study, we aimed to address endothelial cells' (ECs) role in the formation and stability of abdominal aneurysm. We used a multicolour EC lineage tracing in the model of angiotensin II-induced AAA in adult mice. We discovered a clonal expansion of ECs localizing in the different aneurysmal tissue areas, originating from three separate ECs. Furthermore, a detailed inspection revealed a clone-specific proliferative and apoptotic ECs status. Moreover, the formation of AAA was accompanied by significant changes in aortic structure, loss of intimal endothelial cells, their migration within the aortic wall together with the presence of acellular fenestrations. Within the aneurysmal tissue, we evidenced the presence of EC-lined tunnels, the number of which correlate with aneurysm expansion rate. Importantly, their formation was significantly impaired in the ruptured AAAs. Conjointly, this study points out to a pivotal role of ECs in the formation and stability of the aneurysm, which should be further investigated.

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Heme oxygenase-1 colocalizes with G-quadruplexes

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G-quadruplexes (G4) are stacked nucleic acid structures formed by guanine-rich sequences of DNA or RNA, stabilized by monovalent cations. In cells, G-quadruplexes seem to affect DNA replication and gene transcription, and numerous studies suggest that the formation of G4 is regulated through interactions with different proteins. Particular attention has been paid to the helicases that are involved in resolving those structures. In *in-vitro* settings G-quadruplexes (especially DNA-G4) can be stabilized by heme. Since the excess of free heme is known to enhance the generation of reactive oxygen species its availability is strictly regulated by heme oxygenases (Hmox1 and Hmox2). The composition of G4-unwinding complex and its heme sensitivity is unclear. Therefore, we investigated the potential interaction between Hmox1 protein and G-quadruplexes in HEK293 and iPSC model cells. We found that the accumulation of G4 can be affected by Hmox1. Furthermore, using iPSCs expressing only nuclear or only cytoplasmic forms of Hmox1, we showed that nuclear localization promotes G4 removal. Finally, we demonstrated that the proximity ligation assay can detect cellular co-localization of G-quadruplexes with helicases, as well as with Hmox1, suggesting the potential role of Hmox1 in G4 modifications. This co-localization does not mean a direct interaction since using a standard chromatin immunoprecipitation we did not detect G4-Hmox1 complexes, however. Collectively, our results suggest that Hmox1 influences G4 accumulation, but rather as one of the proteins regulating heme availability, not as a rate-limiting factor.

Identification of novel PHLDA1 binding partners in neuroblastoma cells treated with chimeric antibodies recognising GD2 ganglioside

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Neuroblastoma is a cancer derived from neural crest cells. Some of the treatment methods are based on immunotherapy with chimeric anti-GD2 ganglioside antibodies (ch14.18/CHO). Previous studies revealed that PHLDA1 (pleckstrin homology-like domain family A member 1) was increased in IMR-32 cells treated with anti-GD2 ganglioside antibody (14G2a), which correlated with the cells decreasing survival [1]. Our current studies aimed at characterisation of potential molecular pathways in which PHLDA1 may be involved.

To identify PHLDA1 binding partners in IMR-32 neuroblastoma cells treated with ch14.18/CHO antibodies we used co-immunoprecipitation approach and mass spectrometry analysis. The proteins selected by mass spectrometry were further analysed via co-immunoprecipitation and immunoblotting. Six novel PHLDA1 partners were revealed: CaMKII (calcium/calmodulin-dependent protein kinase type II subunits gamma and delta), DCAF7 (DDB1 And CUL4 Associated Factor 7), SCG2 (secretogranin 2), LENG9 (leucocyte receptor cluster member 9), RBM14 (RNA-binding protein 14) and AUTS2 (autism susceptibility gene 2 protein). We observed differential expression levels of the proteins after treatment with ch14.18/CHO. PHLDA1-DCAF7 interaction was confirmed via co-immunoprecipitation. Further research is needed to determine if DCAF7 (involved in neural development), being a binding partner of PHLDA1, might be destabilised by the increased levels of PHLDA1.

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MCPIP1 in skin pathologies

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Monocyte chemotactic protein-induced protein 1 (MCPIP1; also called Regnase-1) is an RNase that negatively regulates the stability of certain stem-loop-containing mRNAs, including those encoding pro-inflammatory cytokines and transcription factors. MCPIP1 function as a fundamental modulator of inflammatory processes, but has been also implicated in the regulation of cell proliferation, differentiation and apoptosis, suggesting tumor-suppressive properties. In the skin MCPIP1 expression is restricted to the suprabasal layers of the epidermis and its activity is crucial for proper proliferation and differentiation of keratinocytes and for the maintenance of skin integrity. Its expression is elevated in human psoriatic skin.

Within this study we investigated the involvement of MCPIP1 in the development of psoriasis-like inflammation in much more detail. For that we utilized mice with conditional knockout of MCPIP1 in keratinocytes. Those mice developed imiquimod-induced skin inflammation faster than the control mice. This phenotype was further related to the accelerated activation of the IL-23/Th17 and Stat3 pathways in their skin. Thus, MCPIP1 appears as an important factor for modulating the onset and progression of psoriasis.

Next, to investigate whether MCPIP1 may be involved in the pathogenesis of other skin diseases, we have analyzed its expression profile in the skin biopsies of patients suffering from hidradenitis suppurativa (HS), actinic keratosis (AK) and squamous cell carcinoma (SCC). Our results indicate that MCPIP1 expression is altered in all: HS, AK and SCC. Future studies are required to understand the molecular mechanisms of how MCPIP1 may contribute to the development of those skin-related disorders.

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Electron transfer paths contributing to oxidation of quinone pool in *Flavobacterium johnsoniae*

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Alternative Complex III (ACIII) is present mainly in organisms that lack *bc1/b6f* complexes, such as *Flavobacterium johnsoniae*, and is predicted to fulfil their function. Recently ACIII structure has been solved, revealing no structural similarity to cytochrome *bc1/b6f*. Interestingly, ACIII is present in a supercomplex with cytochrome *aa3*. But, it is unknown how ACIII operates at molecular level.

To make a step towards gaining the experimental access to explore structure–function relationship of ACIII, we focused on constructing a system for genetic manipulations within this complex. We used this system to delete the genes encoding the heme-containing subunits, ActA and ActE, which were proposed to be involved in the catalytic mechanism.

We show that *F. johnsoniae* is able to live without ActA or ActE, but the bacterial growth is very restricted. This suggests that the absence of either ActA or ActE leads to decreased overall efficiency of the associated electron transport chain. The oxygen consumption measurements showed lack of *aa3* oxidase activity in *actA* deletion, but not in *actE* deletion mutant. Therefore, we assume that *ActE* is not required for electron transfer between ACIII and *aa3* oxidase. We propose existence of several independent electron transfer paths, which contribute to oxidation of quinone pool. One of them involves a split in electron paths at the ActA/ActE interface. The other path is oxygen-independent and involves KCN-sensitive enzyme. Therefore, *F. johnsoniae* seems to use multiple electron transfer paths to secure its flexibility to use different energy sources.

The identification of functional background of an abnormal development of the infant gut microbiome

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A variety of autoimmune and allergy events are becoming increasingly common, especially in Western countries. It was already proven that the gut microbiota may influence those conditions, especially during the early development. The goal of the presented research was to identify the molecular background underlying the abnormal development of the infant's gut microbiome.

Knowing that analysis of bacterial taxonomy may not be sufficient to understand the functional features of the community, we applied functional analysis methods combined with statistical and machine learning analysis.

Our research shows that the differences in the abundances of the two enzymes that are crucial for the utilization of short type 1 oligosaccharides are key factors for the differential development of the infants' gut microbiota.

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Photoexcited particulate matter PM2.5 exhibit phototoxic properties against human keratinocytes

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Smog is a type of intense air pollution and a phenomenon affecting an increasing number of people, which is almost impossible to break away from. Human skin is the organ that alongside with the lungs is the most exposed to the environmental particles. It has been shown that not only ambient PM (particulate matter) can penetrate through barrier-disrupted skin [1] leading to ROS (reactive oxygen species) - dependant inflammatory response *in vivo* but also that PM itself can trigger skin barrier dysfunction [2]. Interestingly, recent *in vivo* studies have shown that the number of pollutants taken up from air in human subjects by inhalation or transdermally was similar [3]. Many compounds found in PM may exhibit photochemical activity and act as photocatalysts of ROS generation or photogenerators of reactive species, e.g., transition metals ions', semiconductors and organic compounds including dyes, porphyrins and aromatic hydrocarbons [4][5]. Therefore, it seems crucial to take light-related toxicity into account when investigating biological implications of particulate matter toxicity to light-exposed tissues. The study demonstrated that the light-induced particulate matter PM_{2.5} decreases the viability of investigated cells (HaCaT), causes oxidative damage to cell organelles and increases the activity of pro-apoptotic and pro-inflammatory factors. PM_{2.5} particles were also found to effectively photogenerate free radicals and singlet oxygen, that may be the crucial factors responsible for the light-related toxicity against human skin.

