22 - 24 FEBRUARY 2022

OMICS RESEARCH
FROM METHODOLOGY TO APPLICATION

Winter School FBBB
Jagiellonian University

ABSTRACT BOOK

Organized by: Laboratory of Metabolomics

Dear Fellow Scientists,

We are pleased to present the abstract book of the XLIX Winter School of FBBB, held in Krakow, Poland, from 22 to 24 February, 2022. Winter School is a periodic summit of our community which promotes active scientific exchange and communication between scientists and students. The theme of this year's XLIX Winter School is "Omics research – from methodology to application".
During this three-day event, trainings and interactive thematic sessions will be held that will allow presentation of the recent research by the scientists from our Faculty related to the theme of the Winter School. Participants will have the opportunity to gain hands-on experience from the experts on the subject and to network with other researchers. We are happy that so many members of FBBB as well as collaborating partners participate in this event.

This year Winter School is different from the previous ones, as it is focused on the methodological aspect. Omics Research is a rapidly evolving, multi-disciplinary, and emerging field that encompasses genomics, transcriptomics, proteomics, and metabolomics. Each of these fields offers the possibility to understand and view biology from a global perspective in a way that was previously unthinkable.
During this edition of the conference, experts in the 4 main topics of Omics Research offered Training Sessions. There are also Oral talks and Poster Sessions. Almost 180 participants present their work, including 31 students and 70 PhD students. There are 24 oral talks and 75 posters presented in the two Poster Sessions.

We believe that the Winter School will inspire to take up Omics methodology into Your research topics. What is equally important, the conference, together with the social events, will provide an excellent platform to build and strengthen our relationships and make this meeting truly unforgettable.
We want to take this opportunity to thank everyone who participated in the successful preparation and realization of the event. Special thanks go to the whole Organizing committee, the Scientific Committee and the Volunteers! Special thanks to the Dean of Faculty of Biochemistry, Biophysics and Biotechnology for hosting this event.

Enjoy the conference!

Dariusz Dziga
Chair of the XLIX Winter School of FBBB, Kraków

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Paweł Golik
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Invited Experts for Training Sessions

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Winter School of FBBB, JU

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9.10 -11.00 **prof. Krzysztof Meissner** (Faculty of Physics, UW) **Prostota i złożoność: od fizyki do biologii**
prof. Paweł Golik (Faculty of Biology, UW) **Czego nie wiemy — wyzwania i problemy genomiki**

11.00 -11.30 Coffee break

TOPICS: GENOMICS & TRANSCRIPTOMICS

11.30 -13.30 Training Session 1: **Genomics**
Wojciech Branicki (MCB & IZBR, JU), **Introduction to human genome variation analysis and beyond.**
Rezvan Noroozi (MCB, JU), **DNA microarray techniques in genomics and epigenomics research.**
Wiesław Babik (IES, JU), **Population-scale whole-genome and targeted resequencing in non-model organisms.**
Piotr Łukasik (IES, JU), **High-throughput characterization of microbiomes, host-microbe interactions, and beyond.**
Agata Jarosz (MCB, JU), **BioS Genomics Core Facility - what we can do for you.**

13.30 -14.30 Lunch break

14.30 -16.30 Training Session 2: **Transcriptomics**
Maja Kosecka-Strojek (FBBB, JU) **RNA processing and preparation of NGS libraries**
Michał Bukowski (FBBB, JU) **From short reads to differential expression**
Sandra Sierankowska (Analityk Genetyka) **Transcriptomics on Illumina platforms**
Guillem Ylla (FBBB, JU) **Small RNAs: when the small rule the bigger**

DAY TWO

Winter School of FBBB, JU

Feb 23, 2022

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11.45 - 13.45	Training Session 4: **Introduction to metabolomics and its application in life-sciences** **Michał Markuszewski** (Medical University of Gdańsk) **Metabolomics in modern bioanalysis** **Mariola Olkowicz** (JCET, JU) **Recent advances and trends in miniaturized sample preparation techniques for MS-based metabolomic analyses** **Simonas Rudys** (Thermo Scientific, Anchem) **Thermo Scientific solutions for untargeted metabolomic** **Mariola Olkowicz** (JCET, JU) **Novel Applications of Metabolomics in Personalized Medicine**
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DAY TWO

Winter School of FBBB, JU

Feb 23, 2022

TOPICS: PROTEOMICS & METABOLOMICS

14.45 -16.30 Oral Session 1: **Nucleic acids**

Mirosław Zarębski Mechanism of induction of single- and double-strand DNA breaks by visible laser light

Katarzyna Łagosz-Ćwik The DNMT inhibitor decitabine has detrimental effects on gingival fibroblasts but sheds light on the role of DNA methylation in periodontitis.

Przemysław Malec The function of photosystem I oligomerization in cyanobacteria: a lesson from transcryptomic profiling of *Synechocystis* PCC 6803 PSAL-mutant

Aleksandra Liszka Transcriptional control of the wood formation process

Jan Łyczakowski Molecular and genetic basis of wood resistance to enzymatic degradation

Gabriela Machaj A novel transcriptomic role of E93 in insect embryogenesis

Natalia Pydyn Multiomic analysis of primary biliary cholangitis-associated pathways in MCPIP1FL/FLALBCRE mice

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*for poster topics please refer to the poster programme

DAY THREE

Winter School of FBBB, JU

Feb 24, 2022

TOPICS: PROTEINS & CELLS

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*for poster topics please refer to the poster programme

DAY THREE

Winter School of FBBB, JU

Feb 24, 2022

TOPICS: PROTEINS & CELLS

13.30 -15.30 Oral Session 3: **Cells**

Krzysztof Szade **How the bone marrow vasculature regenerate? Regeneration of bone marrow endothelial cells at single cell and clonal level**

Elżbieta Karnas **Extracellular vesicles from human ips cells enhance reconstitution capacity of cord blood-derived hematopoietic stem and progenitor cells**

Milena Paw **Cx43 regulates the pro- and anti-fibrotic tgf-β/smad signalling during myofibroblastic transitions in asthma**

Paulina Marona **Tumor initiation – the role of mcpip1 protein**

Paweł Stalica (SHIM-POL) **Selection guide metabolite analysis. metabolomics and proteomics product portfolio**

Elwira Nieboga **The interplay between oral pathogens and inflammatory cytokines in gingival fibroblast activation in periodontitis.**

Aleksandra Wielento **Accessory subunits of *P. gingivalis* major fimbriae potentially modified by ppad are vital tlr2 agonists**

Paweł Żbik, Przemysław Malec **Identification of myxoxantophyll isomers in *Anabaena* (*Nostoc*) PCC7120**

Ariel Kamiński **Searching of bioactive secondary metabolites from five cyanobacterial species**

15.30 - 16.00 Closing ceremony

18.00 Dinner in Old Town

OPENING LECTURES

PROSTOTA I ZŁOŻONOŚĆ: OD FIZYKI DO BIOLOGII

K.A. Meissner[1]

[1]*Wydział Fizyki, Uniwersytet Warszawski, Ludwika Pasteura 5, 02-093 Warszawa*

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Description of physical and biological systems is different in many respects, one of them being the approach to functionality, crucial in biology and (almost) absent in physics. In physics all phenomena are deconstructed to the simplest possible level and the description follows the bottom-up path - interactions of the simplest components lead to more and more complex systems. In biology the path is reverse, top-bottom: the functional complexity seen even in the smallest substructures of a cell is subject to a deconstruction but can be lost in the description if the deconstruction descends to a too low level. Some aspects of these differences will be discussed in the talk.

CZEGO NIE WIEMY – WYZWANIA I PROBLEMY GENOMIKI

P. Golik[1]

[1]*Instytut Genetyki i Biotechnologii, Wydział Biologii, Uniwersytet Warszawski*

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Historia genetyki i genomiki jest stosunkowo krótka. Od upowszechnienia się pierwszych poprawnych koncepcji dziedziczenia w dyskursie naukowym upłynęło niewiele ponad sto lat. Ostatnie dekady przyniosły lawinowy rozwój technik wysokoprzepustowych, umożliwiających pozyskiwanie dużych zbiorów danych opisujących genomy i ich zmienność w populacjach człowieka i innych organizmów. Coraz wyraźniej jednak widać, że wypracowane w ostatnim stuleciu metody opisu zjawisk biologicznych nie wystarczają do zrozumienia poznawanych z coraz większą dokładnością genomów. Na podstawowe pytanie badawcze genetyki – jak genotyp determinuje fenotyp organizmu – wciąż nie możemy w większości przypadków udzielić jednoznacznej odpowiedzi. Język genetyki, opisujący działanie pojedynczych genów, okazuje się niewystarczający do zrozumienia stanowiącego złożony system genu. Problem ten dotyczy nie tylko badań, ale też społecznego odbioru i rozumienia genomiki, co może rodzić potencjalnie groźne nieporozumienia. Wyzwaniem genomiki jest stworzenie nowego w biologii aparatu teoretycznego, który byłby adekwatny do stawianych problemów. Podejmowane obecnie próby stworzenia takiego aparatu teoretycznego, choć pokazują kierunek rozwoju, nie rozwiązują jeszcze większości problemów. Problemy i braki współczesnej genomiki nie tylko wskazują nowe potencjalne kierunki badań, ale też powinny wpłynąć na narrację w dydaktyce i popularyzacji nauki.

ABSTRACTS
TRAINING SESSIONS

TRAINING SESSION 1

GENOMICS

INTRODUCTION TO HUMAN GENOME VARIATION ANALYSIS AND BEYOND

W. Branicki[1]

[1]*Human Genome Variation Research Group, Malopolska Centre of Biotechnology, Jagiellonian University, Gronostajowa 7a, 30-387 Krakow, Poland*

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Genomics is the interdisciplinary study of the genome, including the interaction of the genes with each other and with the environment. Thanks to tremendous advances in DNA sequencing technology, data storage capacity and data analysis, the 1990 challenge of sequencing the first human reference genome has evolved into the challenge of sequencing millions of human genomes in recent times. In my presentation, I will outline the basics of human genome resequencing, highlighting the problems that can be encountered during the subsequent steps, from the planning of the experiment to data analysis. I will draw attention to the great possibilities of targeting the analysis to specific regions of the human genome as well as the possibility of analysing DNA methylation. I will also talk about our experience in human genome variation analysis and the metagenomic analyses we conduct in a forensic context.

ARRAY-BASED TECHNOLOGY FOR GENOMIC AND EPIGENOMIC ANALYSIS

R. Noroozi[1]

[1] *Human Genome Variation Research Group, Malopolska Centre of Biotechnology, Jagiellonian University, Gronostajowa 7a, 30-387 Krakow, Poland*

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Designing a genomic or epigenomic project, researchers must select between sequencing-based or array-based technologies. Practically, questions that might guide choosing the best option are as follows. What and how much information will be provided by each technology per sample? How much computational systems and bioinformatics expertise are required for data analysis? And which option is affordable based on the project's financial resources?

During this presentation, I will go beyond the differences in the goals of each project and discuss the important factors which may affect the decision of choosing one technology such as the accuracy of the outputs, the applicability of the data for novel discoveries, and the coverage of the genomic and epigenomic profiles.

We will have a glance at the different powerful platforms that microarrays provide to efficiently analyze genetic and epigenetic variations in various fields from precision medicine, pharmacogenetics, and cancer research to agricultural applications. I will also briefly mention the pros and cons of the microarray Highlighting its strengths including reliable reproducibility of technical replicates, cross-platform highly correlated data, lower cost, and the less-complicated data analysis steps while also considering its limitations such as predefined content, cross-reactive probes, dye, and batch effects.

Finally, I will discuss the future of array-based technologies in the horizon of multi-omics studies, and we will find microarrays and sequencing technologies as complementary methods rather than competitive alternatives.

POPULATION-SCALE WHOLE-GENOME AND TARGETED RESEQUENCING IN NON-MODEL ORGANISMS

W. Babik[1]

[1]*Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Gronostajowa 7, Krakow, Poland*

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DNA sequencing has become a key tool in ecological and evolutionary studies that aim to characterise biodiversity and to understand the mechanisms of its origin. Recent progress in genomics is closing the (genomic) gap between model- and non-model organisms making genome-scale analyses a standard across the tree of life. I will discuss the approaches that are used to study sequence polymorphism in non-model organisms, compare these approaches with the methods widely used in biomedical sciences, and explore the potential for synergy. Various study designs, their cost, IT requirements, and trade-offs involved will be considered. I will briefly introduce custom genomics solutions that are currently used in the Molecular Ecology Laboratory at the Institute of Environmental Sciences and may be of broader interest. Finally, I will share some thoughts on the strategies of addressing bioinformatics challenges.

HIGH-THROUGHPUT CHARACTERIZATION OF MICROBIOMES, HOST-MICROBE INTERACTIONS, AND BEYOND

P. Łukasik[1]

[1]*Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Gronostajowa 7, Krakow, Poland*

Corresponding author: p.lukasik@uj.edu.pl

Microbial symbionts play essential roles in the biology of multicellular eukaryotes, but their understanding outside of humans and a few model species remains limited. Fortunately, recent developments in sequencing technologies and bioinformatics have greatly facilitated their study.
In this presentation, I introduce the functional diversity of microbial symbionts of animals, and the methods of their study. I focus on the two most used DNA-sequencing-based approaches: marker gene amplicon sequencing and shotgun metagenomics. I explain laboratory and bioinformatics steps, present methodological challenges and artifacts, and discuss these alternative approaches' strengths, weaknesses, and applications. I also present how I have used these approaches in my research on the diversity and evolution of insect microbial symbioses.

BIOS GENOMICS CORE FACILITY - WHAT WE CAN DO FOR YOU

A. Jarosz[1]

[1]*Human Genome Variation Research Group, Malopolska Centre of Biotechnology, Jagiellonian University, Gronostajowa 7a, 30-387 Krakow, Poland*

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W ramach POB BioS działają pracownie typu *core facility*, które świadczą usługi w zakresie analiz omicznych. Pracownia genomiczna – BioS Genomics Core Facility – jest obecnie wyposażona w trzy platformy sekwencjonowania wysokoprzepustowego: Ion Proton (Thermo Fisher), MiSeq (Illumina) i MinION (ONT), a także w aparat do elektroforezy kapilarnej 3500 Series Genetic Analyzer (Applied Biosystems) oraz aparaturę wspomagającą (m. in. QuantStudio 12K, Bioanalyzer, Qubit). Różnorodność dostępnych platform sprawia, że w ofercie pracowni znajduje się wachlarz usług poszerzających możliwości naukowców prowadzących badania w obszarze *life science*. Wśród oferowanych usług znajdują się: sekwencjonowanie fragmentów DNA, sekwencjonowanie małych genomów oraz analizy mikrobiomu i metagenomu, przeprowadzane z wykorzystaniem krótkich lub długich odczytów. Ponadto, pracownia oferuje sekwencjonowanie transkryptomu (RNAseq), różnicową analizę ekspresji genów (panel transkryptomiczny AmpliSeq) oraz sekwencjonowanie miRNA. Wszystkie analizy są wykonywane z zachowaniem najwyższych standardów oraz uwzględniają podwójną kontrolę jakości, co ma na celu zapewnienie wiarygodnych wyników badań. W planach jest doposażenie pracowni w nowe aparaty o większej przepustowości, jak również sprzęt umożliwiający przeprowadzanie analiz *single-cell sequencing*.