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ROS signaling capacity of non-inhibited cytochrome *bc1*

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Cytochrome *bc1*, also known as mitochondrial complex III is considered to be one of the main reactive oxygen species (ROS) producers in electron transport chain (ETC). It is believed that in physiological conditions, certain level of ROS produced by mitochondrial ETC might be beneficial and participate in cellular signaling. On the other hand, elevated levels of ROS might exhibit negative effects resulting in cellular damage.

It is well established that inhibition of the electron flow within cytochrome *bc1* leads to high production of ROS. However, superoxide production in a non-inhibited system remains uncertain. We proposed a novel method for ROS detection in ETC hybrid system comprised of bacterial cytochrome *bc1* and mitochondrial complex IV. In this system an excess of ubiquinol enables accumulation of superoxide-derived signal. In turn, mitochondrial complex IV ensures the oxidation of cytochrome *c*, thus allowing the use of a low concentration of cytochrome *c*.

By using this method it was possible to show that non-inhibited cytochrome *bc1* generates ROS and that adaptive and pathogenic mitochondrial mutations suppress and enhance ROS production, respectively. Moreover, we also noted that cytochrome *bc1* produces ROS in a rate-dependent manner: the rate of ROS production expressed in s^{-1} is continuously growing with increasing enzyme activity. This dependency has not yet been reported, but seems to be crucial when discussing ROS signaling originating from mitochondria.

MicroRNA-378 deficiency mitigates muscular dystrophy in *mdx* mice

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The severity of Duchenne muscular dystrophy (DMD) – an incurable disease resulting in walking and breathing difficulties, might be affected by the dysregulation of microRNAs (miRNAs) expression. Among them, miR-378 is thought to play an important role in muscle development, metabolism and angiogenesis; however, its role in murine model of DMD (*mdx* mice) has not been thoroughly addressed.

Here, we demonstrate that dystrophic mice additionally globally lacking miR-378 (*mdx*/miR-378^{-/-} animals) exhibited better physical performance and improved absolute muscle force compared with *mdx* mice. Accordingly, markers of muscle damage in serum, particularly lactate dehydrogenase and osteopontin were significantly decreased in *mdx*/miR-378^{-/-} mice, what was accompanied by diminished inflammation, fibrosis, and reduced abundance of regenerating fibers within muscles. The lack of miR-378 also normalized the aggravated fusion of dystrophin-deficient muscle satellite cells (mSCs). To shed more light on the molecular pathways responsible for observed effects we performed the comparative transcriptome analysis on gastrocnemius muscle which revealed fibroblast growth factor 1 (*Fgf1*) as one of the most significantly downregulated genes in mice devoid of miR-378, indicating FGF1 as a potential mediator of changes driven by the lack of miR-378.

In conclusion, we suggest that targeting miR-378 has the potential to ameliorate DMD pathology [1].

Acknowledgements

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Role of Mcpip1 in obesity-induced hepatic steatosis as determined by myeloid and liver-specific conditional knockouts

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Monocyte chemoattractant protein-induced protein 1 (MCP1) is a negative regulator of inflammation, acting through cleavage of transcripts coding for proinflammatory cytokines. Moreover, it was demonstrated, that MCP1 regulates lipid metabolism both in adipose tissue and hepatocytes. In this study, we investigated the effects of tissue-specific Mcpip1 deletion on the regulation of hepatic metabolism and development of non-alcoholic fatty liver disease (NAFLD).

We used control Mcpip1^{fl/fl} mice and animals with deletion of Mcpip1 in myeloid leukocytes (Mcpip1^{fl/fl}LysM^{Cre}) and in liver cells (Mcpip1^{fl/fl}Alb^{Cre}), which were fed chow or a high-fat diet (HFD) for 12 weeks.

Mcpip1^{fl/fl}LysM^{Cre} mice fed a chow diet were characterized by reduced hepatic expression of genes regulating lipid and glucose metabolism, which resulted in decreased glucose concentration and dyslipidemia. These animals displayed also systemic inflammation manifested by increased serum levels of Il-6, Il-12, Il-16, Tnf-alpha, Mcp-2, Mip-1 alpha and hepatic leukocyte infiltration. Although we detected a reduced hepatic expression of genes regulating glucose metabolism and β -oxidation in the Mcpip1^{fl/fl}Alb^{Cre} mice, they did not develop any abnormalities related to lipid metabolism. Despite feeding with HFD for 12 weeks, Mcpip1^{fl/fl}LysM^{Cre} mice did not develop obesity, glucose intolerance, nor hepatic steatosis. Mcpip1^{fl/fl}Alb^{Cre} animals, following a HFD, became hypercholesterolemic, but accumulated lipids in the liver at the same level as Mcpip1^{fl/fl} mice.

In conclusion, we demonstrated that Mcpip1 protein plays an important role in the liver homeostasis. Depletion of Mcpip1 in myeloid leukocytes, followed by systemic inflammation, has a more pronounced effect on controlling liver metabolism and homeostasis than its deletion in liver cells.

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Spectroscopic analysis of new fluorescent molecular sensors based on coumarin chromophore core and their applications in imaging living cells

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The use of fluorescent dyes (sensors) to visualize cell structures provides important information about the current state of the cell. Thanks to them, cells can be diagnosed by studying the potential of the cell membrane, intracellular pH, or metabolic activity. Currently, despite a wide selection of sensors, there are still searches for completely new compounds that will simultaneously demonstrate high fluorescence efficiency, photostability, selectivity in action and lack of cytotoxicity. Therefore, it is worth to note compounds whose fluorescence is particularly sensitive to the properties of the microenvironment (e.g., solvent), which can be used, for example, for staining cell organelles. The study aims to assess the possibility of penetration of biological membranes and visualization of cellular structures using new compounds based on coumarin scaffolds. A non-small cell lung cancer line (A549) was used during the study. The cells were incubated in the presence of coumarin derivatives. Analysis performed with the Leica DMiL LED Fluo inverted microscope. In addition, the effect of coumarin derivative compounds on the cytotoxicity of cells was checked. For this purpose, a colorimetric MTT test was used. Cytotoxicity of the tested compounds was compared with the doxorubicin compound, which has proven toxicity to cancer cells. After the MTT test, it was shown that the coumarin derivatives tested are safe for cells and do not affect their physiological properties after both 3 and 24 hours of incubation.

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Glutaminyl cyclase from *Porphyromonas gingivalis*: is it essential or not?

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Porphyromonas gingivalis is a human oral pathogen, which is a major causative agent of periodontitis. Protein secretion is essential for *P. gingivalis* virulence. Many identified virulence factors, e.g. gingipains, are proteins secreted through the type IX secretion system (T9SS), which them through the outer membrane. Beforehand, cargo proteins have to pass the inner membrane and then are modified by the action of a glutaminyl cyclase (QC). The *P. gingivalis* QC is a lipoprotein which enzymatically catalyzes the conversion N-terminal glutamine residue to pyroglutamate, with release

of ammonia as a side product.

The purpose of our research was to demonstrate the essentiality of the QC for *P. gingivalis*. Firstly, we failed to obtain genomic deletion of the QC gene. For this reason we changed our research approach and introduced an additional copy of the QC gene on the plasmid, and deleted the QC in the genome. The obtained mutant strains were viable and presented a wild type phenotype. Next, we tested the QC gene-carrying plasmid stability, which confirmed that *P. gingivalis* needs at least one copy of the QC gene to survive. Moreover, the QC catalytic activity, as well as the enzyme anchorage in the inner membrane, are essential for *P. gingivalis* growth.

In conclusion, we proved that the active and inner membrane anchored QC is necessary for the survival and/or growth of *P. gingivalis*. In the future this knowledge could be used to develop new therapeutic approaches in the treatment of periodontitis.

Loss of keratinocyte Mcpip1 accelerates the development of skin squamous cell carcinoma

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Monocyte chemotactic protein-1-induced protein 1 (MCPIP1), encoded by the *Zc3h12a* gene, possesses a PIN domain that has RNase properties and regulates stability of transcripts coding for inflammatory cytokines (e.g. IL-6, IL-12p40) and transcription factors (e.g. c-Rel, C/EBP β). MCPIP1 functions as an essential regulator of inflammation, but also plays a critical role in cell differentiation, proliferation and cancer development. Accumulating evidences demonstrated that MCPIP1 acts as a potent tumor suppressor and induces cancer cells apoptosis. Importantly, MCPIP1 localizes predominantly in the differentiated suprabasal layers in normal human epidermis. Our recent study showed that keratinocyte-specific Mcpip1-deficient (Mcpip1^{eKO}) mice develop spontaneous skin pathology and systemic inflammation.