TRAINING SESSION 2

TRANSCRIPTOMICS

RNA PROCESSING AND PREPARATION OF NGS LIBRARIES

M. Kosecka-Strojek[1]

[1]*Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, Krakow, Poland*

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Library preparation is a crucial step in every next generation sequencing (NGS) experiment, and it involves multiple steps that have to be carefully carried out. Proper library preparation ensures high number of high-quality short reads that are the usual raw output of NGS. Careful library preparation is particularly important in quantitative techniques such as RNASeq. The first step includes RNA isolation and purification. As for every quantitative technique of expression analysis, obtained RNA must not display signs of degradation. In case of NGS techniques also the amount is important, and that is usually considerably higher than in case of low-throughput techniques, such as RT-qPCR. Since a standard preparation of total RNA consists of roughly 80-95% of ribosomal RNA, substantial depletion of rRNA is critical. It provides samples enriched in mRNA, which is the target for quantitative sequencing. Once RNA is processed as described, it is used for preparation of libraries. In this stage RNA is reverse-transcribed into cDNA. Next, adapters and barcodes are ligated to cDNA in a process that leads simultaneously to cDNA fragmentation. The products are then purified and enriched with PCR to create the final library. That is what the final library consists of, short cDNA fragments flanked by barcodes and adapters. The quality of total RNA, rRNA-depleted RNA and final libraries is accurately analysed by capillary electrophoresis that allows for quantitative assessment of rRNA fraction and undesirable RNA degradation. Now, the library is ready to be used in a sequencing run.

TRANSCRIPTOMICS ON ILLUMINA PLATFORMS

S. Sierankowska[1]

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The aim of the presentation is to discuss next generation sequencing on Illumina platforms using sequencing-by-synthesis (SBS) technology and the Illumina RNA-seq portfolio. Session objectives is to list the major steps in the Illumina sequencing workflow: library preparation, cluster generation, sequencing by synthesis (One-/Two-/Four-channel chemistry) and data analysis. Library preparation will be focused on different RNA library preparation workflows and transcriptomic research. Illumina Stranded Total RNA with Ribo-Zero Plus and Illumina Stranded mRNA preps comparison and overview including assay procedure, kit components, best practices and performance. Guidance on which workflow to choose to address a wide range of research needs and sample types before starting an experiment.

FROM SHORT READS TO DIFFERENTIAL EXPRESSION

M. Bukowski[1]

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High throughput RNASeq sequencing produce short reads on the template of cDNA obtained during library preparation. In the most basic scenario short reads are obtained for three biological replicas for each of two transcriptomes that are being compared. Depending on the type of the library it is possible to infer from such reads a few different kinds of information. However, the most thrilling to a scientist is an opportunity to have an insight into differences in transcription of single transcripts within a whole transcriptome. Prior the differential gene expression (DGE) analysis a quality check of short reads is advisable. Having satisfactory number of good quality reads, the next step is to map short reads to reference transcript sequences. Selection of a proper set of reference sequences as well as the way the mapping process is carried out are of importance. Once short reads are mapped, raw counts are determined. Raw counts can be normalised into human-readable metrics, such as transcripts per million (TPM), that can be roughly compared among single replicas. Nevertheless, raw counts are the metric that is used by DGE algorithms to model differences in transcription. The final output includes two major statistics for every transcript, that is, the fold change and statistical significance of the difference between two transcriptomes. The statistical significance is often expressed as both pvalue and adjusted pvalue, statistics that must not be confused. Finally, DGE analysis results may be visualised in two ways that allow for a comparison of the general scale of differences.

SMALL RNAS: WHEN THE SMALL RULE THE BIGGER

G. Ylla[1]

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Transcriptomics is the study of the transcriptome, and the transcriptome is the whole set of RNA transcripts present in the cells. The advances in high-throughput sequencing techniques facilitated the study of transcriptomes. With the popularization of these techniques, the word "transcriptome" has often been used to refer to solely one specific type of transcripts, those that code for proteins. However, in the transcriptome, there are several other types of RNA molecules. One of these types is the small RNAs, named by their short length, which is typically less than 50 nucleotides.

Within the small RNAs, there are different types of molecules that can be subdivided by their lengths and roles. The most predominant types of small RNAs are the siRNAs, microRNAs, and piRNAs. All of them share one characteristic: the capacity to bind to other RNAs and trigger their degradation or block their transcription. Thus, small RNAs emerged as powerful regulators of gene expression at the post-transcriptional level.

The study of these small RNAs using high-throughput sequencing data is still challenging, especially on non-model organisms. In this session, we will explore the characteristics, functions, and roles of each of these small RNA types. Furthermore, we will look at the methods and techniques available to sequence, identify, and classify them using sequencing data. Finally, we will glance through ways of predicting their functional roles.

All in all, we will see how these small RNAs do it to rule their bigger counterparts, and what methods we have to study them.

TRAINING SESSION 3

PROTEOMICS: PRINCIPLES, TECHNIQUES AND

GEL-BASED COMPARATIVE PROTEOMICS

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Proteomics is a set of analytical tools used for determining protein composition of a sample (e.g. a cell, a tissue, an organism) developed under the influence of genetic or environmental factors. Gel-based comparative proteomics allows for qualitative and/or quantitative analysis of proteins using electrophoretic techniques. The most powerful and widely used one is a two-dimensional difference gel electrophoresis (2D-DIGE). The workflow starts from collection (isolation, fractionation, purification) of protein samples to be compared. Then, pre-experimental optimalization is advised to verify the concentration and purity of proteins as well as to adjust isoelectric point (pI) range of the analysis. Next crucial point is labelling the proteins with three fluorescent dyes, followed by combining the samples in groups to be compared and an internal standard sample. Labelled proteins are loaded on a strip and separated according to their pI by isoelectric focusing (first dimension). Then the strip is transferred on a sodium dodecyl sulphate polyacrylamide gel, and proteins are electrophoretically separated according to their molecular mass (second dimension). Gels are scanned to obtain fluorescent signals corresponding to the samples to be compared and the internal standard. The images are analysed in silico to identify differentiating protein spots. Finally, the spots of interest are cut out of the gel, digested with trypsin and prepared for mass spectrometry analysis which serves to identify the protein within the spots.

MASS-SPECTROMETRY BASED PROTEIN IDENTIFICATION AND QUANTIFICATION - RECENT ADVANCES IN PROTEOMICS

IDENTYFIKACJA I ANALIZA ILOŚCIOWA BIAŁEK W OPARCIU O SPEKTROMETRIĘ MAS - NAJNOWSZE OSIĄGNIĘCIA W PROTEOMICE

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Białka stanowią funkcjonalne składniki komórki, które bezpośrednio wpływają na jej kondycję. Kompleksowe badania proteomiczne prowadzone niezależnie od badań transkryptomicznych są niezbędne dla wyjaśnienia relacji pomiędzy genotypem a fenotypem komórki. Współczesne, oparte na zaawansowanej spektrometrii mas badania proteomiczne dostarczają kompletnych i bezstronnych danych na temat badanego procesu biochemicznego czy też na temat wpływu badanego czynnika na stan komórki/tkanki.

Tandemowa spektrometria mas w połączeniu z ultrawydajną chromatografią cieczową (LC-MS/MS) jest wysokosprawną techniką sekwencjonowania peptydów i w konsekwencji identyfikacji białek. Technika LC-MS/MS pozwala także na analizą ilościową tysięcy białek w pojedynczym eksperymencie. Równolegle rozwijane są znacznikowe i bezznacznikowe techniki analizy ilościowej. Ponadto, stosowane są techniki celowanej proteomiki umożliwiające bardzo dokładną i czułą analizę ilościową wybranego podzbioru białek w skomplikowanej próbce białkowej. Obok techniki zwanej akwizycją zależną od danych, w skrócie DDA (*Data Dependent Acquisition*), w oparciu o najwyższej klasy spektrometry masowe, rozwijana jest technika niezależnej od danych akwizycji tzw. technika DIA (*Data Independent Acquisition*), która pozwala na głęboką i ilościową analizę proteomu.

Sposób przygotowanie próbek do analizy spektrometrycznej zależy od pytania badawczego, rodzaju tkanki i ilości dostępnego materiału i często wymaga optymalizacji. Jakość przygotowania próbki ma bardzo duży wpływ na końcowe wyniki, podobnie jak prawidłowo wykonany pomiar. Bardzo ważna jest również analizy bioinformatyczna i statystyczna.

Powyższe zagadnienia zostaną kompaktowo omówione podczas wykładu.

Ponadto, zostanie udzielona odpowiedź na pytania: 1) od czego zależy jakość uzyskiwanych wyników proteomicznych? 2) w jakim kierunku zmierza oparta na spektrometrii mas proteomika? Czy są to globalne badania modyfikacji potranslacyjnych, badania interaktomów, czy wyznaczanie liczby kopii białka w komórce? a może coś jeszcze bardziej niewiarygodnego?

THE ZENOTOF 7600 SYSTEM – THE NEW, FLEXIBLE TOOL FOR ADVANCED PROTEOMICS

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The ZenoTOF 7600 system, a new high-resolution accurate mass spectrometer is rewriting the rules in proteomic analysis. Driven by the power of the Zeno trap coupled with EAD fragmentation technology, new system provides the ability to acquire key MS/MS features needed to:

- Characterize large molecules including post-translational modifications;
- Elucidate positional isomers on small molecules and lipids;
- Identify and quantify proteins and peptides at unparalleled speed.

For the very first time ZenoTOF 7600 system overcomes QTOF MS/MS duty cycle deficiencies, allowing to analyze >90% ions injected into the TOF. It results with sensitivity gains of up to 5-20x which enables identification and quantification low abundance species. Moreover, build in alternative fragmentation mode - controlled electron activated dissociation (EAD) – allows tunable fragmentation of all molecular types, providing you with yet another level of information like for example identification and localization of phosphorylation or glycosylation, for both N- and O-glycopeptides. Last, but not least, higher MS/MS scan rates of up to 133Hz improve Data Dependent Acquisition (DDA) and high-resolution MRM (MRM^{HR}), for even better quantitative work.

In my short talk I will present several examples illustrating ZenoTOF 7600 benefits in proteomic workflows.

PROTEOMICS CHALLENGES
OVERCOMING SAMPLE COMPLEXITY AND BEYOND

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Proteomics is the study of the proteome, i.e. the totality of all proteins present in a cell or a living organism under defined conditions and at a defined time. In contrast to the (rather) static genome, the proteome is highly dynamic and can therefore change in its qualitative and quantitative protein composition due to changing conditions. Depending on the application, analytical methods have to cope with small sample amounts (e.g. single and sorted cell biology, tissue proteomics or biopsies), high dynamic range (e.g. plasma proteomics), large sample cohorts (e.g. clinical studies) and PTM characterization (e.g. phosphorylation and glycosylation), to name a few. Therefore, the analysis protocol must provide a robust and sensitive system that can handle a high dynamic range without losing resolution or spectral quality.

The Parallel Accumulation - Serial Fragmentation method (PASEF) [1] for trapped ion mobility spectrometry (TIMS) coupled to a quadrupole time of flight (QTOF) instrument, has been described with the promise of achieving five to ten times faster data dependent acquisition of fragment ion spectra with improvements in sensitivity. In recent years, several studies demonstrated the robustness and sensitivity of the instrument.

Here, we like to show latest results on the latter and two case studies. The first giving an overview over biomarker research for COVID-19 with an Evosep One LC system coupled to a timsTOF Pro mass spectrometer. Serum proteomes of COVID-19 patients and symptomatic, but PCR-negative controls, in a time-resolved manner were measured [2]. The second example shows the challenges in the analysis of immunopeptides. For these high sensitivity measurements, a nanoElute LC was coupled to a timsTOF SCP system.

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TRAINING SESSION 4

INTRODUCTION TO METABOLOMICS AND ITS APPLICATION IN LIFE-SCIENCES

METABOLOMIC IN MODERN BIOANALYSIS

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Nowadays, due to widespread access to high-throughput, ultrasensitive bioanalytical methods, metabolomics is the most dynamically developing branch of biomedical research. Taking in mind the vast complexity of the pathophysiological and physiological processes it is not surprising that sets of different metabolites might represent more comprehensively the changes in an organism related to the disease than a single selected compound would.

The leading causes of death globally are cardiovascular diseases (CVD), including hypertension and cancer diseases. There is a need for the development of specific diagnostic methods, more effective therapeutic procedures, as well as drugs, which can decrease the risk of deaths in the course of diseases. For this reason, better understanding and explanation of their molecular pathomechanisms are essential. In the shown studies, the current status of our findings related to those two major health problems would be presented and discussed.

Special emphasis will be on the analysis of transcriptomic and metabolomic data in course of a gastrointestinal stromal tumour (GIST) which is known to be driven in 90% by mutations in KIT or PDGFRA genes. The metabolomics results show that purine, pyrimidine, fatty acyl, and carbohydrate metabolism might be involved in the tumor response to imatinib. As regards the transcriptome, mapping differently expressed gene products to their molecular-level activity relates them to phosphatidylinositol 3-kinase or MAP kinase activity, which is consistent with the imatinib mechanism of action. Integration of metabolomic and transcriptomic data enabled to recognise the gene-metabolite network linked to carcinogenesis, apoptosis inhibition, or tumor invasiveness.

RECENT ADVANCES AND TRENDS IN MINIATURIZED SAMPLE PREPARATION TECHNIQUES FOR MS-BASED METABOLOMIC ANALYSES: NOVEL APPLICATIONS OF METABOLOMICS IN PERSONALIZED MEDICINE

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Solid phase microextraction (SPME) was invented in 1989 to address the need to facilitate rapid sample preparation both in the laboratory and on-site where the investigated system is located [1,2]. The technique involves exposure of a small amount of extractive phase dispersed on a solid support to the sample matrix. Over recent years, new ways of constructing and using the SPME devices have evolved which is evident from developments of needle trap, thin-film microextraction, cold-fibre headspace SPME devices or stainless steel fibres coated with a diversity of thin film polymers particularly suitable for LC/MS-based applications [1,2]. The usefulness of SPME in the analysis of a broad range of metabolites in a variety of matrices have been documented in numerous studies proving its advantages over tedious (frequently multi-step) traditional sample preparation approaches. When coupled with highly sensitive mass detectors permits xenobiotics (such as therapeutic drugs) and specific metabolic pathways profiling or global metabolites profiling providing a snapshot of the entire metabolome or lipidome in 'one go' [1-3]. In the current presentation, the most remarkable and recent biomedical/clinical applications of SPME as a sample preparation tool for MS-based targeted and untargeted metabolomic and lipidomic investigations will be demonstrated. In addition, special attention will be given to SPME's suitability for use with *in vivo* and non-destructive sampling in order to capture a representative snapshot of the intact metabolome/lipidome. Finally, future directions for further SPME's applicability in the metabolomics field will be highlighted with indication of the role of metabolomics in advancing the practice of precision medicine [3 4].

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THERMO SCIENTIFIC SOLUTIONS FOR UNTARGETED METABOLOMIC

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Untargeted metabolomics describes an unbiased approach that globally analyzes the metabolome in any given sample. Comprehensive metabolome coverage is obtained by orthogonal sample preparation, different chromatographic separation techniques and ionization modes, collecting all measurements indiscriminately with a fundamental notion “collect everything and leave nothing behind.” At the same time, unbiased data collection results in an exhaustive list of spectral features or signals, making data analysis laborious and cumbersome.
Here we present how Dual UHPLC separation system Transcend™ Duo LX-2 coupled to an Orbitrap IQ-X Tribrid mass spectrometer with AcquireX Intelligent data acquisition is used to increase analysis throughput while maximizing metabolome coverage and how Compound Discoverer™ software helps to reduce redundancies and accelerate data analysis.

ABSTRACTS
ORAL PRESENTATIONS

ORAL SESSION 1

NUCLEIC ACIDS

MECHANISM OF INDUCTION OF SINGLE - AND DOUBLE-STRAND DNA BREAKS BY VISIBLE LASER LIGHT

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Various aspects of DNA damage induction and repair can be studied in situ, in live cells, by inducing localized DNA damage, and detecting recruitment and dissociation of repair factors. We have shown that low-power visible laser light focused on a small region of the nucleus, in the absence of exogenous photosensitizers, induces DNA breaks. This phenomenon can be exploited in studies of the repair of individual DNA lesions. We detected light-induced DNA breaks indirectly, by imaging phosphorylation of histone H2AX [1] and recruitment of proteins involved in repair of SSBs (XRCC1) and DSBs (53BP1), and directly, by imaging free DNA ends (DSBs and SSBs) using STRIDE method [2]. We show that this method of inducing lesions does not lead to the oxidation of DNA bases, as the OGG1 glycosylase is not recruited to damage. Despite this, oxygen is necessary for induction of DNA breaks. The results of mass spectrometry studies of oligonucleotides exposed to light in the presence of photosensitizers which are known to exist in nuclei of mammalian cells suggest that the DNA breaks can occur as a result of destruction of guanine.

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THE DNMT INHIBITOR DECITABINE HAS DETRIMENTAL EFFECTS ON GINGIVAL FIBROBLASTS BUT SHEDS LIGHT ON THE ROLE OF DNA METHYLATION IN PERIODONTITIS

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Periodontitis is a chronic inflammatory disease caused by dysbiosis of the oral microbiota. Interactions between key periodontopathogen *Porphyromonas gingivalis* and gingival fibroblasts (GFs) significantly contribute to the chronicity of inflammation in periodontitis. Epigenetic changes in DNA methylation play an important role in periodontitis pathogenesis and recent studies indicate that DNA methyltransferase (DNMT) inhibitors may have a protective role in disease progression. However, little is known about the impact of DNMT inhibitors on GF inflammatory and antimicrobial responses.

GFs were cultured with decitabine for 12 days in order to induce DNA hypomethylation. We observed a plethora of potentially detrimental effects of decitabine on GF biological functions. First, decitabine significantly reduced GF proliferation and was cytotoxic after extended incubation. Second, decitabine treatment strongly increased expression and secretion of the Th17 chemokine CCL20 and matrix metalloproteinase-1, suggesting that DNA hypomethylation in GFs may promote Th17 cell infiltration and collagen degradation in the gingival tissue. Third, we observed increased *P. gingivalis* adherence to GFs treated with decitabine, which may contribute to bacterial dissemination. Interestingly, we also observed a trend towards decreased global DNA methylation in a chronic model of infection, raising the possibility that DNMT inhibitors may partly mimic the effects of the inflammatory environment on dysregulation of epigenetic processes in GFs.

Collectively, these results show that decitabine is a useful tool to study the role of DNA methylation in cells relevant to the pathogenesis of periodontitis. However, therapeutic potential of decitabine in periodontitis may be limited due to its detrimental effects on GF viability and inflammatory responses.

THE FUNCTION OF PHOTOSYSTEM I OLIGOMERIZATION IN CYANOBACTERIA: A LESSON FROM TRANSCRIPTOMIC PROFILING OF *SYNECHOCYSTIS* PCC 6803 *PSAL*$^{-}$ MUTANT

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In thylakoid membranes of the mesophilic cyanobacterium *Synechocystis* PCC6803, photosystem I reaction centers (PSI) are organized as monomers and trimers. In a *Synechocystis* mutant deficient in small hydrophobic 16 kDa subunit of the PSI (*psaL*$^{-}$), PSI monomers are the only PSI complex forms [1]. It was found that in cyanobacteria, the increase of growth temperature caused a decrease of the ratio of monomer/trimer in PSI complexes and the level of lipid unsaturation [1,2]. The monomerization of PSI induced alterations in the accumulation of carotenoids and in the functioning of photosynthetic electron transport chain [1,3]. However, little is known on the molecular mechanisms accompanied by PSI oligomerization in cyanobacteria. To identify genes associated with *psaL* deficiency in *Synechocystis*, we performed an RNA-seq analysis of the wild-type strain and the *psaL* loss-of-function mutant grown under standardized conditions. Among the total of ~3500 sequenced mRNAs, the disruption of *psaL* significantly upregulated the expression of 172 genes and downregulated 141 genes, related to various cellular functions including photosynthesis, metabolism, transport and signaling. Interestingly, most of the differentially expressed genes (DEGs) were identified as encoding ATP-dependent membrane (ABC) transporters and elements of two-component signaling systems. We have also observed differential expression of over 40 predominantly novel non-coding RNAs. Our results suggest that the *psaL*-dependent oligomerization of PSI might be crucial for the stabilization of membrane function in *Synechocystis*. They provide key information for uncovering molecular factors involved in the regulation of photosystem I monomer/trimer ratio and could facilitate identification of the mechanisms responsible for cyanobacterial unique potential for adaptation to changing environmental conditions.

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TRANSCRIPTIONAL CONTROL OF THE WOOD FORMATION PROCESS

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Conifers form an important part of a global ecosystem since they dominate boreal forests and constitute the majority of commercially planted trees. Wood made by coniferous trees, known as softwood, is commonly applied in industry.
Despite the importance of softwood, little is known about the variation in the structure of secondary cell walls which build it. A key characteristic of softwood is that it is made of two distinct types of tracheid cells which form earlywood and latewood. Interestingly, our biochemical analysis indicates that the structure of earlywood and latewood cell walls differs. With molecular phylogenetics and transcriptional profiling we attempted to identify the genetic basis of the observed biochemical diversity. We uncovered three spruce genes with high similarity to *Arabidopsis thaliana* Vascular related NAC-Domain (*VND*). These genes are transcription factors which could be involved in the control of secondary cell wall deposition. To model earlywood to latewood transition in spruce we performed controlled growth experiments in long and short-day conditions. With qPCR analysis we showed that the expression pattern of the putative spruce *VNDs* changes depending on the light regime applied. Moreover, we conducted *in vivo* assays to establish if spruce *VNDs* can stimulate production of secondary cell wall polysaccharides. After detailed scrutiny, we concluded that the biomass obtained shows typical secondary cell wall features.
Our experiments provide insight into the molecular basis of wood formation. This may enable identification of breeding or genetic engineering targets for future attempts at wood improvement.

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MOLECULAR AND GENETIC BASIS OF WOOD RESISTANCE TO ENZYMATIC DEGRADATION

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Wood is the largest biological repository of carbon and an important source of polymers with potential industrial applications. Despite this significance of wood, we have a limited understanding of the basis for its resistance to enzymatic degradation – a process that is central to most applications of this biological material. On a molecular level, wood is primarily composed of plant secondary cell walls which are made of polysaccharides, such as cellulose and hemicelluloses, and a polyphenolic compound known as lignin. We previously demonstrated [1] that branching xylan, one of the hemicelluloses, with glucuronic acid (GlcA) is essential for the maintenance of secondary cell wall resistance to enzymatic degradation. In this presentation, I will discuss our recent work [2] in which we identified and characterised conifer genes encoding enzymes responsible for the addition of GlcA to softwood xylan. Using molecular phylogenetics and transcriptomic analysis we identified candidate genes encoding GlucUronic acid substitution of Xylan (GUX) enzymes in spruce and pine genomes. Through *in vitro* and *in planta* assays we demonstrated that these candidate GUX enzymes are capable of adding GlcA onto xylan. We were also able to characterise the specific pattern of GlcA branches generated by spruce and pine GUX enzymes, what has implications for the assembly of secondary cell wall polysaccharides in softwood. We are now moving towards application of these discoveries to the modification of softwood biomass, what can result in formation of wood with superior performance in biorefinery processing.

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A NOVEL TRANSCRIPTOMIC ROLE OF E93 IN INSECT EMBRYOGENESIS

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E93 is a transcription factor known as a trigger for the pupa to adult of the metamorphosis in holometabolous insects, which are those with complete metamorphosis [1, 2]. We have observed that in the cockroach *Blattella germanica*, which undergoes the ancestral incomplete metamorphosis mode called hemimetabolous metamorphosis, *E93* has an additional expression peak in embryo during the maternal-to-zygotic transition [3]. Our hypothesis is that the expression of embryonic E93 could be a determinant factor of the metamorphosis type.
To elucidate the role of E93 in hemimetabolous insect embryos, we knocked down *E93* expression via RNAi in two days-old embryos followed by mRNA-seq. In total, we generated mRNA-seq from 6 control and 5 *E93* depleted embryo pools. For each sample, we obtained a minimum of 60 million reads from which around 90% of them were mapped to the reference genome. Using differential gene expression analysis, we identified 98 down- and 47 significantly up-regulated genes in E93-depleted samples compared to controls. Significantly downregulated genes were enriched by essential functions for insect development.
Furthermore, to show that the embryonic *E93* expression is a general feature of hemimetabolous insects and is absent in holometabolous insects, we queried all NCBI-SRA for transcriptomic datasets of embryos and pre-adult stages of insects. This yielded the identification of appropriate transcriptomic data for 4 hemimetabolous and 10 holometabolous species in which we examined the expression of *E93*.
Our results suggest that the *E93* plays a fundamental role in the embryogenesis of hemimetabolous insects differently from holometabolous in which there is no embryonic expression peak of E93. Our findings shed light on the *E93* role in the regulation of the development and evolution of metamorphosis.

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MULTIOMIC ANALYSIS OF PRIMARY BILIARY CHOLANGITIS-ASSOCIATED PATHWAYS IN MCPIP1$^{FL/FL}$ALB^{CRE} MICE

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Primary biliary cholangitis (PBC) is a chronic liver disease, that results from a progressive destruction of the intrahepatic bile ducts. PBC progression leads to the development of fibrosis, cholestasis and liver cirrhosis. Recently, we demonstrated, that mice with Mcpip1 RNase deficiency in the liver epithelial cells recapitulate features of human PBC [1]. In current study, we investigated proteome and transcriptome of livers from Mcpip1$^{fl/fl}$Alb^{Cre} mice, in order to analyze the molecular mechanisms responsible for PBC development.
We used 6 and 52 weeks old control Mcpip1$^{fl/fl}$ mice and animals with deletion of Mcpip1 in liver epithelial cells (Mcpip1$^{fl/fl}$Alb^{Cre}). Liver transcriptome was analyzed by Next Generation Sequencing and proteome by Mass Spectrometry.
We found that lack of Mcpip1 in liver epithelial cells led to upregulation of genes and proteins involved in e.g. collagen fibril organization, organ regeneration and cell proliferation (2024 genes and 569 proteins in young mice; 509 genes and 116 proteins in old mice). Moreover, downregulated genes and proteins in livers of Alb^{Cre}Mcpip1$^{fl/fl}$ mice are involved in e.g. steroid metabolic process, lipid homeostasis and retinoic acid metabolic process (1377 genes and 475 proteins; 142 genes and 137 proteins, respectively). For further analysis, we chose 21 potential targets of Mcpip1 (e.g. Cxcl5, Cxcl2, Krt7, Sox9, Tgfb2) among 100 the most upregulated genes in the livers of Mcpip1$^{fl/fl}$Alb^{Cre} mice.
In conclusion, we have identified potential targets of MCPIP1 associated with development of primary biliary cholangitis, which require further, detailed investigation.

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ORAL SESSION 2

PROTEINS

ASSAYING COVID-19 – FROM HIT TO LEAD TOOLBOX

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COVID-19 is an infectious respiratory disease caused by SARS-CoV-2 virus with 5.5 M deaths and over 318 M cases world-wide. Due to its pandemic nature, the understanding of the viral proteins' interactions and mechanism is of utmost importance. Here, we investigate various enzymes and their inhibitory mechanisms, which play an essential role in the life cycle of the virus, and its molecular and cellular interactions. We present a unique program to rapidly discover lead compounds for clinical applications through a combination of structure-assisted drug design, virtual drug testing and high-throughput screening, and in vitro experiments. The primary focus is on identifying drugs that target critical viral proteins, such as the SARS-CoV-2 main protease Mpro and papain-like protease PLpro liberating other viral enzymes from the polyprotein, and nsp16 and nsp14 methyltransferases involved in mRNA capping. Our techniques comprise of primary enzymatic assays for initial screens, "hits" cross-verification with orthogonal assays such as nanoDSF combined with DLS, MST or NMR, cell-based assays using virus inducted cytopathic effect (CPE) inhibition test, RT-qPCR analyses using A549 overexpressing ACE2 and TMPRSS2 (A549ACE2/TMPRSS2) cells and co-crystallization of inhibitors with target proteins. The comprehensiveness and robustness of the described toolbox promotes synergistic effect of biochemical and biophysical characterization of potential inhibitors. Designing effective inhibition strategies requires knowledge of drug action and host response. Our approach to biochemical and virological studies provides immediate answers about their activity, interactions, structural relationships, toxicity, binding mode, and cellular response. This concedes effective exclusion of non-specific inhibitors and identification of putative candidates.

AN OMICS APPROACH TO INVESTIGATE CYANOPHAGE INFECTION IN FRESHWATER CYANOBACTERIA

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Cyanophages infecting cyanobacteria play a significant role in shaping the interactions between cyanobacteria and other organisms, hence they impact the circulation of nutrients in the microbial food web and, also entire biogeochemical cycles. Marine cyanophages are capable of transforming the host's metabolism which concerns mainly energy metabolism and the flux of carbon, nitrogen, and phosphorus compounds. The infection strategy of freshwater cyanophages has been however poorly studied.

We aimed at understanding the impact of cyanophages on freshwater species *Aphanizomenon* and *Microcystis* by proteomic and metabolomic approaches. Differences between infected and non-infected cells were examined by employing LC-MS/MS methods. The proteomic approach revealed alterations in the abundance of proteins involved in crucial metabolic pathways connected to energy metabolism, carbon flux and nucleotide synthesis. The level of proteins involved in CO_2 assimilation has been significantly reduced in favour of the pentose phosphate pathway. The expression of proteins related to glycolysis as well as the synthesis of nucleotides, amino acids, pigments and chaperones was also stimulated. All these alterations are related both to the formation of viral nucleic acids and proteins and to supply energy and reducing agents necessary for the generation of progeny viruses.

To understand the impact of cyanophage infection on the host's metabolome a global non-targeted analysis of metabolites extracted with 75% methanol was applied. Preliminary findings revealed several compounds with significantly different abundance between infected and non-infected cells. Some of them have been annotated to known metabolites of masses ranging 100-1000 Da.

The results deepen the insight into host-phage interactions and contribute to understanding bloom dynamics and interactions within aquatic environments.