To deepen our knowledge on the role of MCPIP1 in skin pathophysiology, we utilized a well characterised DMBA/tPA approach to induce skin carcinogenesis in mice. Our studies showed that Mcpip1^{eKO} mice developed tumors faster and then with increased quantity, compared to the control mice. At the histological level, we observed more aggressive tumors. This was indicated by abundant keratinization, infiltration of tumors cells into dermis and the presence of keratin pearls, the most characteristic feature of invasive SCC tumors. Based on immunofluorescence staining, we noticed increased expression of proliferation and vascularization markers. Furthermore, RNA-seq analysis showed significant changes between tumors.

Based on our data, we hypothesize that MCPIP1 functions as a significant factor in the development and progression of skin cancer.

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Spectroscopic investigation into binding interactions of bovine and human serum albumin with fluorescent molecular probes

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Bovine serum albumin (BSA) and human serum albumin (HSA) are components of blood plasma and the most abundant plasma proteins found in mammals. BSA shows almost 80% sequence homology and 76% structural similarity with the HSA. Steady-state fluorescence at 298 K of the interaction of bovine serum albumin (BSA) and human serum albumin (HSA) with fluorescence molecular probes have been carried out to visualize the location of the binding position and perturbation of the binding location of the tryptophan (Trp)/tyrosine (Tyr) of the protein(s) by monitoring the emission maxima of Trp residue(s) in proteins. The binding constant for protein-fluorescent sensor complexes and the number of binding positions were estimated from the Trp fluorescence quenching assay. These studies reveal distinct binding of the compounds to both proteins, and the values of the binding constants are evaluated by various methods. The fluorescence study also provides insight into the changes occurring in the microenvironment of the protein after sensor association.

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MCPIP-1 participates in macrophage-dependent endotoxin tolerance through restraining NF- κ B signaling

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Endotoxin tolerance is a state of hyporesponsiveness of immune cells to LPS, triggered by their previous exposure to endotoxin. Such an immunosuppressive state enhances the risks of secondary infection and sepsis. The strict regulation of endotoxin tolerance process is fundamental since it impairs the immune response and in consequence abolishes the elimination of the invading pathogen by macrophages. Although the phenomenon of LPS tolerance is extensively studied, its molecular mechanism is not fully explained. Among candidates that play a crucial role in this process are negative regulators of TLR signaling. The contribution of one of such regulators MCPIP-1 (Regnase-1) has not been studied yet. Therefore, we aimed to examine whether macrophage expression of MCPIP-1 participates in endotoxin tolerance. We used both murine and human primary macrophages devoid of MCPIP-1 expression showing, that MCPIP-1 strongly contributes to LPS tolerance and macrophage reprogramming. We evidenced that its mechanism depends mainly on the inhibition of ERK1/2 and p38 kinase phosphorylation and further NF- κ B activation, whereas RNase activity of this protein plays a marginal role in the endotoxin-tolerance process. Finally, using myeloid-MCPIP-1-deficient mice we showed their reduced tolerance to endotoxin manifested by increased mortality when compared to wild-type animals. Moreover, we found that deficiency of MCPIP-1 is compensated by increased expression of other negative regulators crucial in homotolerance process. Taken together, we showed for the first time that MCPIP-1 ensures endotoxin tolerance. Moreover, we evidenced that macrophages are a crucial cell population responsible for LPS-mediated hyporesponsiveness.

Hairy citrullinated *Porphyromonas gingivalis* enhances Toll-like 2 receptor activation

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Porphyromonas gingivalis (*Pg*) is the keystone oral pathogen implicated in development and progression of periodontitis. One of virulence factors of *Pg* is peptidylarginine deiminase (PPAD), an enzyme citrullinating host- and bacterium-derived proteins and peptides. Importantly, PPAD is essential to stimulate the proinflammatory response in host cells and our results suggested citrullinated fimbriae as the vital factor enhancing inflammation. This *Pg* surface appendage works as a ligand for Toll-like Receptor 2 (TLR2). Using a reporter system in transfected cell line overexpressing the TLR2 receptor we investigated other factors involved in the TLR2 activation. Cells were infected with various *Pg* strains and their mutants deficient in different surface proteins. The highest activation of the TLR2 receptor was observed for the ATCC33277 strain, while the W83 and 381 strains elicited a weak response. Catalytic inactivation of PPAD or fimbriae deletion in the ATCC33277 strain caused significant decrease in their ability to activate TLR2-dependent pathway. TLR2 was also activated by outer membrane vesicles (OMV) isolated from ATCC33277 (citrullination dependently) and W83 (citrullination independently) *Pg* strains. Interestingly, treatment with purified ATCC33277 major fimbriae induced TLR2 activation and co-stimulation with *Pg* LPS did not enhance the signal. In conclusion, the presented results imply that major *Pg* virulence factors activating TLR2 are bacterial cell surface proteins citrullinated by PPAD, especially major fimbriae. Furthermore, *Pg* activated TLR2 in the strain-dependent manner, which suggests that type of fimbriae plays the key role in this process.

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First case of staphylococcal linezolid-resistant infection in laryngological patients in Poland

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Linezolid has emerged as synthetic drug for treatments against Gram-positive pathogens. Both, linezolid-resistant *Staphylococcus aureus* and *Staphylococcus haemolyticus* strains have recently emerged worldwide.

The purpose of this study was to genetically characterize three linezolid-resistant isolates recovered from patients with chronic sinusitis.

Phenotypic and four Sanger-based methods were used for the species identification. The antibiotic resistance profiles were detected by phenotypic susceptibility testing and PCR-based methods. Genetic profiles including spa typing, SCCmec typing, MLST, MLVA, detection of virulence genes and *ica* operon were characterized.

All of isolates were multidrug resistant. The *S. haemolyticus* strain was identified as ST-42 and positive for *mecA* and hemolysin genes. Both *S. aureus* strains were recovered from the same patient and shared their genetic profile, identified as ST-398, spa type t4474 and SCCmec cassette type IVe. Moreover, *S. aureus* strains harbored adhesin, hemolysin genes and *ica* operon. The linezolid resistance in *S. haemolyticus* strain was associated with the presence of *cfr* gene and both *S. aureus* strains demonstrated acquisition of T314C and G362A mutation in the ribosomal protein L3 and C575T change in the L4 protein.

Presented results suggest that linezolid resistance may be related to acquisition of mobile genetic elements or mutations in ribosomal proteins. Moreover, the detection of livestock-associated ST-398 in patient indicate ability of crossing borders by *Staphylococcus* strains. As, both coagulase-negative staphylococci and *S. aureus* are significant etiological factors in laryngological infections, their genetic characterization is of great importance in patients diagnostics and treatment.



POSTERS

Novel approaches for diabetes management

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Small molecules targeting protein kinases are among the best tools for advanced therapy. They allow us to understand the mechanisms of disease. In recent years, the role of DYRK1A kinase in beta-cell proliferation and its possible use as a therapeutic target in the treatment of diabetes has been demonstrated [1]. Consequently, novel compounds dedicated to this kinase are being sought. The inhibitors under investigation are potential regulatory agents that restore pancreatic β -cell mass, secretory, and organ regulatory functions. Moreover, patient-derived induced pluripotent stem cells (iPSCs) allow screening for various diseases and facilitate the discovery of new drugs [2, 3]. The optimal inhibitor will restore the blood glucose levels and provide the conditions necessary for the growth and function of the host and new beta cells derived from iPSCs. The presented study proved that tested inhibitors increase the beta-cell proliferation, homing, engraftment, and thus regulate the blood glucose levels. The functionality of pancreatic islets derived from iPSC was also confirmed after treatment with selected inhibitors. Islets were isolated from BALB/c mice, and insulin secretion was determined after treatment with the tested compounds.

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Sumoylation of AtUVR3 (6-4 PP photolyase) from *Arabidopsis thaliana*

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Sumoylation is a posttranslational modification based on a covalent attachment of Small Ubiquitin related MOdifier (SUMO) to a target protein. It has been shown to play a crucial role in variety of biological processes including DNA repair. Ultraviolet Repair Defective 3 (AtUVR3) is the photolyase responsible for the UVA/blue light-dependent photorepair of UV-induced 6-4 photoproducts (6-4 PPs) in *Arabidopsis* DNA. According to in silico studies several amino acid of the AtUVR3 protein were identified as potential sumoylation sites. The aim of this study was to examine the AtUVR3 sumoylation and its physiological role.

AtUVR3 sumoylation was analysed using an *Arabidopsis* sumoylation system reconstituted in *Escherichia coli*. Protein-protein interactions and their subcellular localization in planta were tested using Bimolecular Fluorescence Complementation (BiFC) analysis and transient transformation of *Nicotiana benthamiana* epidermis cells. To confirm observed interactions the Split-Ubiquitin-based Yeast Two-Hybrid (YTH) system was used. Photorepair efficiency of 6-4 photoproducts was measured by ELISA assay.

The obtained results showed that AtUVR3 was sumoylated in bacteria overexpressing the reconstituted *Arabidopsis* sumoylation system. Interactions of AtUVR3 with SUMO proteins and SUMO ligases were observed in *Nicotiana* nuclei. These interactions were confirmed with YTH assay. Finally, some of *Arabidopsis thaliana* T-DNA mutants of SUMO proteins and SUMO ligases showed lower efficiency of 6-4 PP photorepair in comparison to the wild type plants. The results presented here suggest the possible contribution of the sumoylation pathway in the functioning of AtUVR3. Further studies are necessary to establish mechanism and molecular consequences of AtUVR3 sumoylation on photorepair of *Arabidopsis* DNA

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A Photodynamic Inactivation of Antibiotic-Resistant Pathogens with Porphyrin-Based Photosensitizers

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Scientists estimate that due to the increasing antibiotic-resistance, almost 700k people die every year worldwide. In recent years, the development of antimicrobial agents has not progressed sufficiently to control infections. The pathogens may cause infections of the respiratory system (*S. aureus*), gastrointestinal tract (*E. coli*, *C. albicans*) and cause food spoilage (*A. niger*). To limit this emerging public health problem, there is a need to develop non-incurative, non-toxic, and new antimicrobial techniques that act more effectively and quicker than the current antibiotics. Photodynamic inactivation (PDI) represents an alternative method of treating bacterial infections in response to the growing multidrug-resistance [1]. Designing of the new photosensitizers, as well as their immobilization on the semiconductors, is concerned with their biocompatibility, high accumulation in bacteria cells, selectivity, and phototoxicity[2].

In this work, we investigated porphyrin-based photosensitizers for antimicrobial photoinactivation. We determined the broad-spectrum killing of Gram(+) and Gram(-) bacteria and fungi. These porphyrins are activated with blue light and highly active at micromolar concentration. Moreover, no killing of mammalian cells under the same conditions with a short incubation time was observed. It was also indicated that the mechanism of action is primarily via singlet oxygen but hydroxyl radicals may also be involved. Overall, the results suggest that PDI may be useful as a promising tool for industrial and clinical purposes where savings in time is a critical point to achieve efficient inactivation of microorganisms.

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Determination of the role of CCL2 and biomechanical changes in the model of cerebral ischemia

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Stroke is the second most common cause of death in developed countries. There are two types of strokes, hemorrhagic stroke, which accounts for 15%, and ischemic stroke, which accounts for 85% of all strokes. This process activates the immune system and changes the mechanical properties of the ischemic tissue.

The aim of my work was to determinate the role of CCL2 and biomechanical changes in the processes of cerebral ischemia in oxygen and glucose deprivation (OGD) model of stroke.

The research model used in these studies were organotypic hippocampal cultures (OHC), which were obtained from 6-7 day old rat pups. Then, on day 7 of culture, the hippocampal slices were transferred to the hypoxic chamber for 40 minutes. After it, slices returned to the standard condition. All biochemical and atomic force microscopy analyzes were performed 24 hours after OGD.

The use of the OGD procedure on OHC results in a significant increase in mortality of nervous tissue. Oxygen-glucose deprivation induces decrease in the stiffness (Young's modulus) of the OHC and increase the level of CCL2. The use of Irbesartan as an antagonist of the CCL2 receptor reduced OHC mortality after OGD.

To sum up, activation of the CCL2-CCR2 pathway is unfavorable in the process of ischemia, and the CCR2 antagonist has a neuroprotective effect, which also coincides with biomechanical changes.

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DYRK1A, potential therapeutic target for diabetes

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Diabetes is a lifelong disease and recently has become one of the leading causes of death. Type 1 diabetes (T1D) and type 2 diabetes (T2D) are defined by insufficient insulin production. Restoring functional β -cell mass is an important goal in the process of curing diabetes. Recently, new evidence has emerged on the role of Dual-specificity tyrosine phosphorylation-Regulated Kinase 1A (DYRK1A) in β -cell proliferation, which expanded the list of potential therapeutic targets in T1D and T2D. DYRK1A suppresses the proliferation and activity of β -cell. Kinase inhibition leads to a higher proliferation of β -cell and insulin production.

In the current study, we report the progress of our search for new inhibitors against DYRK1A. The gene fragment encoding kinase domain of DYRK1A was cloned and expressed in *E. coli* and the protein was purified using various chromatography methods and its activity was confirmed by *in vitro* assay. Then we screened the library of natural small-molecule compounds, and out of them, curcumin and gossypin showed the highest inhibitory effect. Additionally, in a cell-based assay, both inhibitors were able to restore NFAT activity, the transcriptional factor known to be involved in β -cell proliferation. Furthermore, we set DYRK1A – curcumin/gossypin complexes for crystallization trials, in order to decipher inhibitor-enzyme interaction on the molecular level.

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In vitro analysis of the immunomodulatory properties of polyethylene glycol

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Polyethylene glycol (PEG) is a polymer widely used in medicine, primarily for the modification of small molecule drugs, proteins and nanomaterials. PEGylation of therapeutic agents provides increased solubility, limited protein interaction, and favorable biodistribution of the modified drugs. PEG modified compounds have been used in medicine for many years in the form of subcutaneous, intramuscular or oral administration. Therefore, the use of PEG in humans is considered safe, regardless of how it is administered to a patient. However, dangerous side effects (infusion reactions, e.g., hand and foot syndrome and stomatitis) have been observed in patients following the intravenous administration of PEG-modified liposomes. The mechanism of this phenomenon is not fully understood, but the results of recent reports indicate that after intravenous exposure, PEG interacts with components of the immune system, followed by complement activation or the production of anti-PEG antibodies. Additionally, the results of my previous research showed that PEG was responsible for the systemic activation of cytokine production in mice after intravenous exposure to PEGylated nanocapsules [1].

While there is strong evidence that PEG interacts with immune cells, there is no information as to which cell subpopulations are activated upon exposure to PEG. Therefore, in my research, I assessed the effect of free PEG chain on various human or mouse immune cells (e.g., macrophages and neutrophils). I paid attention to the ability of PEG to activate cytokine production by immune cells. Understanding the mechanism of PEG's toxicity on immune cells is essential for the safe use of PEGylated therapeutics.

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The *psm-mec* gene impact on biofilm formation in *Staphylococcus aureus* clinical strains

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Staphylococcus aureus bacteria are opportunistic pathogens present within a natural human microbiota. Although, as opportunistic pathogen, *S. aureus* is also often resistant to antibiotics and responsible for many infections in humans. The antibiotic resistance is related to the presence of specific genetic elements, that can be transferred between strains and species as a result of horizontal gene transfer. One of such mobile genetic elements, the staphylococcal chromosome cassette *mec* (*SCCmec*) encodes antibiotic resistance genes and can also contain the *psm-mec* gene. The PSM-mec protein encoded by the *psm-mec* gene is one of phenol-soluble modulin proteins which take part in biofilm's structure formation. The aim of presented study was to link the presence of *psm-mec* gene with the production of biofilm structures by *S. aureus* clinical strains. First, the presence of *psm-mec* gene in 26 methicillin-resistant *S. aureus* (MRSA) strains collected from pediatric patients treated in University Children's Hospital in Prokocim, Krakow, was confirmed by PCR reaction specific for targeted gene. Furthermore, the control group of 10 MRSA strains without *psm-mec* had been selected. Then, phenotypic analysis of staphylococcal strains based on biofilm development rate was performed. The biofilm formation had been examined by measuring the absorbance of each sample, and classified on its basis into 5 groups of biofilm formation rate. The obtained results showed that strains with *psm-mec* gene, produced biofilm at a much higher level, compared to ones with no *psm-mec* present. This may suggest, that *psm-mec* promotes biofilm formation in *Staphylococcus aureus* strains.

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Studies on LPORC transcriptional activity in dark-grown seedlings of *Arabidopsis thaliana*

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In angiosperms, the light dependent NADPH:protochlorophyllide oxidoreductase (LPOR) catalyzes the conversion of protochlorophyllide to chlorophyllide, enabling chlorophyll biosynthesis only under illumination. The accumulation of LPOR and its substrate, protochlorophyllide, in etiolated seedlings is beneficial and ensures rapid biosynthesis of initial chlorophyllide/chlorophyll pool from the beginning of light exposure. However, a part of protochlorophyllide stay unbound to LPOR and increases a risk of reactive oxygen species production in light. In *Arabidopsis thaliana*, LPORA and LPORB are believed to form protochlorophyllide-LPOR complexes in etiolated seedlings, which formation is regulated by as yet unknown mechanisms.