STAPHYLOCOCCAL TOXIN-ANTITOXIN SYSTEMS IN PROTEOMIC STUDIES

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Toxin-antitoxin (TA) systems are small genetic elements widespread in bacteria, including the pathogens. TA systems are composed of two elements, a stable toxin and a labile antitoxin, and are known to be engaged in the maintenance of genetic elements as well as involved in cellular processes associated with pathogenesis, stress response, bacterial survival in adverse environmental conditions and biofilm formation. In this research s*prF1/sprG1, mazE/mazF* and *pemI/pemK* TA systems in the opportunistic pathogen *Staphylococcus aureus* were examined. Their influence on intracellular, extracellular and biofilm protein profiles was studied. Moreover, we looked at in which cellular processes TA systems are involved with special emphasis on examination whether the systems play a role in the regulation of staphylococcal virulence. Wild type and recombinant *S. aureus* strains with the deletion of the whole TA system or only of its parts (toxin or antitoxin) were compared. In the research the proteomic approach was applied, with the engagement of two dimensional difference gel electrophoresis followed by mass spectrometry analysis. Results revealed that the deletion of the whole TA system or its elements resulted in various modifications of the *S. aureus* proteome profile. The obtained results allow to infer that TA systems influence the level of proteins that implicated in basic metabolic processes such as glycolysis, tricarboxylic acid cycle, as well as nucleotides and amino acids metabolism. It was also recognised that TA systems influence the pool of secreted virulence factors and moonlighting proteins which also are linked to staphylococcal virulence.

MICROBIAL ABILITY TO METABOLIZE HOMS AS A FUNCTIONAL BACKGROUND OF DISTINCTIVE PROFILES OF THE INFANT GUT MICROBIOME IN NORTHERN EUROPE

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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 that results in distinctive development of the infant's gut microbiome in different populations.
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 among key factors for the differential development of the infants' gut microbiota.

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PROTEOMIC CHARACTERISTICS OF EXTRACELLULAR VESICLES PRODUCED BY *CANDIDA* PATHOGENIC YEASTS

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Extracellular vesicles (EVs), produced by cells representing all known kingdoms of life, are spherical, nanometer-sized structures surrounded by a lipid bilayer, carrying diversified and plentiful load, and involved in cell communication with environment. Fungi from genus *Candida*, widespread opportunistic human pathogens, also release EVs transporting numerous proteins, polysaccharides, lipids, nucleic acids and signaling molecules. Both, fungi existing as free-floating cells, as well as those living in the form of a complex community in biofilm, produce EVs that have a significant impact on other microbial cells in the same niche and on the host organism during infection. This effect is often related to the functionality of vesicular proteins. Therefore, with the use of tandem mass spectrometry coupled with liquid chromatography, we analyzed proteome of EVs produced by selected *Candida* species, including the most common *C. albicans*, as well as emerging non-albicans *Candida* species – *C. glabrata*, *C. tropicalis* and *C. parapsilosis*. Moreover, the location of individual proteins within the vesicles was also assessed, particularly including proteins exposed on the surface of EVs and available for the direct contact with other interacting molecules or cells. There was observed a noticeable vesicular presence of proteins responsible for organization of the fungal cell wall, typical adhesins and hydrolases related to candidal virulence. The group of proteins involved in cellular metabolism and performing at the same time distinct functions in diverse locations–known as "moonlighting proteins"–was also very abundant, what may confirm their important role in the pathogenesis of fungal infections.

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THE ROLE OF FIBRILLIN-1 AND TGFβ IN THE FORMATION OF ABDOMINAL AORTIC ANEURYSM

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An 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. Its incidence is closely related to environmental factors but could also be driven by genetic factors, as in Marfan syndrome, where the mutation in cysteine-rich fibrillin-1 (FBN-1) occurs, leading to increased TGFβ signaling. Our research indicates a high level of cysteine modification S-nitrosation (SNO) and altered ultrastructure of the aortic endothelial layer in the aortas of Nrf2 transcriptional knockout (tKO) mice, which are susceptible to AAA formation. The structural anomalies of the endothelial layer are characteristic of Marfan syndrome aortas. Therefore, we aimed to address the status of FBN-1 and TGFβ signaling during AAA formation in Nrf2 tKO mice.

We used an angiotensin II (AngII) based AAA model in tKO mice. Upon AngII infusion, we observed an altered endothelial layer, which was more pronounced in the formed aneurysms. These structural anomalies localized to the cadherin 5-positive endothelial junctions. Nrf2 tKO mice are characterized by packed elastin fibers, in the proximity of which we evidenced FBN-1 presence. We observed significantly higher levels and activation of TGFβ signaling in aneurysm-susceptible mice and upon AngII infusion. Moreover, TGFβ neutralization abrogates AAA formation. W evidence that loss of FBN-1 triggers endothelial cells activation and angiogenesis *in vitro*.

Summing up, here we propose an alternative, genetically-independent mechanism of AAA formation. Moreover, we emphasize the significance of TGFβ signaling and endothelial ultrastructure alterations during AAA formation.

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THE EXPRESSION OF DOMINANT-NEGATIVE SIGIRR$^{\Delta E8}$ PROMOTES COLORECTAL CANCER BY INCREASING CELL METABOLISM

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The tumor suppressor SIGIRR (single immunoglobulin IL-1-related receptor) is frequently inactivated in human colorectal cancer (CRC) by the increased expression of a novel SIGIRR$^{\Delta E8}$ isoform. SIGIRR$^{\Delta E8}$ functions as a dominant negative mutant that traps the full-length SIGIRR protein in the endoplasmic reticulum (ER) via interaction with the ER resident protein ribophorin 1 (RPN1), preventing its modification by complex glycan and membrane localization.
The aim of this study was to investigate whether SIGIRR$^{\Delta E8}$ confers other novel function(s) in addition to trapping full-length SIGIRR resulting in loss of SIGIRR's inhibitory effect on TLR/IL-1R signalling.
BrdU incorporation assay showed that SIGIRR$^{\Delta E8}$ overexpression resulted in an increased cell proliferation rate of HT-29 colon adenocarcinoma cells. In the proteome profiling experiment, we identified a mitochondrial protein, the alpha subunit of ATP synthase (ATP5A1), as an interacting protein of SIGIRR. We confirmed these results in the overexpression system and also by pulling down endogenous SIGIRR. By using the Seahorse Analyzer, we found that SIGIRR$^{\Delta E8}$ expression led to the increase of the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) as compared to the control cells. Consistently, SIGIRR$^{\Delta E8}$ promoted the survival and proliferation of colon cancer cells in xenograft model.
SIGIRR$^{\Delta E8}$ may exert a direct impact on cell metabolism via interaction with the ATP5A1 in the CRC cells. Further studies are required to evaluate the accurate impact of the SIGIRR$^{\Delta E8}$ expression on metabolism of CRC cells.

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UNEXPECTED SPECTRAL AND REDOX PROPERTIES OF HEMES *b* IN CYTOCHROME b_6f

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Cytochrome b_6f (cytb_6f) is a structural and functional homodimer involved in photosynthetic energy conversion of cyanobacteria, algae and higher plants. It plays a major role in electron transfer (ET) from membrane soluble plastoquinone pool to water soluble plastocyanin [1]. The efficiency of ET catalyzed by cytb_6f strictly depends on the properties of the redox-active prosthetic groups (PG) embedded in the protein structure: hemes c_n, b_n b_p, forming the low-potential chain and the Rieske cluster and heme *f*, forming the high-potential chain. Crucial parameters determining the electron relay function of cytb_6f are the redox midpoint potentials (E_m) of PG. It means that E_m values of PG need to be properly tuned to foster ET from substrates to products [2].

Despite many years of studying of cytb_6f, redox properties of hemes b_n and b_p are still not fully understood [3]. Here, we addressed the long-standing problem of uncertainty in determining the E_m values of these two hemes. We performed a large-scale equilibrium redox titrations of isolated spinach cytb_6f followed by the analysis of the samples by cryogenic: i) optical spectrophotometry, ii) continuous wave, and iii) pulse EPR spectrometry. Careful analysis of experimental data obtained for cytb_6f and their comparison to the analogically obtained data for cytochrome bc_1 led us to the conclusion that, contrary to the current assignment, the E_m of heme b_n is lower than that of heme b_p. This calls for reexamination of the thermodynamic profile of this ET pathway in cytb_6f.

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FLUORESCENCE LIFETIME IMAGING AS A TOOL FOR SENSING NUCLEAR PROTEIN ASSEMBLING

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The role of molecular crowding in the formation and maintenance of membraneless nuclear organelles has been actively studied in recent years. However, these studies are highly challenging when performed in live cells, therefore the principles underlying assembly and disassembly of nucleolar proteins in such organelles are still poorly understood. Here, a non-invasive fluorescence lifetime imaging (FLIM) approach was employed for quantitative monitoring of protein condensation cycles in nuclear compartments of live cultured cells [1]. This approach is based on an inverse correlation between the fluorescence lifetimes of fluorophores (e.g. fluorescent proteins) and the refractive indices of their local environment [2].

The real-time monitoring of fluctuations in protein concentrations in major cellular organelles was assessed by FLIM. It was demonstrated that the rates of recruitment and the release of proteins from phase-separated nuclear organelles (e.g. nucleoli, nuclear speckles and Cajal bodies) is not constant and drastically fluctuates over time. In studied organelles, the detected fluctuations of concentration were in the range of ~20-100 mg/ml, with a significant level of correlation between the rates of protein local concentration in different nuclear organelles of the same cell. Moreover, this FLIM-based approach has recently been successfully applied to unveil the process of concentrating of nuclear proteins in DNA damage response.

Overall, the obtained results provide unexpected insight into cellular metabolism and cellular protein reorganization processes in eukaryotic cells.

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ORAL SESSION 3

CELLS

HOW THE BONE MARROW VASCULATURE REGENRATE? REGENERATION OF BONE MARROW ENDOTHELIAL CELLS AT SINGLE CELL AND CLONAL LEVEL

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The bone marrow endothelial cells (BM-ECs) are the key part of bone marrow niche critical for regulation of hematopoiesis. The rapid regeneration of endothelial network in BM after conditioning-induced injury determines the success of hematopoietic cell transplantation. However, the cellular mechanisms of BM-ECs regeneration remain unclear. Our aim was to understand how the BM-ECs regenerate at the single cell and clonal level.

First, we performed single cell RNA sequencing of mouse BM-ECs and combined our data with published datasets. The analysis revealed that sinusoidal ECs express high levels of Fcgr2b, but are negative for Ly6c, while the arterial type ECs express Ly6c, but no Fcgr2b. We also identified transition cells that were double positive for Fcgr2b and Ly6c (DP-ECs). Imaging of the BM niche revealed that DP-ECs localize between the sinusoidal and arterial ECs. Next, using FACS we prospectively isolated different BM-ECs and developed single cell-derived EC organoid assay to study their clonogenic potential. Single sorted ECs from all fractions formed multicellular vessel organoids on BM stromal monolayer with similarly high efficiency (1/4.3±2.7 sorted cells), indicating the broad regeneration potential of BM-ECs regardless of their phenotype.

Finally, we used Cdh5-CreER-Rainbow mice to study clonality of BM-ECs regeneration after irradiation. We observed highly polyclonal pattern of BM vasculature after irradiation. Modeling based on local assortativity and machine learning supports contribution of many BM-ECs, rather than rare progenitor fraction, to vascular regeneration.

Concluding, our novel single-cell clonogenic assay and fate mapping indicate broad and polyclonal contribution of BM-ECs to regeneration of BM vascularization.

EXTRACELLULAR VESICLES FROM HUMAN iPS CELLS ENHANCE RECONSTITUTION CAPACITY OF CORD BLOOD-DERIVED HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Human cord blood (CB) represents a rich source of several stem cell (SCs) types including hematopoietic stem and progenitor cells (HSPCs). Thus, clinical application of CB cells has become an alternative for the bone marrow transplantation. However, successful application of CB-HSPCs in adult patients requires the development of effective strategies improving their *ex vivo* expansion, homing and regenerative potential. One of the promising approaches for enhancement of SCs functionality includes their treatment with extracellular vesicles (EVs), which were shown to harbor and transfer bioactive content. Thus, in our study, for the first time we have evaluated an impact of human induced pluripotent SCs (hiPSCs)-derived EVs (hiPSC-EVs) on selected functions of CB-HSPCs, important for their hematopoietic potential *in vitro* and *in vivo*.

Our results revealed that hiPSC-EVs may transfer their bioactive content and improve functional properties of CB-HSPCs including metabolic activity, hematopoietic and clonogenic potential, as well as survival, chemotactic response to stromal cell-derived factor 1 (SDF-1) and adhesion to the model components of hematopoietic niche *in vitro*. Importantly, hiPSC-EVs enhanced homing and engraftment of CB-HSPCs *in vivo*. Additionally, we have demonstrated that the treatment with hiPSC-EVs may activate signalling pathways in CB-HSPCs on both gene expression and the protein level.

In conclusion, our findings suggest that the "priming" with hiPSC-EVs may improve several functions of CB-HSPCs important for their homing and hematopoietic activity following the transplantation. These results support the new concept envisioning hiPSC-EVs as next-generation tools enhancing future applications of CB in hematology.

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CX43 REGULATES THE PRO- AND ANTI-FIBROTIC TGF-β/SMAD SIGNALLING DURING MYOFIBROBLASTIC TRANSITIONS IN ASTHMA

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Subepithelial fibrosis in the bronchi of asthmatic patients (AS) is associated with the enhanced potential of human bronchial fibroblasts (HBFs) to the myofibroblastic transition (FMT). Increased levels of the proinflammatory cytokines, such as TGF-β_1, activate Smad2/3 signalling, the canonical profibrotic pathway, in HBFs AS. It leads to the enhanced expression and secretion of fibrosis markers. Our recent reports indicate that connexin43 (Cx43) regulates FMT by the modulation of TGF-β_1/Smad2/3 axis[1]. In our other study, we have shown the disturbed balance between the activity of (profibrotic) Smad2/3 and (antifibrotic) Smad1/5/(8)9 pathway in HBFs AS[2]. While the Cx43-Smad2/3 interactions have been previously described in this model, the Cx43-Smad1/5/(8)9 interactions remain unknown. In this study, HBFs AS and their non-asthmatic (NA) counterparts isolated from bronchoscopy sections were transfected with siRNA or plasmid to induce the Cx43 silencing in HBFs AS or Cx43 overexpression in HBFs NA, respectively. Then, the cells were cultured in serum-free conditions in the absence or presence of TGF-β_1 for 1h, immunostained for pSmad2/3 and pSmad1/5/(8)9 and analysed by confocal microscopy. Our results demonstrate that Cx43 overexpression in HBFs NA causes the activation of profibrotic TGF-β/Smad2/3 signalling and suppression of antifibrotic TGF-β/Smad1/5/(8)9 pathway. In turn, Cx43 silencing in HBFs AS led to the suppression of profibrotic TGF-β/Smad2/3 signalling and to the activation of antifibrotic TGF-β/Smad1/5/(8)9 pathway. These observations confirm a regulatory role of Cx43 in FMT and indicate that it acts as a "molecular switch" between the profibrotic Smad2/3 pathway and the antifibrotic Smad1/5/(8)9 pathway.