In our work, we analysed the accumulation of mRNA encoding LPORC in *A. thaliana* during 12 days of seedling growth in darkness. Surprisingly, we detected LPORC transcripts already in dark-grown 4-day-old plants. The expression of LPORC gradually decreased in older seedlings. LPORC is considered to be accumulated preferentially in illuminated plants and little is known about its biosynthesis in dark. Our observation point to the contribution of the LPORC isoform to the well-known LPOR pool accumulation in young etiolated seedlings and, in consequence, to the initial chlorophyll biosynthesis. Moreover, we observed that LPORA and LPORB expression also decreased during etiolation whereas the content of unbound protochlorophyllide increased. No symptoms of plant stress (based on ion leakage), nor senescence (*SAG12* and *SAG13* marker genes) were detected. Thus, the observed decrease in LPORs transcripts were not due to seedling senescence. Prolonged etiolation is not detrimental for seedlings but it might influence the chlorophyll biosynthesis in greening seedlings.

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Optimisation of muscle satellite cells generation from human induced pluripotent stem cells

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Duchenne muscular dystrophy (DMD) is a neuromuscular disorder originating from mutations in *DMD* gene which result in complete loss of *DMD*-encoded dystrophin [1]. Our and others studies demonstrated that DMD significantly affects not only muscle fibers but also satellite cells (SC), the muscle stem cells [2,3]. Limited availability of human SC and mature muscle fibers, however, hinders better understanding of DMD-related molecular changes occurring in these cells [1]. Therefore, the aim of this study was to evaluate the potential of described protocols for myogenic differentiation of human induced pluripotent stem cells (hiPSC) to generate SC (hiPSC-SC). First approach, reported by Shelton *et al.* [4], relied on induction of mesoderm development from hiPSC by activation of WNT pathway and further culture in Essential 6 medium. Analysis performed from day 8 to 14 revealed the presence of cells expressing SC surface markers (CXCR4, $\alpha 7$ integrin and CD82), however after sorting, they did not sustain expression of PAX7, a canonical intranuclear SC marker. In contrast, long term differentiation (until day 30) provided higher level of $\alpha 7$ integrin+CD82+ population with partial PAX7 expression maintained under proper culture conditions post sorting. Second approach was based on Caron *et al.* [5], however, it failed to provide reproducible generation of hiPSC-SC. The last method relied on commercially available Skeletal muscle differentiation kit (Ambio) and provided the highest efficiency of PAX7-expressing cells generation. Importantly, initial studies indicated the delayed hiPSC-SC development from DMD hiPSC line in comparison to their isogenic, control counterparts. In summary, hiPSC technology can provide novel model for disease modelling in hiPSC-SC.

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Chaos Game Representation and its application in free-alignment methods

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The numerical representation of biological sequences plays an important role in bioinformatics and has many practical applications. One of most popular approaches is the Chaos Game Representation, where a sequence is represented on a plane inside a unit square. Despite the simplicity, it provides reliable results. There are also two especially interesting methods namely the Recurrence Quantification Analysis and the Cross-Recurrence Quantification Analysis which may be applied to both nominal and numerical sequences. They measure repetitions of elements within one or two series and may be useful in pattern searching. They were successfully employed to many biological problems such as protein classification or transitions detection in protein dynamics. One advantage is the possibility to apply them for short sequences, which is a problematic issue in other methods. Additionally, they may be computationally inexpensive. The Recurrence Quantification Analysis and the Cross-Recurrence Quantification Analysis may be utilized alternatively to Chaos Game Representation, however, some authors combined them to get better results. In our paper we compared these methods and employed them to free-alignment approach. Additionally, we indicated some criteria to find the optimal parameters for them.

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Temozolomide increases the invasive potential of glioblastoma multiforme cells in the Snail-1-dependent, MGMT-independent manner

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Effective treatment of glioblastoma multiforme (GBM) remains one of the greatest challenges of the current neuro-oncology. Current standard of care for patients with GBM is surgical resection, followed by concurrent radiotherapy and temozolomide (TMZ) – based chemotherapy. However, it increases the median survival of GBM patients only up to 15 months. In many cases GBM becomes unresponsive to TMZ during treatment, predominantly due to the up-regulation of O-6-methylguanine-DNA methyltransferase (MGMT) [1]. Here, we compared the effect of long-term TMZ treatment on the invasive potential of TMZ-sensitive MGMT-low U87 and TMZ-resistant MGMT-high T98G cell line. In our hands, TMZ treatment augmented the invasiveness of T98G and U87 cells (estimated by time-lapse video microscopy and transmigration assay), increased Snail-1 and connexin 43 (Cx43) levels in these cells and induced morphological changes reminiscent of epithelial-mesenchymal transition. Additionally, U87 cells reacted to TMZ with hypertrophy correlated with their decreased apoptotic response. Inspired by our previous data concerning the role of Cx43 in the formation of tumor invasive fronts [2], we also analyzed the effects of Cx43 overexpression/knockdown on the responsiveness of GBM cells to TMZ to show the function of Cx43 in TMZ-induced pro-invasive responses of T98G but not of U87 cells. These data show that long-term TMZ treatment can prompt the progression of GBM by evoking Snail-1 and/or Cx43-dependent glial-mesenchymal transition (GMT). MGMT is not necessary for this process, however it can modulate its progress and efficiency.

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Bioactive compounds from *Lactarius deterrimus* interfere with invasive potential of gastric cancer cells

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Stomach cancer is 4th most common cancer among men. Despite the years, stomach cancer treatment methods do not differ from those used fifty years ago. Still the most effective methods of treatment are surgeon's tools and chemo-/radio-therapies. Mushrooms as rich in potentially therapeutic substances seem to be forgotten and omitted. Studies conducted by asian researchers confirm anticancer properties of many native mushrooms i.a. *Coriolus versicolor*. In this study, anticancer working of *Lactarius deterrimus* ethanolic extract on AGS (human stomach cancer cells) were examined. Firstly, HPLC test showed richness of potentially anticancer chemical compounds in this extract. Application of LDE (*Lactarius deterrimus* extract) in AGS cell culture induced significantly proliferation inhibition, cell viability decreasing and reduction of migration activity of these cells. Furthermore, LDE caused increased oxidative stress level and intensified autophagy process, which lead a significant amount of cells to apoptosis. What is more, LDE affected on F-actin cytoskeleton arrangement of cancer cells by stress fibers reduction and actin degradation. Moreover, metabolic analysis showed that LDE decreased ATP production in AGS cells. Besides, in all conducted experiments, LDE enhanced cytostatic drugs (which are commonly used in stomach cancer therapy- cisplatin and 5-fluorouracil) effects. Studies on LDE require following stages. However, LDE seems to be promising supplement of stomach cancer therapy.

MyD88s, a splice variant of MyD88

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MyD88 is one of the key players in native immunity. It acts as a cytosolic adaptor protein for IL-1R and all TLRs except TLR3 upon their recognition of PAMPs. After TLR activation and dimerization, MyD88 is recruited via its TIR domain and then binds to downstream IRAK kinases through death domain (DD) interactions and this protein complex is called myddosome. The signaling pathway mediated by MyD88 results in NF-κB activation and production of variety of proinflammatory agents. However, immune responses must be tightly controlled and adjusted as any aberration and excessive reaction could lead to pathogenesis. MyD88s is a splice variant of MyD88 that inhibits NF-κB activation and is observed in cells after a prolonged stimulation with LPS, a ligand for TLR4.[1,2] MyD88s lacks an intermediate domain (ID) that is mostly consider to be a linker between DD and TIR domains. Yet, the fundamental differences between MyD88 and MyD88s in their ability to activate NF-κB indicate that ID is essential for signal propagation. It is proposed that MyD88s is not able to form myddosomes and hence inhibits the pathway. However, the detailed explanation of myddosome formation is still necessary. We investigated whether MyD88 and Myd88s are able to interact with each other as dimer formation seems to be crucial for initiating myddosome formation. We also wanted to examine if stimulation of other TLRs could induce expression of MyD88s as it is not known whether MyD88s regulates the pathway only when TLR4 is activated or other TLRs likewise.

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Copy number variation analysis in selected lines of Leghorn chickens

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Copy number variation (CNV) is considered as one of the major sources of genetic variation. In this study CNVs were detected and analysed within four Leghorn layers chicken lines using next generation sequencing and CNVnator and DELLY programs. Using CNVnator a total of 23017 CNVs including 20577 deletions and 2440 duplications were identified. Using DELLY a total of 41681 CNVs including 33621 deletions and 8060 duplications were identified. Between all four lines we found 32 CNVs which were confirmed by both programs. This CNVs harbored 82 genes. Some of these genes coded proteins that can be phenotypically important. In our study we catalogued CNVs in four examined lines, confirmed CNVnators and DELLYs capacity for detection of CNVs and their application for choosing candidates for further analysis by microarrays and qPCR. CNVnator was proved to be more accurate in terms of CNV detection.

Vemurafenib and dabrafenib, the BRAF inhibitors, selectively interact with RIPK4

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Small-molecular weight inhibitors of mutant BRAF kinase (vemurafenib and dabrafenib) are used for the treatment of malignant melanoma patients carrying V600E BRAF mutation. However, melanoma cells develop resistance for both drugs when used as monotherapy. Therefore, mechanisms of drug resistance are investigated and new molecular targets are sought that could completely inhibit the development of melanoma. Because of suggested oncogenic function of the receptor-interacting protein kinase (RIPK4) kinase in melanoma and its structural similarity with BRAF protein, we analyzed the impact of vemurafenib and dabrafenib on RIPK4 in melanoma cells [1]. The *in silico* study confirmed the high similarity of the kinase domains of the BRAF mutant to the RIPK4 protein on both the sequence (BLAST) and the structural (PyMOL) level. Molecular docking analysis revealed that both drugs could directly bind to RIPK4. Besides these, drug - RIPK4 interactions are 50-65% stronger than interactions of ATP with RIPK4. Furthermore, we demonstrated that both vemurafenib and dabrafenib inhibits ERK1/2 activity and lowered RIPK4 kinase level in melanoma cells by Western blot analysis.

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Common errors committed by experimentators during in vivo studies on mice: unaware or aware? Dermatologic perspectives

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Among the animals models used in biological and biomedical research murine ones have remained to be invaluable. Over the years the mouse strains become more and more sophisticated and demanding. But neither widely available sensitive methods and instruments, nor animal models designed for particular purposes, guarantee the success of the correct interpretation of the *in vivo* data without a deep knowledge of the animal biology.

Murine skin is its biggest organ. In the lifetime the skin undergoes repeatedly enormous physiological changes within a relatively short time, concerning its thickness, pigmentation, vascularization, innervation, hormone level, expression of numerous growth factors and receptors, activation of different metabolic pathways, or activity of the immunologic system [1]. All these changes are connected with cyclic growth of hair and the cycle-associated remodeling of hair follicles. Both the skin itself, and the intensity of its changes affect the whole organism. Moreover, the presence of melanin in mice fur finds its effect in skin color, spleen melanosis, pigmentation of the aortic and pulmonary valves, and lymph nodes [2, 3]. Also the strain- and age-related changes just like ulcerative dermatitis (UD) for skin of C57BL/6 background mice [4] must be taken into account. Although the hair cycle and its influence on the skin biology is known for years, it is still neither known nor understood commonly enough. It needs to be emphasised that the lack of this knowledge may lead to the misinterpretation of the *in vivo* experiments. We try to shortly characterize this situations in this poster.

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Photoreactivity of natural melanins from different skin phototypes

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Melanin pigments are produced in a multistage process by specialized cells - melanocytes. In the skin, melanin once produced is quickly transferred to neighbouring keratinocytes. Melanin, particularly brown-black eumelanin, is perceived as an effective photoprotective agent against solar radiation due to its ability to absorb and dissipate light and scavenge reactive oxygen species. While eumelanin does it very efficiently, the yellow-reddish pheomelanin, on the other hand, seems to be less photoprotective and even photoreactive. Recently, photoreactivity of melanin has become a major focus of research for the suggested involvement of the pigment in UVA-induced melanoma. Available research demonstrates that pheomelanin can generate reactive oxygen species, in particular singlet oxygen much more efficiently than eumelanin. However, most of the studies were carried out using synthetic melanin models, therefore, photoreactivity of natural melanins is yet to be systematically analyzed. In this research, we examined photoreactive properties of natural melanins isolated from hair samples obtained from donors of skin phototypes (I, II, III and V). X-band and W-band electron paramagnetic resonance (EPR) spectroscopy were used to determine the paramagnetic properties of the pigments. EPR oximetry and EPR spin-trapping were used to examine oxygen photoconsumption and photo-induced formation of superoxide anion during UV-A and blue light irradiation. Finally, time-resolved singlet oxygen phosphorescence was employed to demonstrate the generation and quenching of singlet oxygen by natural melanin pigments.

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Spheroids Cell Cultures as the promising *in vitro* tumor model in pancreatic ductal adenocarcinoma (PDAC) studies

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Standard neoplastic cells grown using the two-dimensional method lose crucial properties that affect their further development. First of all, cell-to-cell communication, the possibility of differentiation and polarization, and contacts with the extracellular matrix are lost. That means that the *in vitro* experimental data have limitations. It is significant to recreate more complete conditions to study tumor growth *in vitro*. The alternative method is to culture cells in 3D, where the cells form into spheroids. Spheroids mimic physiological conditions to certain degree, which is of great importance in research on neoplastic tumors in the context of a better understanding of the basic neoplastic processes and testing antitumor therapies. [1,2]

The goal of the study is to determine the optimal conditions for the formation and characterize the spheroids from human and mice pancreatic ductal adenocarcinomas and their growth in Matrigel®. For this purpose, two cell lines were used: Pan_O2 and Panc-1, which were grown using the hanging drop method. The obtained spheroids were inserted into the Matrigel®. Subsequently, the spheroids were stained by H&E and immunohistochemistry and imaged using a fluorescence microscope, and biochemical analysis using the Western blot method was also performed.

These data are the basis for further research related to the recognition of morphological and structural features of 3D culture. A thorough understanding of the interactions between cells and individual components of the extracellular matrix, as well as the impact of the microenvironment on neoplastic cells, will help to understand the molecular mechanisms occurring within pancreatic ductal adenocarcinoma (PDAC) tumors.

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Influence of environmental factors on proliferation, cell cycle and genome editing in HEK293T cells

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Environmental conditions, such as temperature and oxygen concentration are one of the key components of *in vitro* cell culture, affecting vital cellular processes, such as growth and metabolism. These can impact genome editing results, which are dependent on cell cycle stage and the activity of DNA-damage repair machinery.

The major goal of this project was to examine the effect of hypothermia (32 °C) and hypoxia (5% O₂) on proliferation, cell cycle, and genome editing efficiency in HEK293T cells, which constitute a model cell line for genome editing studies. Proliferation and cell cycle were measured by hemocytometer and flow cytometry, respectively. To measure genome editing rates, we edited WT1 and OCT4 loci and performed PCR-based genotyping using single cell-derived clones.

Our results show that both hypoxia and hypothermia significantly decreased the proliferation rate of HEK293T cells. Moreover, these conditions affected the cell cycle of HEK293T cells. The most significant change was observed in hypoxia, which induced S phase-arrest. This could be beneficial for increasing the efficiency of precise genome editing based on homologous recombination. However in our study, we achieved the highest efficiency of precise genome editing in standard cell culture conditions, based on the number of positively targeted cell clones. Interestingly, hypoxia and hypothermia favored precise bi-allelic integration of the transgenic cassette.

In summary, we have shown that environmental conditions impact on the proliferation and cell cycle of HEK293T cells. The combination of hypoxia and hypothermia increased the efficiency of bi-allelic but not the monoallelic targeted integration in these cells.

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Spectral intensity-dependent Monte Carlo simulations of light transport in scattering and absorbing media

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Successful photodynamic therapy (PDT) requires knowledge of the photodynamic dose that is a function of tissue oxygen availability, photosensitizer concentration, and light fluence, these which are difficult to measure. The PDT uses incoherent light sources or lasers. Daylight photodynamic therapy (DPDT) uses sunlight as an alternative light source for PDT. DPDT offers many advantages over conventional PDT in terms of tolerability, treatment duration, and cost [1].

A dedicated Python software was developed to simulate the transport, scattering and absorption of light through the skin tissue during illumination by sun or artificial white light source. The application extends classical Monte Carlo Radiation Transfer simulation programs MCLM and CONV into an integrated system for performing MC calculations for spectral irradiance in all wavelengths. The developed system requires two parameters, the optical properties of the tissue layers and the light intensity profile, as input data. The different light sources are simulated by sampling wavelengths from the corresponding light spectra. The values of the optical parameters (absorption, scattering, refractive indices, and anisotropy factor) of the layer must be known for all wavelengths, the experimental values are only available for certain wavelengths. These must be supplemented by theoretical calculations and extrapolation of experimental data. In this paper we show the results of MC simulation of multi-wavelength light transport in synthetic melanin (from DOPA) and Intralipid solutions. The obtained results reproduce the distribution of the light field (as a function of wavelength) inside the studied solutions with good accuracy.