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TUMOR INITIATION – THE ROLE OF MCPIP1 PROTEIN

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Developing new treatment strategies for cancer patients is extremely difficult due to the problem of identifying cancer stem cells (CSCs) which are responsible for tumor initiation and progression. A growing number of publications suggest that the MCPIP1 protein may influence the development of cancer by direct or indirect regulation of factors involved in the processes of angiogenesis, proliferation and cell death.

The main aim of our research is to study the importance of MCPIP1 for the presence of CSCs markers, factors involved in tumor initiation as well as acquirement of stemness features.

We have shown that the low level of MCPIP1 or D141N mutation in PIN domain, responsible for the endonucleolytic properties of MCPIP1, increases the proliferation and clonogenicity of normal epithelial TCMK-1 cells compared to control. Moreover, cells with decreased level of MCPIP1 protein or with the D141N mutation are characterized by higher levels of the c-Met receptor, CD44 and c-Myc phosphorylation. In addition, low level of MCPIP1 results in increased expression of vimentin, a major mesenchymal marker, and transcription factors involved in epithelial-to mesenchymal transition. Next, we checked whether low level of MCPIP1 in normal cells predisposes them to proliferate after injection into immunocompetent mice. We found that, D141N mutation caused the growth of large tumors, while the control cells injected into mice developed only local fibrosis.

We believe that the MCPIP1 protein may be a marker of tumor initiation and play a key role in neoplastic transformation by regulating the changes in cell phenotype and levels of CSCs markers.

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SELECTION GUIDE METABOLITE ANALYSIS. METABOLOMICS AND PROTEOMICS PRODUCT PORTFOLIO

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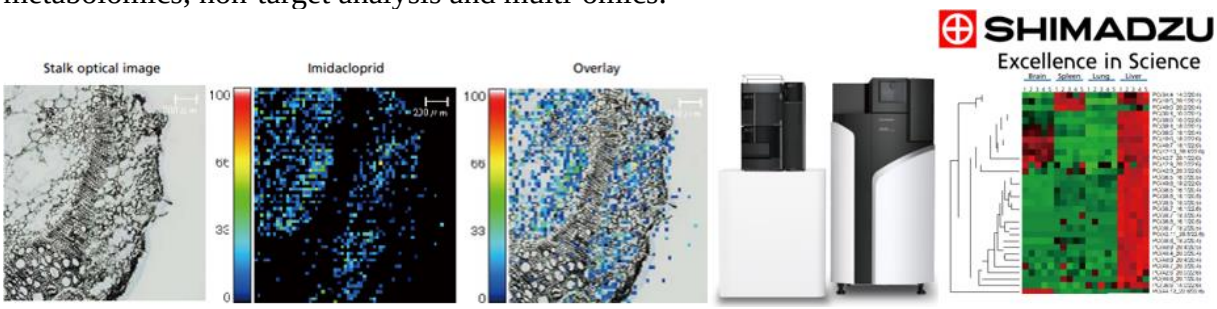
Metabolomics refers to an array of techniques used to comprehensively detect and analyze various metabolites formed in vivo during biological activity. The qualitative and quantitative changes in metabolites reflect the ever-changing biological phenomena and are widely used for diagnosis, biomarker discovery, and drug discovery research. In recent years, metabolomics has been used in the food industry to improve taste and quality and to develop functional foods. It is also used in the biotechnology industry to improve fermentation and biofuel productivity. Shimadzu supports the development and proliferation of metabolomics technologies by providing solutions combining mass spectrometers, imaging mass microscops, databases, ready-to-use method packages, and software (including DDA/DIA approach), which cover quantitative metabolomics, non-target analysis and multi-omics.



References:
[1] Shimadzu brochure number: C146-E280D. SELECTION GUIDE METABOLITE ANALYSIS. METABOLOMICS PRODUCT PORTFOLIO.

THE INTERPLAY BETWEEN ORAL PATHOGENS AND INFLAMMATORY CYTOKINES IN GINGIVAL FIBROBLAST ACTIVATION IN PERIODONTITIS

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Periodontitis is a chronic inflammatory disease of the periodontium caused by microbial imbalance. Pathological changes are driven by a failed attempt of the host immune system to eliminate pathogenic bacteria [1]. Pathogens managed to develop strategies to evade the antimicrobial activity of immune cells, while maintaining inflammation [2]. However, it is still unknown how oral pathogens affect inflammatory responses of gingival fibroblasts (GFs) in the context of the local microenvironment of the inflamed gingival tissue.

We show that infection of primary human GFs with a laboratory strain of *P. gingivalis* during stimulation with tumor necrosis factor (TNF) resulted in synergistic increases in the expression and production of IL-6 and IL-8 without any changes in cell viability. Similar results were observed during infection with *Fusobacterium nucleatum.* Sensitization of GFs to inflammatory activation by *P. gingivalis* was significantly less pronounced in the absence of direct contact between cells and bacteria, indicating that soluble factors released by *P. gingivalis* are not sufficient for full amplification of TNF-induced GF responses. It was also shown that *P. gingivalis* modulates TNF-induced GF activation mainly through the TLR2 pathway. Finally, similar experiments performed on macrophages demonstrated that the synergy between proinflammatory cytokines and oral bacteria is characteristic only for GFs and does not occur in monocyte-derived macrophages.

Collectively, these results demonstrate that oral pathogens synergize with the inflammatory environment to amplify GF activation, which may lead to excessive cytokine accumulation in the gingival tissue. This knowledge could facilitate identification of ‘host modulation therapeutic agents’ for the treatment of periodontitis.

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ACCESSORY SUBUNITS OF *P. GINGIVALIS* MAJOR FIMBRIAE POTENTIALLY MODIFIED BY PPAD ARE VITAL TLR2 AGONISTS

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Porphyromonas gingivalis, a keystone oral pathogen implicated in development and progression of periodontitis, may also contribute to the pathobiology of such diseases as arthritis, atherosclerosis, and Alzheimer's. Using large variety of virulence factors, *P. gingivalis* is a master manipulator of host immune responses. Among these factors, *P. gingivalis* peptidyl arginine deiminase (PPAD), an enzyme unique to *P. gingivalis*, converts C-terminal Arg residues in bacterium- and host-derived proteins and peptides into citrulline. PPAD activity is essential for stimulation of proinflammatory responses in host cells, especially in gingival fibroblasts. Given that Toll-like 2 receptor (TLR2) is the main pathogen-associated molecular pattern recognition receptor for *P. gingivalis*, in our previous work we determined the role of PPAD activity on TLR2-dependent host cell responses. We found that PPAD activity was required for TLR2 activation by *P. gingivalis* and proposed major fimbriae as a potentially citrullinated TLR2 ligand. Here we aimed to identify, which subunit encompassing fimbriae is citrullinated and responsible for this signalling. All the attempts to determined citrullination in FimA, the major fimbriae subunit failed. Therefore, we focused on accessory fimbriae subunits (FimCDE) and their influence on TLR2-dependent host cell responses. The preliminary results obtained using reporter cell lines as well as primary cells indicate that accessory fimbriae subunits not FimA are crucial for TLR2 activation. In conclusion, our data strongly suggest that accessory fimbriae subunits are modified by PPAD and are important for TLR2-dependent cell responses to *P. gingivalis* infection.

Acknowledgements:
This work was supported by grants from the National Science Centre of Poland (grant numbers: 2019/35/B/NZ5/01823 to AMG and 2018/31/B/NZ1/03968 to JP).

IDENTIFICATION OF MYXOXANTOPHYLL ISOMERS IN *ANABAENA* (*NOSTOC*) PCC7120

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The cyanobacteria from *Anabaena* genus have recently been recognized as a potential source of secondary metabolites of pharmacological and biotechnological importance. In particular, myxoxanthophylls – specific carotenoid glycosides accumulated in cyanobacterial cells, attract emerging interest.

Anabaena (*Nostoc*) sp. PCC7120, a filamentous, mesophilic, nitrogen-fixing cyanobacterium, originally isolated from paddy fields, is a model organism used in biochemical and genetic studies. Recent findings show that the functioning of the carotenoid biosynthesis pathway in *Anabaena* cells is affected by environmental factors. Specifically, the balance between β-carotene and ketocarotenoids is altered according to temperature conditions [1]. The carotenoid pool of *Anabaena* 7120 consists of five main species of pigments: β-carotene, echinenone, canthaxanthin and two myxoxanthophyll derivatives: myxoxanthophyll ((3R,20S)-myxol 20-fucoside) and keto-myxoxanthophyll ((3S,20S)-4-ketomyxol 20-fucoside) [2].

In tnis study, a new method, based on single-step liquid adsorption chromatography has been developed and applied to separate a fraction containing myxoxanthophylls from *Anabaena* 7120 cells. It was found that this method allowed to obtain high-purity (>95%) myxoxanthophylls from pigment pools as previously extracted from cyanobacterial cells, independently of the extraction technique used. The subsequent analysis by HPLC and LC/MS methods demonstrated that the myxoxanthophyll fraction consists of the mixture of compounds with different retention times. Based on their fragmentation spectra and optical properties, these compounds were identified as geometrical isomers of myxoxanthophylls, including the dominant *all-trans*forms and less abundant *cis* forms. The structures of myxoxanthophyll isomers are proposed.

Our results show for the first time the existence of isomeric forms of myxoxanthophylls in cyanobacterial cells. The formation and cellular function of isomers remains to be elucidated.

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Acknowledgements:

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SEARCHING OF BIOACTIVE SECONDARY METABOLITES FROM FIVE CYANOBACTERIAL SPECIES

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Cyanobacteria are a great source of unexplored secondary metabolites, potentially with harmful (cyanotoxins) or beneficial (drugs) effects on other organisms. In this work we investigated the impact of the five cyanobacteria strains *Jaaginema* sp., *Trichormus variabilis*, *Komarekiella chia*, *Nodularia mediterranea*, and *Iphianassa zackieohae* with unknown metabolome on *Lemna trisulca* macrophyte. The experiments were prepared in short- (up to 24h) and long-term (co-cultivation in BG11 medium, 2 weeks) periods. Co-cultivation of macrophyte with *I. zackieohae* cells initially stimulated plant growth, but after 14 days the value was close to the control, while the remaining cyanobacteria slightly inhibited the accumulation of macrophyte biomass. After 14 days of co-cultivation, *K. chia*, and *N. mediterranea* significantly increased the total amount of protein in the plant [$mg \cdot g^{-1}$ dry weight], by 33% and 44%, respectively. The extracts of all analyzed cyanobacteria significantly increased the respiration process after 24-hour exposure. The tested cyanobacteria strains, except *I. zackieohae*, decreased the content of chlorophylls *a* and *b* by an average of 48% and 53%, respectively. *Jaaginema* sp. extract temporarily inhibited plant photosynthesis just within 7 minutes. Oxidative stress induced after treatments is also discussed.

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4. B. Janusz, S. M. Elnagdy, D. M. Ghaith, V. Savini, J. Międzobrodzki, M. Kosecka-Strojek*
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The yeast-neutrophil war – mechanisms of interaction between *C. albicans* and neutrophils

75. A. Gąsiorek*, E. Dobosz, T. Hutsch, J. Kozieł

The role of MCPIP-1 in gingival keratinocytes during alveolar bone remodeling

ABSTRACTS
POSTER PRESENTATIONS

IS THERE A LINK BETWEEN HEME OXYGENASE-1 AND REPLICATION STRESS?

P. Chudy[1], P. Nguyen[1], D. Klóska[1], J. Kochan[2], L. Muchova[3], D. Cysewski[4], A. Grochot-Przęczek[1], K. Szade[1], A. Szade[1], A. Józkowicz[1], W. Nowak[1], W. Krzeptowski[1]

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Heme oxygenase-1 (Hmox1) is a heme degrading enzyme, present both in the cytoplasm and in the nucleus. Nuclear localization is observed in proliferating cells and can be enhanced by heme overload. To shed a light on the enigmatic role of Hmox1 in the cell nucleus we used genetically modified HEK-293 cells, murine induced pluripotent stem cells and murine hematopoietic stem cells that lack Hmox1.

We found the enhanced G-quadruplexes (G4s) immunostaining in the cell nuclei and the upregulation of genes involved in G4s unwinding in Hmox1-deficient cells. Moreover, using a proximity ligation assay we demonstrated that in wild-type cells Hmox1 localizes in the vicinity of G4 structures. G-quadruplexes are four-stranded structures formed by guanine-rich DNA (or RNA) sequences stabilized by several ligands, including heme. We demonstrated that nuclear form of Hmox1 is enzymatically active, thus it might be involved in the removal of a free heme to help unwind G4.

Importantly, G4s formed during DNA replication can disrupt the replication forks and, if not resolved, can cause replication stress which leads to DNA breaks. Indeed, our data showed a higher proportion of stalled replication forks in Hmox1-deficient cells. What is more, Hmox1-deficiency was accompanied with increased DNA breaks and enhanced DNA repair response. We also observed that PARP1, which can interact with G-quadruplexes, is a binding partner of Hmox1 and its level is higher in Hmox1-deficient cells. On the other hand, our proteomics analysis indicates that DNA-repair complex is not modified significantly in the absence of Hmox1. Instead, it suggests that apart from a putative direct effect of the heme removal and G4 formation, Hmox1 can indirectly modify the G1/S and G2/M cell cycle checkpoints, influencing the nucleus-mitochondria cross-talks.

To sum up, Hmox1 protects cells from replication stress, possibly not only through direct regulation of free heme availability and G4 unwinding.

APPLICATION OF THE LSTM NEURAL NETWORKS FOR GENES ESSENTIALITY PREDICTION IN BACTERIA

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Essential genes constitute a set of genes that is crucial for an organism's survival and reproduction [1]. It is worth noting that a gene's essentiality depends on the considered environment. In some conditions, a gene may be classified as essential, but not in others. The research of essentialoms, which are sets of genes crucial for an organism's survival in a certain environment, has been conducted since the 1990s [2]. The expansion of our experimental knowledge on the full Bacteria's genome and its functionality has enabled the essential gene cognition. This may be used during computer predictions and model buildings. One may be interested in the essentiality of a particular gene in considered Bacteria. To this aim, various approaches are applied. In this study, the Long-short-term memory (LSTM) neural network and the corresponding representation inspired by natural language processing have been used for the bacterial gene essentiality prediction. Conducted experiments have demonstrated the high accuracy of predictions and showed the significant usefulness of LSTM in this issue.

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POSSIBLE LUNG FIBROBLAST-TO-MYOFIBROBLAST TRANSITION UPON CONTACT WITH *CANDIDA ALBICANS* BIOFILM

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The fungi *Candida albicans* is a pleiotropic commensal that under not fully understood conditions turns into a life-threatening pathogen. Among its virulence factors, the role of Secreted Aspartic Proteases (SAPs) – ten endopeptidases with wide substrate specificity, can be underlined. Sap6, mainly expressed by the filamentous form of fungal cells composing biofilm poses an optimal pH close to physiological levels and amino acid motifs RGD/KGD involved in the binding to integrins. In the present study, we investigated the interplay between the biofilm secretome and human fibroblasts - the main stromal cell type. Human fibroblasts were challenged with biofilm supernatants, presenting a significant upregulation of alpha-smooth actin (*ACTA2)* and cellular communication network factor 2 (*CCN2)* genes. Focus on biofilm components – isolated Sap6 showed no cytotoxicity at physiologically relevant concentrations, even a proliferative effect was observed. Thereby, the analysis of a cluster of pro-, anti-inflammatory and pro-fibrotic gene expression was performed, in which upregulation on *ACTA2*, *CCN2*, tumor growth factor-β1, and proteinase-activated receptor-2 was identified. In addition, the level of fibroblast colonization was assessed using mutant strain lacking *SAP6* gene but no statistical difference was detonated, suggesting a marginal role of this virulence factor in fibroblast colonization. This observation suggests that different elements of the secretome could act synergically to promote fibroblast-to-myofibroblast (FMT) transition.

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CHARACTERIZATION OF CLINICAL METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS FROM ITALY AND EGYPT

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Staphylococcus aureus bacteria possess a collection of virulence factors and the ability to acquire drug resistance to variety of antibiotics, leading to the spread of methicillin-resistant *S. aureus* MRSA strains. Establishing transfer pathways of genetic elements responsible for this phenomenon is crucial for epidemic detection. Seventy MRSA clinical strains were characterized, 20 and 50 isolates from Italy and Egypt, respectively. MRSA phenotype was confirmed by defying MIC values for cefoxitin. The following genetic elements were detected using PCR: hemolysins: *hla/hlb/hld/hlg/hlg-2* and leukocidins: *pvl/lukAB/lukDE*. Moreover, both clonality and in-depth characterization of SCC*mec* cassettes within collection was performed by *spa* and two independent SCC*mec* typing methods. Within Italian strains, 9 *spa* types were observed with dominating European types: *t032*, *t002* and *t008*. Additionally, SCC*mec* type IV and II were found. Within Egyptian strains two genetic profiles were found: European *spa* type *t002* with mix of SCC*mec* type I with V and African *spa* type *t037* with mix of SCC*mec* type III with V. The *hla* gene was found in all Italian strains and in Egyptian strains with *t002*, while *hlb* gene was found in all collected strains. The l*ukAB* gene was present in all strains, *lukDE* was found in all Egyptian and majority of Italian strains. In comparison to Egyptian strains study shows genetic diversity within Italian ones. The presence of European *spa* type *t002* in isolates from Egypt shows, how genetic elements are transferred between previously established regional clonal complexes. Monitoring these changes is crucial for precise pathogen identification.

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MATRIX REPRESENTATIONS COMBINED WITH THE DISCRETE FOURIER TRANSFORM IN BIOLOGICAL SEQUENCES STUDIES

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There are many representations of biological sequences such as Chaos Game Representation (CGR), Z-curve or genomatrix. In the first approach, a sequence is projected on a unit square. Subsequent corners represent nucleotides or amino acids groups. In many real cases, some fractal patterns are observable and may indicate characteristic compositions within sequences. Similar results are obtained by analysing the genomatrix representation. They both were successfully applied in many areas, for instance, free-alignment methods, gene recognition and others. The authors compared mentioned representations combined with the Discrete Fourier Transform (DFT) in the nucleotide sequences analysis, including the avian influenza viruses case study. We indicate the adequacy of this combination applied in the free-alignment approach and phylogenetic trees construction. Moreover, we proposed the application of these methods for visual inspection of the RNA's secondary structure, mainly by checking the corresponding paired bases in structural elements.

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THE IMPACT OF DGAT1 DEFICIENCY ON EPIDERMAL TRANSCRIPTOMIC LANDSCAPE

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The skin is one of the largest and most complicated organs of the body. As it is constantly exposed to the external environment, its main function is to provide a protective barrier for internal organs. The outermost layer – epidermis – is stratified and each of the strata represents different stages of keratinocyte differentiation. Keratinocytes in the deeper layers are the major producers of keratin, which helps to resist mechanical stress, but they also secrete lamellar bodies containing various lipids, antimicrobial peptides and proteolytic enzymes. The topmost epidermis layer is formed by keratinized and anucleated corneocytes that are cross-linked and are embedded in a lipid-rich matrix delivered by lamellar bodies [1].

Among the enzymes involved in the production of epidermal extracellular lipid matrix, DGAT1 (acyl-CoA:diacylglycerol acetyltransferase 1) is suggested to play a vital role in maintaining the proper barrier function of the skin, due to its ability to catalyze the synthesis of a broad spectrum of lipids, retinoids and wax. Although DGAT1-deficient mice show skin abnormalities as a consequence of the disturbance in the lipid synthesis, it is unclear how absence of DGAT1 affects keratinocytes [2]. It has been shown that retinoids can regulate keratinocyte proliferation and differentiation and DGAT1 also plays a role in maintaining retinoid homeostasis [3,4]. This lead us to focus on the role of DGAT1 in regulating the function of keratinocytes.

In this study we performed the RNA-Seq analysis of epidermis isolated from DGAT1WT and DGAT1KO mice and identified differentially expressed genes, suggesting the influence of DGAT1 on regulating keratinocyte function.

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PORPHYROMONAS GINGIVALIS AND IL-1β DIFFERENTIALLY MODULATE mRNA TURNOVER AND STABILITY IN GINGIVAL FIBROBLASTS

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Changes in mRNA stability are one of the key mechanisms that regulate gene expression during inflammation. This study aimed to investigate the kinetics of the expression rate and mRNA stability of inflammatory mediators involved in periodontitis pathogenesis: interleukin-6 (IL-6), IL-8, and cyclooxygenase-2 (COX-2). Moreover, changes in the expression of ARE (AU-rich elements) binding proteins were analyzed. Primary human gingival fibroblasts were stimulated with IL-1β or infected with *Porphyromonas gingivalis*, the key pathogen in the development of periodontitis. IL-1β strongly induced the expression of inflammatory mediators, which was associated with a significant increase in their mRNA stability. At the same time, stimulation with IL-1β regulated mRNA expression of several ARE binding proteins, particularly tristetraprolin (TTP), which is a destabilization factor for, among other transcripts, *IL6*, *IL8* and *COX2*. TTP was highly induced both at the mRNA and protein levels already after 1 hour of stimulation with IL-1β. While TTP protein remained highly expressed after IL-1β stimulation, the relative intensity of bands with different molecular weights, indicative of changes in TTP phosphorylation status, changed over time. In contrast, TTP expression was regulated to a much lesser extent after infection with *P. gingivalis* compared to IL-1β stimulation and, consequently, cell infection was not accompanied by significant changes in the stability of *IL6* and *COX2* transcripts. Interestingly, destabilization of the *IL8* transcript was observed after infection with *P. gingivalis*. These results suggest that mRNA stabilization plays an important role in gingival fibroblast activation by cytokines, but not by *P. gingivalis*.

NOVEL TOOL FOR ANNOTATING piRNA CLUSTERS

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Piwi-interacting RNAs (piRNA) are small non-coding RNAs ranging between 28 and 31 nucleotides. PIWI proteins bind to piRNAs and guide them to complementary RNA sequences blocking their transcription or triggering their degradation. By these means, piRNAs silence transposable elements acting as key protectors of the genome [1]. Recent studies have shown that piRNAs might also have a functional role beyond the protection of the genome [2].

Currently, one of the limiting factors to study piRNAs across organisms is the lack of robust computational methods to reliably identify them. Here, we present a Python package that uses small RNA-seq mapped reference genome to identify and annotate piRNA clusters in any animal species based on piRNA biogenesis criteria.

The biogenesis of piRNAs is not completely understood, but the main steps are well defined. First, a long RNA precursor is transcribed and subsequently cleaved into small fragments producing the piRNAs. The genomic locus is called a piRNA cluster loci [3]. To identify such clusters, our tool first identifies the length of the piRNAs on the given species based on the read length distribution of the small RNA-seq reads. Then, scans the genome with the density-based clustering approach to find genomic regions with a high density of mapped reads of the appropriate piRNA length, and marks them as potential cluster candidates.

We annotated piRNA clusters with the use of our tool and then assessed its performance in comparison to the two most widely used methods.

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IMPACT OF HYPOXIA ON HUMAN INDUCED PLURIPOTENT STEM CELLS TRANSCRIPTOME

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Induced Pluripotent Stem Cells (iPSCs) constitute an extremely useful source of cells for both research purposes and potential therapies in human patients as a part of personalized medicine approach. There is growing evidence indicating that one of the key factors impacting iPSCs physiology and differentiation potential is oxygen tension in their microenvironment. iPSCs are usually cultured in ambient oxygen conditions (21% O_2), however, lower oxygen concentrations (3-5% O_2), termed hypoxia, were shown to support their pluripotency profile. To investigate the influence of various oxygen concentrations (3, 5, 21% O_2) on global gene expression profile in human iPSCs (hiPSCs), we performed whole transcriptome analysis on three hiPSC lines. RNA sequencing was performed on Ion Torrent platform. The obtained sequencing reads were aligned to a reference human genome using the Salmon software, while the differential gene expression (DGE) step was conducted using the edgeR program and Bioconductor's packages. We identified a number of genes overexpressed or underexpressed in hypoxia, compared to normoxic conditions. Functional analysis of DE genes showed enrichment in gene ontology (GO) terms related to glycolytic process, response to hypoxia, cell stress, catabolic processes, or regulation of pyruvate metabolic process, in cells cultured in low oxygen concentrations. Regulatory RNA molecules, including miRNAs and lnc-RNAs, constituted a significant part of the molecules detected as DE genes at the transcriptome level, and their role will be further investigated. Concluding, our study supports the important role of oxygen condition in the regulation of hiPSCs metabolism, as shown by differences in gene expression profile of hiPSCs cultured in different oxygen concentrations.

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THE EMERGING PROBLEM OF LINEZOLID-RESISTANT STAPHYLOCOCCUS IN POLAND

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The increasing antibiotic resistance among *Staphylococcus* is an emerging problem worldwide. The clinical linezolid-resistant *Staphylococcus epidermidis* strains are observed in Poland since 2015 [1]. Nasal microbiota, hence bacterial strains from laryngological patients, can become a threat for immunocompromised patients and is a reservoir for opportunistic pathogens which possess resistance and virulence genes.

The aim of this study was to genetically characterize two *Staphylococcus* strains potentially carrying linezolid resistance genes, recovered from patients with sinusitis.

Bacterial strains were identified at the species level by Sanger-based methods, and antimicrobial resistance patterns were analyzed. For characteristics and detection of virulence and antimicrobial determinants, PCR-based methods for targeted genes, MLST, *spa* and SCC*mec* typing were used.

Both identified *Staphylococcus aureus* and *Staphylococcus haemolyticus* isolates showed resistance to more than ten antibiotics. The linezolid susceptible *S. aureus* strain was identified as t4474/ST398/IVe and harbored adhesin and hemolysin genes. The linezolid resistant *S. haemolyticus* strain was identified as ST42/*mecA*+ and possessed hemolysin genes. For *S. aureus* strain, potential linezolid resistance genes *optrA*, *poxtA* and mutations in the ribosomal proteins L3/L4 were detected. In *S. haemolyticus*, resistance was associated with the presence of *cfr* gene.

Obtained results show the increasing linezolid resistance of *Staphylococcus* isolates is an emerging problem in Poland, not only in the hospital environment. Both tested *Staphylococcus* strains were recovered from ambulatory patients who were not treated with linezolid [2]. The obtained results suggest ability to the acquisition of mobile genetic elements from other bacterial isolates, leading to the spread of drug-resistant strains.

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CHARACTERISTICS OF THE INTERACTION BETWEEN GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) OF *CANDIDA ALBICANS* AND *CANDIDA GLABRATA* AND THE PROTEIN OF HUMAN EXTRACELLULAR MATRIX – VITRONECTIN

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Candida is a genus of yeast-like fungi numbering more than 200 species, several of which having an ability to infect humans. The course of these infections can be mild or dangerous, depending on the patient health condition. *Candida albicans* and *Candida glabrata* are among the most common and virulent species of *Candida*. Their high virulence is determined by multiple virulence factors, of which one of the major is an ability of pathogens to adhere to host proteins and cells.

The aim of this study was to characterize the interaction of the cytoplasmic enzyme – glyceraldehyde 3-phosphate dehydrogenase (Gapdh), also found on the surface of the *Candida* cell wall, with the human extracellular matrix protein – vitronectin.

The binding between these two proteins was determined by a microplate ligand-binding assay with a biotin-labeled Gapdh – streptavidin system and by surface plasmon resonance measurements. Recombinant Gapdh of *C. albicans* and native Gapdh isolated from the cell wall of *C. glabrata* were used in the assays. Additionally, the results were compared with the enzyme from a non-pathogenic yeast species – *Saccharomyces cerevisiae*.

The obtained values of dissociation constant (K_D) confirm the high strength and specificity of the interaction between vitronectin and candidal Gapdh, in contrast to insignificant binding strength observed for Gapdh from the non-pathogenic yeast. These results suggest a role of the pathogen surface Gapdh in adhesion to host proteins and, more generally, candidal pathogenesis.

HOST PROTEASES AS POTENT NETs INDUCERS DURING ASEPTIC INFLAMMATION

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Neutrophil extracellular traps (NETs) are structures composed of chromatin fibers decorated with components of azurophilic granules. They immobilize pathogens preventing their dissemination. Our recent data showed that the activation of Proteinase-Activated Receptor-2 (PAR-2) plays an important role for the generation of NETs induced by periodontal pathogens [1]. In the current study we aimed to expand the initial observation and examined the role of host proteases. Obtained data revealed that trypsin, kallikrein 14 and blood coagulation factor X classified as the most potent activators of PAR-2 induce the significant release of NETs. Of note, neutrophils from mice lacking PAR-2 expression did not generate NETs after treatment with tested enzymes, which clearly indicates that these receptors are involved in the formation of NETs. In addition, blocking PAR-2 with an antagonist abolished the formation of NETs caused by proteases. Moreover, studying the signal transduction pathway we found that activation of Erk and Rho is required for the PAR-2 mediated NETosis. Taken together, obtained data indicate the crucial role of host proteases in NETs formation induced during aseptic inflammation.

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IDENTIFICATION OF PROTEASES PRODUCED BY *STREPTOCOCCUS ANGINOSUS*

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Streptococcus anginosus are gram-positive bacteria, which belong to *Streptococcus anginosus* group (SAG, *Streptococcus milleri)*. *S. anginosus* has been classified as commensal bacteria of the colon, oral cavity, and vagina, however, nowadays it is considered as a human pathogen leading to brain or liver abscesses. Despite the increased number of clinical reports, the molecular mechanisms of *S. anginosus* pathogenesis remain unknown. Recently we investigated the pathogenicity of different SAG clinical isolates (n=41) using *Dictyostelium discoideum* and *Galleria mellonella,* selecting strains with high virulence (n=4). As we revealed that their pathogenicity positively correlates with high proteolytic activity, therefore, the current study aimed to identify their molecular targets. The analysis of their genome and proteolytic activity revealed the presence of two classes of proteases serine and metalloproteases. We examined their potential substrates including the crucial components of the immune system, including AMPs, immunoglobulins and complement systems. We found that *S. anginosus* significantly affects the stability and antibacterial function of the complement system. Taken together, SAG proteases are important virulence factors, which allow bacteria to avoid elimination by the host. In the future, we will expand the project trying to define precisely their role in human infection.