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The effect of pan-PPAR and duo-PPAR agonists on the effectiveness of myofibroblastic transitions of human bronchial fibroblasts from asthmatics

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Bronchial wall remodeling is observed in respiratory tract of patients with diagnosed asthma (AS) and is characterized among other by subepithelial fibrosis. In this process, human bronchial fibroblasts (HBFs) are exposed to the locally high concentration of transforming growth factor- β_1 (TGF- β_1) and following acquire the highly contractile myofibroblast phenotype with stress fibers enriched with α -smooth muscle actin (α -SMA) anchored in the supermature focal adhesions (sFA). Our previous studies suggested that peroxisome proliferator-activated receptors (PPARs) may regulate the FMT potential of HBFs AS. In this study we investigated the effect of dual-PPAR and pan-PPAR ligands on the FMT potential in HBFs AS isolated from bronchoscopy sections. Cells were cultured in serum-free conditions with dual-PPAR agonist (GFT505) or pan-PPAR agonist (IVA337) in the absence or presence of TGF- β_1 for 5 days. The non-cytotoxic concentrations for both compounds were determined using crystal violet and MTT assay. FMT potential was determined by the percentage of myofibroblasts using immunofluorescence and/or in-cell ELISA assay. Our results demonstrate the inhibitory effect of GFT505 and IVA337 on the both percentage of myofibroblasts and levels of myofibroblast-related proteins (α -SMA and collagen1) in TGF- β_1 treated HBFs AS. Moreover, TGF- β_1 -induced maturation of FA was significantly diminished by the IVA337 in HBFs AS. Exerted the strong inhibitory effects of both compounds on FMT and/or architecture of actin cytoskeleton in TGF- β_1 -treated HBFs AS show their ability for the suppression of subepithelial fibrotic changes in airway wall, but extensive studies are needed to clarify the therapeutic potential of dual/pan-PPAR agonists in asthma.

The involvement of p38 kinase in the myofibroblastic transitions of human bronchial fibroblasts from asthmatic patients

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Subepithelial fibrosis is a component of complex process termed remodeling observed in bronchial wall of asthmatic patients. In this process, human bronchial fibroblasts (HBFs) drive the phenotypic shifts into highly contractile myofibroblasts (FMT) in response to the locally high levels of the inflammatory cytokines, such as transforming growth factor- β_1 (TGF- β_1). Myofibroblasts express the α -smooth muscle actin (α -SMA) incorporated into stress fibers anchored in the supermature focal adhesions (sFA). On the other hand, enhanced secretory activity of myofibroblasts leads to the increased deposition of extracellular matrix proteins as collagens. Exposition of HBFs on TGF- β_1 leads to the activation of TGF- β_1 /Smad2/3 axis, known as the canonical profibrotic signaling pathway. However, pleiotropic properties of TGF- β_1 to the activation of others non-canonical signaling pathways may also promote FMT. Several reports suggest that TGF- β /MAPK pathways participate in the progression of fibrosis in many tissues. However, little is known about the role of p38-dependent signaling in the effectiveness of phenotypic shifts of TGF- β_1 -treated HBFs. In this study we investigated the effect of p38 kinase inhibition on the FMT potential of HBFs derived from asthmatic patients. Our results demonstrate that the chemical inhibitor of p38 activity (SB203580) used in non-cytotoxic concentration significantly reduces TGF- β_1 -induced FMT measured by percentage of myofibroblasts and levels of myofibroblast-related genes and/or proteins (α -SMA, collagen1, fibronectin) which correlate with the limitation of FA maturation. These observations justify future research on the role of p38 kinase on FMT efficiency and bronchial wall remodeling in asthma.

Mitochondrial rearrangements participate in the microevolution of GBM cell resistance to doxorubicin

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Recent progress in elaboration of new clinical strategies of glioblastoma multiforme (GBM) chemotherapy is related to the introduction of doxorubicin (DOX) as a drug, which relatively effectively interferes with GBM development. Nevertheless, the mechanisms of GBM resistance to DOX have not been described so far. Taking into account the significance of drug-evoked microevolution of GBM drug-resistance and its links with tumor cells metabolism, we investigated the mitochondria-related “metabolic flexibility” of human T98G GBM cells subjected to DOX stress. Using fluorescence microscopy, ImageStream® cytometry, enzymatic assays and Seahorse® XF-assisted metabolic profiling, we estimated short- and long-term GBM cell responses to DOX treatment, with the special emphasis on the metabolic profile of cells that survive DOX-induced stress. Short-term cellular responses of T98G cells to DOX included impairment of glucose (2-NBDG analog) uptake and the induction of mitochondrial reactive oxygen species (mtROS) production. Attenuating effect of ATP-synthase inhibition by Oligomycin A on DOX-treated cells survival was correlated with the intensified oxidative phosphorylation (OXPHOS) and mitochondrial hyperfusion in the cells undergone long-term DOX treatment. Both processes are apparently related to the function of the regulators of mitochondrial dynamics (MFF, Drp-1, March-5). Collectively, we hypothesize that mitochondrial fusion facilitates OXPHOS in DOX-treated GBM cells, which is crucial for the metabolic “compensation” of DOX cytotoxicity. These data shed the new light on the role of “metabolic flexibility” in the microevolution of GBM drug-resistance.

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An old inhibitor with a new target

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Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) is a constitutively active serine/threonine kinase, which activity is regulated by the autophosphorylation. Initially DYRK1A has attracted the therapeutic attention due to its genetic locus in the human chromosome 21, called Down syndrome (DS) critical region, and its association with both neuropathological phenotypes and cancer susceptibility in patients with DS. More recently high throughput screens have identified DYRK1A inhibitors as inducers of pancreatic beta-cell proliferation, and thus potential therapeutic candidates to promote islet regeneration in diabetic patients. The proliferative effect is driven by the induction and nuclear translocation of NFAT transcription factors, which act to transactivate the cell cycle. Thus the need for DYRK1A inhibitors motivates scientists for search for new therapeutic compound with potent application in DS, cancer and diabetes.

Here, we report that CX-4945, a well-characterized inhibitor of casein kinase 2 (CK2) currently in phase I/II clinical trials for cancer treatment, inhibits DYRK1A as off-target kinase in ATP-competitive manner. *In vitro* validation, including Microscale Thermophoresis (MST) and drug affinity responsive target stability (DARTS) technique, proved the specific interaction between CX-4945 inhibitor and DYRK1A kinase. Additionally CX-4945 targeting DYRK1A was able to restore NFAT activity in dose-dependent manner in cell based assay. The presented crystal structure of DYRK1A-CX4945 explains the binding mode at atomic level and empowers the comparative analysis for further selective inhibitor design.

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Developing [60]Fullerene Nanomaterials for Better Photodynamic Treatment of Non-Melanoma Skin Cancers

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Skin cancer is the most common cancer in the U.S.A. and Europe. Its subtype, squamous skin carcinoma (SCC), if allowed to grow, has the potential to metastasize and can become deadly. Currently, carbon nanomaterials are being developed to treat cancer due to their attractive physicochemical and biological properties such as an enhanced permeability effect and their ability to produce reactive oxygen species. Here, we describe the synthesis of two water-soluble aminofullerenes (MonoaminoC60 and HexakisaminoC60), which were evaluated as novel [60]fullerene based photosensitizers exhibiting anticancer properties. Moreover, the previously described neutral glycofullerene GF1 and its peracetylated lipophilic precursor MMS48 were compared with the aminofullerenes for their ability to generate reactive oxygen species and oxidize lipids. Remarkably, the generation of singlet oxygen and a superoxide radical by HexakisaminoC60 was found to be markedly elevated in the presence of bovine serum albumin and NADH, respectively. Mechanistic studies of lipid peroxidation using cholesterol as a unique reporter molecule revealed that although all four fullerene nanomaterials primarily generated singlet oxygen, superoxide anion was also formed, which suggest a mixed mechanism of action (in which Type I and Type II photochemistry is involved). The [60]fullerene derivative HexakisaminoC60 was also studied for its phototoxicity in squamous skin cancer cell line (A431) using the MTT test and propidium iodide staining.

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The Sleeping Beauty and MS2 combined systems as a tool for identifying transcript-regulating proteins and transcript tracking

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The Sleeping Beauty (SB) transposon system originates from the reactivated transposon in salmonid fish. Its enzymatic activity was improved by obtaining a hyperactive mutant transposase SB100X. The system employs the DNA transposons flanked by inverted terminal repeats (ITRs), recognized by the transposase. The SB transposon integrates into the genome in a limited number of copies thus allowing for avoiding recombinant protein aggregates and overloading of specific biological pathways.

The MS2 tagging system is a method based on the natural interaction of coat protein (MS2-BP) of bacteriophage MS2 with stem-loops structures that occurred in the phage genome. The MS2 system is based on two vectors that are transfected into recipient cells. The first vector encodes the mRNA of interest with MS2 loops added at its 3' end. The second vector encodes a fusion protein consisting of the MS2-binding protein fused with the fluorescent protein. Upon introduction of both vectors into cells the fluorescent MS2-BP binds the MS2-loops in the investigated transcript allowing for the direct visualization of the mRNA in living or fixed cells.