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THE MECHANISM OF POSTTRANSLATIONAL MODIFICATION OF MCPIP-1 INDUCED BY INFECTION WITH PERIODONTAL PATHOGENS

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Monocyte chemotactic protein-induced protein 1 (MCPIP-1) is a negative regulator of the inflammatory response, playing a key role in the maintenance of oral epithelial homeostasis [1]. It was shown that MCPIP-1 protein undergoes posttranslational modifications (PTM), including phosphorylation, ubiquitination with subsequent proteasome degradation, and proteolysis catalyzed by paracaspase MALT-1 (*mucosa-associated lymphoid tissue lymphoma translocation protein-1*). The process of bacterial infection promotes the intensity of PTM in the host, therefore, we decided to examine MCPIP-1 protein modifications during periodontitis. Using an *in vitro* model of the immortalized human gingival keratinocytes we found decrease of MCPIP-1 protein upon long-term infection with periodontal pathogens. The molecular analysis of observed phenomenon revealed that the process depends on enzymatic activity of MALT-1. Moreover, we found that infection with periodontal pathogens lead to different pattern of ubiquitinated proteins and significant activity of proteasomal degradation. Therefore, we also examined this pathway, but our data revealed that proteasomal degradation is not responsible for decrease in MCPIP-1. Taken together our studies expanded our knowledge about the molecular basis of MCPIP-1 modification in periodontal disease. Collectively, we showed that at the initial stage of *P. gingivalis* infection (hours), the mechanism of MCPIP-1 proteolysis depends on cysteine proteases - gingipains [2], but then (days) is taken over by the LPS-activated MALT-1 protease. These results shed light on the future of understanding the pathology of periodontal disease.

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THE ANTIVIRAL ACTIVITY OF TEMPORIN ANALOGUES AGAINST *HERPES SIMPLEX* VIRUS

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Herpes simplex virus type 1 (HSV-1) is classified among the most widespread human pathogens. It is estimated that 67% of the worldwide population suffer from prevalent HSV-1 infection. It is treated mainly with nucleoside analogues, such as acyclovir and its derivatives, which interfere with viral DNA synthesis reducing virus replication and shedding. Most of these drugs neither prevent reactivation of the latent HSV-1, nor eliminate mature form of the virus. Therefore, there is a need for the development of novel anti-herpes agent. Antimicrobial peptides (AMPs), are classified as innate immune effectors in a variety of species, including plants, insects, amphibians, and mammals. They act as potent antimicrobial molecules against a broad spectrum of microorganisms, including viruses, bacteria and fungi. As natural compounds they are a promising target for antiviral drugs. Here, we designed a lysine-rich derivative of amphibian temporin-1CEb conjugated to peptides penetrating the host cell membrane. We examined their antiviral activity using a variety of techniques, including titration assay, qPCR and confocal imaging. Importantly, no toxicity was observed for these peptides in the range of active concentrations. We showed that temporins analogues hamper the different stages of the HSV-1 infection, including the interaction between the virus and the cellular attachment receptor, as well as the subsequent post-infection phase. Moreover, their efficacy was confirmed using 3D organotypic model of the oral human mucosa, suggesting temporins analogues as a novel antiviral medication for the prevention and/or treatment of HSV-1 infection.

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THE ROLE OF BRADYKININ IN LIPID METABOLISM – AN ALLY OR AN ENEMY?

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Obesity is a complex civilization disease, the prevalence of which has rapidly grown in recent decades. Disturbances in the lipid and carbohydrate metabolism are usually associated with the appearance of systemic inflammation, in which fatty tissue plays an important role. Bradykinin, a well-known pro-inflammatory peptide, abundantly occurs in most tissues and biological fluids. The aim of the present study was to determine the adipocyte ability to produce bradykinin and to characterize the effect of this peptide on lipid metabolism.

The studies were carried out on a differentiated human pre-adipocyte line Chub-S7, which showed adipose tissue cell-specific properties, such as the fat accumulation and increased expression of adiponectin and lipoprotein lipase genes. It was demonstrated that adipocytes were able to produce bradykinin, since the genes for kallikreins, prolylcarboxypeptidase and kininogen were expressed in these cells. Moreover, the gene expression of kinin receptors was also confirmed. Different responses to bradykinin action were observed in adipocytes depending on the peptide concentration and the presence of kininase inhibitors. High bradykinin concentrations in the absence of kininase inhibitors led to increased lipid accumulation, increased pro-inflammatory cytokine release and disturbed gene expression of proteins involved in cell metabolism, such as lipoprotein lipase, Cell Death-Inducing DFFA-Like Effector Protein C and **CCAAT-Enhancer-Binding Protein alpha.**

This study has supported a hypothesis on the involvement of kinin peptides in the development of obesity. The obtained results can enrich knowledge on the mechanisms underlying obesity-related disorders.

THE ROLE OF HEMAGGLUTININ/ADHESIN DOMAINS OF *PORPHYROMONAS GINGIVALIS*

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Porphyromonas gingivalis is one of the main pathogens associated with chronic periodontitis. The most important virulence factors of *P. gingivalis* are gingipains, which are responsible for the vast majority of its proteolytic activity. In addition to catalytic cysteine peptidase domain, gingipains RgpA and Kgp contain repeating hemagglutinin/adhesin (HA) domains. The HA domains are also present in the sequences of the gingipain paralogs, namely hemagglutinins. Both groups of proteins are involved in adhesion, agglutination and hemolysis of erythrocytes, which results from the presence of the HA domains and proteolytic activity of gingipains.

The aim of the research was to produce recombinant protein constructs containing the HA domains of gingipains of high purity and homogeneity. We have successfully obtained seven constructs variants, differing in number of the HA domains. Using dynamic light scattering technique, we analyzed their particle size and polydispersity index. To assess biological properties of the protein variants, we performed hemagglutination and hemolysis assays. Moreover, we tested the influence of gingipain proteolytic activity on the maturation (processing) of these domains. Considering that the HA domains of gingipains and hemagglutinins play crucial role in several biological processes such as invasion of the host cells, biofilm formation and nutrient acquisition, our results may contribute to the development of therapeutic agents that reduce the virulence of *P. gingivalis*.

UBIQUITIN AND UBIQUITINYLATED PROTEINS IN CELL WALLS OF *SCENEDESMUS OBLIQUUS* 633 AS IDENTIFIED BY IMMUNOCHEMISTRY AND LC/MS/MS PROTEOMICS

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Cell walls of green algae (*Chlorophyta*) are complex polymeric structures consisting of cellulose, hemicelluloses, pectins, proteins and, in many cases, a hydrocarbonaceous polymer – algaenan [1]. Although cell walls are fundamental structures of algal cells, their protein composition in biotechnologically important genera of microalgae remains poorly understood. It was shown previously that the multilayered cell wall of *Scenedesmus obliquus* 633 contains a polypeptide related to the cell-wall glycoprotein *gp3* of *Chlamydomonas reinhardtii* [2].
In this work, the fraction of residual cell wall material has been isolated from the long-lasting cultures of *Scenedesmus obliquus* 633 and subjected to immunofluorescence microscopy using anti-ubiquitin antibodies. For LC-MS/MS proteomics and immunoblotting, cell wall-associated polypeptide fraction was extracted from cell walls to water at temperature of 80^{o}C.
NCBI databases were used to analyze the results obtained from LC-MS/MS analyses. Matches were searched for in the protein sequences of three databases: the *Scenedesmaceae* family (35 proteins identified), the *Chlorophyceae* class (22 proteins), and *Chlamydomonas reinhardtii* (9 proteins). Additionally, it was noticed that 11 of the listed proteins occur in both the *Scenedesmaceae* and *Chlorophyceae* databases. The LC-MS/MS analysis showed that heat shock proteins and proteins involved in plant-type cell wall organization were present among identified proteins. In *Scenedesmus obliquus* cell walls, immunoblotting analysis, immunofluorescence microscopy, and LC-MS/MS proteomics collectively demonstrated the presence of ubiquitin and several ubiquitin conjugates of higher molecular mass. These results suggest the significance of protein ubiquitinylation for the formation and functioning of cell walls in green algae.

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THE INFLUENCE OF THE MCPIP1 PROTEIN ON THE LEVEL AND FUNCTION OF N-CADHERIN

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The MCPIP1 (Monocyte Chemoatractant Protein-Induced Protein 1) protein is encoded by the *ZC3H12A* gene and it's called the negative regulator of inflammatory response. As a result of RNase activity MCPIP1 protein regulates levels of many transcripts, including pro-inflammatory cytokines: IL -1β and IL-6 as well as several miRNAs important in tumor growth and metastasis. Recent studies have shown that the MCPIP1 protein plays an important protective role in the development and progression of breast cancer and clear cell renal cell carcinoma, while its reduction leads to an increase in the proliferation of cells and changes their phenotype to mesenchymal. Then, the level of Snail or Slug transcription factors increases, as well as other mesenchymal markers, such as: N-cadherin and β-catenin. N-cadherin has mainly structural functions, but it may be cut to intracellular fragments (CTFs) and transport to the nucleus. CTFs may participate in the regulation of the expression of certain genes, e.g. metalloproteinase 9 (MMP9). Our results indicate that MCPIP1 may regulate level of CTFs of N-cadherin which can fulfill important functions other than being just the structural protein.

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HOW TO REMODEL PHOTOSYNTHETIC MEMBRANES WITH CURTs

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Curvature Thylakoid 1 (CURT1) is a family of thylakoid membrane proteins which are the major contributors to the shaping of the chloroplast thylakoid membranes and stabilizing the sharply curved membrane at the grana margin. It is postulated that CURT1 proteins can participate in deetiolation by modulating the geometry of highly packed lipid membranes and by modulating the geometry of highly packed lipid membranes. Protochlorophyllide oxidoreductase (LPOR), an enzyme that carries out the penultimate reaction of the chlorophyll synthesis pathway, is also involved in this process. The aim of our research is to observe the interactions between CURT1 proteins and LPORs.

In *Arabidopsis thaliana*, CURT protein family is represented by four thylakoid membrane-anchored proteins named CURT1A, CURT1B, CURT1C, CURT1D with molecular weights between 11 and 15 kDa. The main isoform, CURT1A is a small (11.2 kDa), integral membrane protein. It has two stroma-facing amphipathic helices linked by two transmembrane helices, which are required for membrane targeting and insertion.

The subject of our study was to examine how CURT1A protein is able to remodel photosynthetic membranes. Using AlphaFold2, we generated plausible protein oligomer models. Based on them, we assume that the protein possibly assembles into oligomers on the membrane leading to the membrane remodelling, thus we want to verify it experimentally. For this purpose CURT1A protein was cloned and its expression optimized in the bacterial system. We cloned the protein using RNA isolated from *Arabidopsis thaliana* WT and then amplified the gene and cloned it into pET15b vector using the Gibson ligation method.

The optimization of the expression of the CURT protein has been performed in *E. coli* expression system and the soluble protein has been successfully isolated.

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UNCOVERING THE MYSTERY OF AT4G25290 – A NOVEL *ARABIDOPSIS THALIANA* PROTEIN

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AT4G25290 is an uncharacterized Arabidopsis thaliana protein which belongs to the cryptochrome/photolyase family (CPF). The common feature of CPF proteins is a photolyase domain which binds two cofactors. Despite structural similarities, cryptochromes and photolyases perform different functions. Photolyases use blue light and UV-A to repair UV-B-induced pyrimidine dimers in DNA in a process known as a photoreactivation. Cryptochromes in plants function as blue light photoreceptors regulating various processes, e.g. circadian rhythm, flowering and phototropism. Within CPF only cryptochromes posses an extended C-terminal domain enabling protein-protein interactions. This extension is also present in AT4G25290, suggesting that this protein may act as a photoreceptor.

AT4G25290 is localized in chloroplasts and consists of two domains: an *N*-terminal photolyase domain and a C-terminal hydrolase domain of an unknown function. The hydrolase domain is homologous to a pheophytinase – a crucial enzyme of chlorophyll degradation pathway during leaf senescence in *A. thaliana*. This allowed to put forward another hypothesis - that AT4G25290 may be involved in a chlorophyll degradation.

The aim of the research is to examine the role of AT4G25290 in light and phytohormone signaling and abiotic stress responses in plants. We also want to determine more precisely the AT4G25290 localization in the cell and how it affects its function(s).

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ActA MOBILE DOMAIN' HEME IS THE ONLY DONOR OF ELECTRONS FOR *aa*$_3$ OXIDASE IN ACIII - *aa*$_3$ SUPERCOMPLEX

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Bioenergetic systems convert energy using diverse proteins, usually assembled into larger complexes. These complexes often form even larger structures, called supercomplexes. The function and mechanisms of action of the supercomplexes are intensely debated. An example of proteins which form supercomplex are *aa*$_3$ oxidase and alternative complex III (ACIII). ACIII is an enzyme that can functionally replace mitochondrial complex III and its role is to oxidize quinone and transfer electrons to *aa*$_3$ oxidase [1].

The molecular structure of ACIII, resolved recently by cryo-EM, revealed a highly intriguing spatial arrangement of cofactors [2]. Especially, the membrane-anchored segment of one of the subunits (**m**obile **d**omain of ActA, mdActA) was suggested to serve as a possible mobile element, functionally connecting ACIII with its partner, *aa*$_3$ oxidase [2, 3].

Our recent work described first genetic system for manipulations within ACIII of *Flavobacterium johnsoniae*, which allows structure – function studies [3]. We used this system to verify the proposed role of mobile domain of ActA. We obtained mutant that lacks mdActA (ΔmdActA) and examined whether and how the absence of mdActA alters the activity of the supercomplex.

The results show that in ΔmdActA mutant the electron transfer to the cytochrome *aa*$_3$ does not occur, and the consumption of oxygen by this strain is residual. This confirmed that mdActA heme is the only donor for electrons for *aa*$_3$ oxidase.

Results of this work help us to define the electron transfer paths connecting ACIII with *aa*$_3$, and provide first insight into the molecular mechanism of action of the supercomplex.

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HOW DOES TOMATO SYNTETHEZE ITS CHLOROPHYLL? FOCUS ON THE LIGHT DEPENDENT STEP

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Our scientific work is focused on light-dependent protochlorophyllide oxidoreductase (LPOR), which uses NADPH to catalyze protochlorophyllide reduction. Light induces its activity, what is an unusual property of enzymes.
Recent studies were conducted on: *A. thaliana*, *Pisum sativum*, *Helianthus annuus*. These plants have different number of isoforms of this enzyme *A. thaliana has three isoforms*, whilst *Pisum sativum* has only one. And that is why the next object of studies is *Solanum lycopersicum*, which similarly to *A. thaliana* has three isoforms out of which one has mutations in the crucial regions of an enzyme. Moreover *S. lycopersicum* is a member of a vital plant family in agriculture. Understanding its biochemistry is important, since it can be useful in its cultivation and future food production improvements.
Our studies are focused on determining the activity and the substrates binding properties of the tomato isoforms using low temperature fluorescence measurements.
Our experimental work began with an RNA isolation, from ethiolated seedlings of tomato; amplification of the genes of interest, which were cloned in expression vectors, using Gibson ligation. In order to achieve the best possible conditions of the expression, optimization was performed.
Spectrophotometrical analysis of substrates binding and the activity of each isoform was performed in the presence and absence of lipids at the temperature of liquid nitrogen, in order to prevent sudden activation of an enzyme by light.
Measurements showed that isoforms differ in the affinity towards NADPH, however the lipids can stimulate this process. In addition, lipids promoted the oligomerization of every isoform.