Using the SB system we have introduced MS2 system elements into cells avoiding stress responses resulting from the high expression of recombinant protein. The obtained cell line is characterized by the continuous expression of

IL-6-MS2 stem-loops mRNA and doxycycline-inducible expression of the MS2-BP-EGFP fusion protein. Implementation of this cell line will allow us to identify proteins engaged in *IL-6* mRNA half-life regulation and analyze the co-localization of the *IL-6* transcript with proteins and cell structures.

Analysis of growth and pigmentation of melanoma tumors cultured on the chorioallantoic membrane of a fertilized chicken egg

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Melanoma is an aggressive form of cancer that exhibits metastasis to various critical organs, including the liver, lungs, bones, and brain. One of the characteristic features of melanoma is the ability to synthesize melanin pigment. In normal tissues, melanin has a protective function against solar radiation thanks to its physicochemical properties such as broadband absorption and its ability to scavenge free radicals. In melanoma, melanin synthesis is highly dysregulated, and the exact role of melanin on tumor growth, remains unknown. The loss of the ability to synthesize melanin by most melanoma cells in *in vitro* culture and the limitations of *in vitro* culture make it difficult to analyze the effect of melanin pigmentation on the growth of melanoma tumors.

In this work, we present a unique model of melanoma cell culture on the chorioallantoic membrane of a fertilized chicken egg (CAM) [1]. In the analysis we employed melanoma cells of different origin, including mouse, hamster and human, of either melanotic or amelanotic origin. The obtained results indicated a correlation between localization of melanoma tumors on the CAM membrane and their melanin pigmentation. Tumors located 'below' the membrane were heavily pigmented, whereas tumors 'above' the membrane were amelanotic. Results of electron paramagnetic resonance spectroscopy analysis indicated that the composition of melanin in melanoma tumors grown on the CAM was almost identical to that of cells cultured *in vitro*. The obtained results indicate that both the size and morphology of melanoma tumors may depend on the pigmentation of cells.

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The role of protein Regnase-3 in *Porphyromonas gingivalis*-induced neuroinflammation

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Neurodegenerative disorders are a growing problem associated with the aging society, strongly connected with chronic neuroinflammation. Recent studies demonstrate, that also periodontitis and infection with *Porphyromonas gingivalis* may contribute to the development of inflammation in the brain and neuroinflammatory disorders for example Alzheimer's disease. Bacterial infection activates inflammatory responses contributing to the disease progression. Inflammation is controlled on different levels, including the regulation of inflammatory transcripts stability. MCPIP/ZC3H12 protein family has been recently described as RNases regulating mRNAs turnover. One of the family members - Regnase-3 (Reg-3), is an active RNase capable of destabilization of inflammation-related transcripts such as IL-6, Regnase-1 and IER3 mRNAs. According to the data from the public database (Human Protein Atlas) and our preliminary results, Reg-3 is highly expressed in the brain. We have also shown, that infection of astrocytoma cell line U251-MG with IL-1 β , as well as infection with *P. gingivalis* induces synthesis of Reg-3 mRNA. Induction of Reg-3 expression results in the inhibition of the level of pro-inflammatory transcripts upregulated upon *P. gingivalis* infection. Simultaneously, infection with *P. gingivalis* results in downregulation of Reg-3 protein level. All these data suggest the potential involvement of Reg-3 in the regulation of neuroinflammation induced by *P. gingivalis* infection.

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Development and optimization of new orthotopic model of pancreatic cancer in mice

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Pancreatic cancer is an extremely aggressive type of disease with a poor prognosis and a five-year survival rate of less than 7%. Even in the case of early diagnosis, the neoplasm is very difficult to surgically resect. Gemcitabine is a commonly used treatment, however, often has little impact on patient survival because of chemoresistance. Novel therapeutic options of pancreatic cancer are urgently needed, as well as the establishment of reliable and reproducible animal models.

The aim of the study was to optimize a murine, immunocompetent pancreatic cancer model in mice. The orthotopic model of pancreatic cancer has clinical relevance better than subcutaneous models, and reflects the relationship between the cancer and its tissue environment. In pancreatic tumors, their location significantly affects their matrix and vascularization.

We compare the different tumour inoculation techniques using both cell suspensions and spheroids, also the ultrasound-guided and surgical inoculation. The most effective approach was to use spheroids closed in Matrigel® plugs, which prevent leakage of cancer cells into the peritoneal cavity. Plugs were delivered into the pancreas during surgical inoculation.

New orthotopic model is designed to allow non-invasive imaging of tumor progression in real time, allowing to a significant advancement in the drug screening and a high predictive value for the response to treatment of patients with pancreatic cancer.

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From first light to first stress – how young greening wheat seedlings respond to light stress

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Greening is an especially important process in plant development. It includes well-choreographed cellular changes, including the synthesis of chlorophyll and proteins, and the formation of photosystems. Greening is induced by light, which can generate stress. However, it stays unknown which protective mechanisms are active during the initial phases of greening.

We used 6-day-old etiolated wheat seedlings and illuminated them with white light (100 or 500 μ moles of photons per m² per s) for 1 or 6 hours to induce greening. Then, they were treated with high light intensity for 40 minutes to induce stress, and left in darkness for 6 hours. At certain time points we analysed the pigment composition of seedlings' tissues, with special regard to pigments of light-protecting violaxanthin cycle. *In vivo* chlorophyll fluorescence was measured to monitor the activity of the photosynthetic apparatus.

The seedlings which were de-etiolated for 6 hours had more completely assembled photosynthetic systems, as shown by the presence of chlorophyll *b* compounds. When it comes to light intensity used for de-etiolation, the higher one seemed to be more stressful for seedlings. After de-etiolation, such seedlings contained more zeaxanthin and its content did not increase during high-light condition. This indicates high initial activity of the violaxanthin cycle. Moreover, these seedlings greened with higher light intensity exhibited low values of maximum quantum yield of photosystem II.

In conclusion, this model for studying the earliest stages of plant greening is capable of discerning the differences in regards to how greening plants react to sudden light stress.

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How the lack of lutein or chlorophyll b affects the greening process?

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Emerging angiosperm seedlings undergo greening to become photosynthetically active. The development of photosynthetic membranes includes biosynthesis of pigments and proteins, and their assembly in photosynthetic complexes. We have compared greening of 5-7 day old *A. thaliana* seedlings, including *lut2*, *CaO* mutants and Wild Type (WT). These mutants have disrupted biosynthesis of lutein (*lut2*) and chlorophyll *b* (*CaO*) - pigments of LHC photosynthetic antennae involved in light harvesting. Additionally, lutein contributes to the chlorophyll triplet state quenching in the Photosystem II (PSII).

Etiolated seedlings were exposed to white light (100 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) for up to 24 hours. We have measured their photosynthetic pigment profile (HPLC and fluorescence), Maximal PSII Quantum Yield (F_v/F_m) and Non-Photochemical Quenching (Chl *in vivo* fluorescence), plastid ultrastructure (TEM) and the expression of multiple genes (RT-PCR).

After 24-hours of greening, PLBs (characteristic for etiolated seedlings) were still present in *lut2*; *CaO* mutants developed only a lamellar membrane system, whereas small grana were evident in WT. Reduced level of all photosynthetic pigments was observed in *CaO*, whereas *lut2* mutants over accumulated xanthophylls (excluding lutein). Both mutants had lower F_v/F_m value than WT and differed in NPQ kinetics. The transcript level of several genes, involved in chlorophyll metabolism and LHC complexes assembly, was altered in *lut2* and *CaO* mutants when compared with WT. At the end of the etiolation, the level of the photoactive protochlorophyllide was higher in *lut2* compared to *CaO* and WT. The initial chlorophyllide production was also significantly higher in *lut2* than in WT and *CaO*.

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Production of antibodies specifically recognizing MCPIP2 and their use for studying MCPIP2 posttranslational modifications induced by IL-1 β

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MCPIP2 is a recently identified RNase engaged in the regulation of the decay of a set of inflammation-related transcripts. MCPIP2 possesses a NYN/PIN and a CCCH-type zinc finger domains which together allow this enzyme to degrade specific mRNA substrates. We found that the activity of this protein is controlled at the post-translational level. IL-1 β signaling causes a temporary inactivation of MCPIP2 that results in increased levels of its substrates. However, our results are based on overexpression studies and as overexpression may cause intracellular pathways overload and cell stress the physiological relevance of our findings needs confirmation at the level of endogenous MCPIP2 protein. Because specific MCPIP2 antibodies are not available commercially we decided to produce in-house antibodies using hybridoma method. As MCPIP2 is highly similar to another MCPIP protein family member, MCPIP3, and both proteins are of similar mass (94 and 99 kDa respectively) we decided to use a novel approach for antibody production in terms of the antigen design. Instead of using full length protein we selected the most unique MCPIP2 peptides joined by linkers with low immunogenicity constructing a chimeric protein which after purification will be used for immunization and should allow for production of antibodies specifically recognizing MCPIP2.

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