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INTERACTION OF FERREDOXIN AND CYTOCHROME *b*$_6$*f* STUDIED WITH EPR SPECTROSCOPY

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The problem of possible electron flow pathways during the Cyclic Electron Transfer (CET) process has long been a source of debate in bioenergetics of photosynthesis. It has already been proposed that FNR attached to cytochrome *b*$_6$*f* transfers electrons back from NADPH pool to plastoquinone pool supporting CET. We propose that ferredoxin may also be a key player involved in returning of the electrons during CET. Using the methods of EPR spectroscopy designed to detect protein-protein interactions, we investigated the binding of spin labeled ferredoxin to cytochrome *b*$_6$*f*. Observed change in the CW-EPR spectrum of spin-labeled ferredoxin in presence of cytochrome *b*$_6$*f* suggest that this interaction occurs. This observation was further supported by pulse EPR experiments of relaxation of spin-label. This method is based on detecting the magnetic interactions between a ferredoxin spin-label and a fast-relaxing paramagnetic iron cofactors of cytochrome *b*$_6$*f*. Our findings show a significant increase in spin label relaxation, implying the presence of a stable complex between ferredoxin and cytochrome *b*$_6$*f*.

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STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF INTERACTION BETWEEN RagAB PEPTIDE TRANSPORTER AND TonB

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Porphyromonas gingivalis, a gram-negative, anaerobic and asaccharolytic bacterium, is the major etiological factor of periodontitis, the most prevalent chronic, inflammatory disease in the developed world [1, 2]. *P. gingivalis* requires peptides derived from extracellular proteins for growth [3]. Those peptides are imported through the outer-membrane protein complex, RagAB in a TonB-dependent manner via a 'pedal bin' mechanism [4]. Interestingly, *P. gingivalis* encodes three proteins with homology to TonB. In this project, we identified RagAB-cognate TonB that energizes RagAB and provided structural and functional insights into the interaction between RagAB and TonB. To this aim, we purified C-terminal domains (CTDs) of three TonB proteins and carried out size exclusion chromatography (SEC) of complexes and microscale thermophoresis (MST) to determine the dissociation constants. Using single particle cryo-electron microscopy (cryoEM) we obtained structural data showing dynamics of the interaction between RagAB and cognate TonB. Finally, we performed pull-down assays with membrane fraction of *P. gingivalis* and purified TonBs to determine the interaction network between different TonBs and TonB-dependent transporters (TBDTs). Since the interaction of RagAB with TonB is indispensable for the uptake of nutritional peptides by *P. gingivalis*, the data obtained in this work can serve in the future to develop potential therapeutic compounds inhibiting the growth of *P. gingivalis* and thus attenuating *P. gingivalis*-related diseases.

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THE COMPLICATED RELATION BETWEEN LIGHT DEPENDENTPROTOCHLOROPHYLLIDE OXIDOREDUCTASE IN *SYNECHOCYSTIS* AND THE LIPIDS

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Chlorophyll is an inherent component of photosynthesis, one of the most important processes carried out by plants and other photosynthetic organisms. One of the enzymes involved in the synthesis of this pigment is protochlorophyllide oxidoreductase, which reduces protochlorophyllide to chlorophyllide. We became interested in the light-dependent form of this enzyme (light-dependent protochlorophyllide oxidoreductase, abbreviated as LPOR) in *Synechocystis* bacteria. We asked ourselves how LPOR binds substrates and releases products. It is known that NADPH is the coenzyme of LPOR, but the effect of lipids has not been fully understood. By measuring low temperature fluorescence spectra, we investigated the effects of some lipids and their mixtures on the activity of the enzyme and the reagents binding. By comparing the spectra, we noticed that the protein binds the substrates the most strongly outside the membrane, but the presence of the membrane promotes the products release. Moreover, the oligomerization properties of the enzyme were also investigated with the use of electron microscopy. Possible biological consequences of the findings are discussed.

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DETERMINATION OF CELLULAR FUNCTIONS AND LOCALIZATION OF N4BP1 PROTEIN AND IDENTIFICATION ITS BINDING PARTNERS

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Nedd4 Binding Protein 1 (N4BP1) is an RNase that consists of K-homology domain (*KH domain*), NYN (N4BP1, *YacP-like Nuclease domain*) domain and CoCUN (*Cousin of CUBAN*) domain. N4BP1 was proved to be an essential factor in immunological response, signal transduction to NFκB and degradation of viral mRNA.

The aim of this research was the identification of binding partners of human N4BP1. We assessed the role of N4BP1 complexes in cells by determination of cellular localization of this protein and verification of the impact of stress conditions on N4BP1 levels.

Plasmids coding for sfGFP and Fc-IgG1-tagged versions of N4BP1 were generated. These vectors were used for transfection of HEK293 cells followed by immunoprecipitation. The samples were analyzed with mass spectrometry to identify N4BP1 binding partners. Cellular localization of N4BP1 was determined using sfGFP-tagged variant of N4BP1 and fluorescence microscopy. Western blot was used to measure N4BP1 protein level after sodium arsenite stimulation.

The mass spectrometry revealed numerous N4BP1 binding protein partners. Some of them are non-coding RNA-binding proteins which means that the role of N4BP1 is ncRNA processing. Overexpressed sfGFP-tagged N4BP1 is localized in granular structures of the cytoplasm as part of stress granules or processing bodies complexes. Under stress conditions induced by $NaAsO_2$ N4BP1 is effectively degraded.

In conclusion, our results shed a new light on the RNase activity of N4BP1 by revealing its interaction partners important in binding of mRNA and ncRNA.

ANALYSIS OF PARACRINE SIGNALLING BETWEEN MESENCHYMAL AND SENESCENT ENDOTHELIAL CELLS

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Senescent cells accumulate with age and are implicated in age-related pathologies including progression of atherosclerosis [1]. Besides losing their function and ability to divide, senescentcells induce inflammation and impact surrounding cells in a paracrine manner.
The aim of this study was to investigate the interaction and paracrine communication between senescent human aortic endothelial cells (sHAEC) and human adventitial cells (hAdv) in an in vitro model of oxidative stress-induced vascular senescence.
Senescence of HAEC was induced via exposure to hydrogen peroxide. To investigate the paracrine effects of these cells, hAdv cells (shown before as drivers of tissue fibrosis and vascular calcification [2]) were stimulated with conditioned medium (CM) from sHAEC. Mass spectrometry analysis was applied to analyse the proteome of both cell types.
InsHAEC, among the 66 upregulated proteins, we identified proteins involved in oxidative-stress response (HMOX1, GSTK1) and cell-cell interaction (CD44, ITA5, TSP1). The most highly upregulated in sHAEC was growth differentiation factor 15 (GDF-15) – serum cytokine with predictive value for cardiovascular diseases, known to impair autophagy and lipid homeostasis [3]. As a secreted protein, GDF-15 may affect cells in a paracrine manner. Interestingly, in hAdv cells stimulated with CM from sHAECs, we observed upregulation of autophagy-related SQSTM1 protein and proteins involved in lipid binding and metabolism (APOL2, LDLR, APOB).
In summary, senescent endothelial cells intensively communicate and exert paracrine effects on neighboring mesenchymal cells, what may lead to changes in metabolism and survival of the later ones and promote the development of vascular pathologies.

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ANALYSIS OF THE ABILITY OF THE KHNYN PROTEIN TO FORM DROPLETS AS A RESULT OF LIQUID-LIQUID PHASE SEPARATION

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KHNYN (KH and NYN domain-containing protein) is the most enigmatic protein belonging to the family of proteins containing the characteristic NYN domain with RNase activity. Few studies devoted to this protein indicate its involvement in the defense of cells against viruses [1]. Our research suggests that KHNYN may be a novel regulator of cellular stress response. Our experiments show that the KHNYN protein creates structures in cells that morphologically resemble stress granules. However, the granules formed by the KHNYN protein do not colocalize with SG and do not contain the G3BP1 protein - a key marker of these non-enveloped organelles. Our observations allow us to hypothesize that the KHNYN protein participates in the formation of a new, so far undescribed class of membraneless cytoplasmic organelles.
The aim of the research is to raise a completely unexplored issue, which is the formation of granules by the KHNYN protein, and to verify whether these structures are formed as a result of liquid-liquid phase separation (LLPS) - a phenomenon responsible for the formation of membraneless organelles in cells. This research will also investigate the role of the NYN domain on the process of granule formation by the KHNYN protein in vitro and check whether, similar to the formation of SG and G3BP1 protein droplets, the formation of KHNYN granules depends on the presence of RNA.

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REGULATION OF SIGNALING PATHWAYS BY ZC3H12B

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The ZC3H12/MCPIP family of proteins consists of 4 members, called ZC3H12A-D/ MCPIP1-4. All of them have a highly conservative NYN/PIN domain and a CCCH zinc finger in their structure [1]. The most studied member of this family is the protein ZC3H12A (MCPIP1), which plays an important role in the regulation of inflammation. The control of inflammatory processes by ZC3H12A takes place at many levels, including the degradation of pro-inflammatory cytokine transcripts and protein deubiquitination resulting in modulation of signal transduction pathways [1,2]. Despite limited information on the remaining ZC3H12 proteins, studies published so far indicate that they also have RNase activity and are involved in inflammation control. For ZC3H12C and ZC3H12D, their role in the regulation of the activation of signal pathways was also demonstrated. So far, no studies focused on the role of the ZC3H12B protein in the regulation of signal transmission have emerged [3,4].

Taking into account the high homology in the structure of the NYN/PIN domain and the zinc finger domain between proteins from the ZC3H12 family and the similarity in the regulated pathways signaling and proinflammatory transcripts, we hypothesized that one of the biological roles ZC3H12B protein is the regulation of signal transmission in the cell. The research carried out in our laboratory shows that ZC3H12B regulates the NFκB signal transmission path, but does not affect the activation of the AP-1 pathway. Additionally, we have observed a decrease in global protein ubiquitination in wild-type ZC3H12B overexpressing cells.

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IL-1β - DEPENDENT REGULATION OF ZC3H12B ACTIVITY

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ZC3H12B is the most enigmatic member of the ZC3H12 protein family. These proteins are primarily involved in maintaining the homeostasis of the immune system [1,2]. We have shown that ZC3H12B is involved in the regulation of inflammation through the degradation of pro-inflammatory transcripts (eg IL-6). Interestingly, overexpression of ZC3H12B inhibits proliferation, and the level of ZC3H12B increases during cell differentiation [3]. The latest studies show that a decrease in the ZC3H12B mRNA level may promote the development of colon cancer [4]. Data from publicly available databases and our experiments demonstrate significant expression of ZC3H12B in the brain [5]. The Oncopression database also shows that the ZC3H12B level is reduced in brain tumors (gliomas) compared to healthy tissue. These data and our preliminary studies suggest that ZC3H12B may play an important role in the regulation of inflammation and central nervous system (CNS) neoplastic processes.
To better understand the biological role of ZC3H12B, we investigated how its activity is regulated at the protein level. Our studies show that in cells of nervous origin (U251-MG) with inducible overexpression of ZC3H12B, stimulation with the pro-inflammatory cytokine IL-1β reduces the level of the investigated protein. Using the Western Blot technique and its modification (Phos-Tag), we discovered that ZC3H12B undergoes post-translational modification (phosphorylation), ubiquitination, and proteasomal degradation. We also determined which pathway is involved in the observed effect and narrowed down modified residue to threonine.

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DETERMINING THE ROLE OF FUNGAL ADHESIN ON INTERACTION OF *CANDIDA ALBICANS* WITH HUMAN LUNG EPITHELIAL CELLS

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Candida albicans, fungal pathogen living on human mucosa, has considerable ability to form biofilms – well-organized structures connecting cells with different geno- and phenotypes. The first step of biofilm formation is the adherence of fungal cells to artificial or natural surfaces. Specific proteins, embedded in the membrane or associated with the microbial cell surface, are involved in adhesion. One of the important fungal adhesins is Mp65 - mannoprotein that possesses enzymatic activity of β-glucosidase, and is involved in the rebuilding of candidal cell wall during phenotypic switching.

Presented studies concern the contribution of Mp65 in the effective binding of *C. albicans* to human lung epithelial cells BEAS-2B. Mp65, purified from the supernatant of *Candida* liquid culture was tested for interaction with lung cells, as well as with *C. albicans* cells. Both were identified as a good target for Mp65 binding, suggesting that Mp65 might play the function of linking protein between both cell types. To verify the proper role of Mp65 in the adhesion process, its binding properties were also analyzed in the competition test between both cell surfaces. Obtained results confirmed the significant contribution of this protein in the modulation of *C. albicans* interactions with lung epithelia and its possible role in the development of aspiration pneumonia as a consequence of oral fungal infection.

The study of possible mechanisms involved in the interaction between fungal pathogen and human host is important for a better understanding of infection development and may result in the improvement of treatment for fungal infectious diseases.

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PROTEIN EXCHANGE WITHOUT COMMISSION

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Porphyromonas gingivalis is gram-negative bacteria from *Bacteroidetes* phylum and it is regarded as a keystone pathogen of periodontal diseases. Protein secretion in *P. gingivalis* may take several routes of which the most important for its virulence leads through Type IX Secretion System (T9SS). However, before virulence factors pass through the periplasm and outer membrane with T9SS complex they need to cross inner membrane via Sec translocon where subsequently the N-terminal signal peptide is cleaved off by the signal peptidase I (SPI). A cargo protein between those two translocons encounters a certain enzyme: periplasmic but IM embed, small but essential for survival that modifies cargos newly formed N-terminus- the glutamyl cyclase (QC).

QC enzymes transform N-terminal glutamine residue in polypeptides to pyroglutamate and ammonia. Generally, we recognize two evolutionary not related classes of this enzyme: animal- and plant-type, which differ in protein fold, active site architecture and mechanism of catalysis. In *P. gingivalis* (carrying the animal type version) above 60% of SPI substrate proteins are potential targets for this modification, with the most potent virulence factor between them [1]. Taking together above information – *P. gingivalis* QC appears to be an interesting subject of research. In presented work we challenged *P. gingivalis* cell by exchanging its PgQC enzyme for other animal and plant-type QC from different *Bacteroidetes.*

In conclusion, the research course undertaken proved to be a valuable tool in deciphering the function of otherwise essential enzyme that could not be mutated with other standard procedures. We believe, the acquired knowledge could be used to invent a novel, innovative inhibitor that will attenuate virulence of *P. gingivalis*.

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ENOLASE ON THE *CANDIDA ALBICANS* CELL WALL AS A RECEPTOR FOR HUMAN KININOGEN: MAPPING THE BINDING SITES

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Human kininogen can adhere to *Candida albicans* cell wall, followed by the adsorption of other components of plasma kinin-release system. Kinins – bradykinin-related peptides – contribute to the development of candidal infection (candidiasis). The biological significance of kininogen interaction with putative receptors on fungal surface cannot be fully appreciated until the underlying molecular mechanisms are characterized. In the present study, we showed a "moonlighting" protein - enolase - to bind high molecular weight kininogen (HK) at the candidal cell surface. We characterized the enolase-binding ability of various parts of HK molecule and showed that the protein utilizes the light chain for enolase attachment. Subsequently, using synthetic peptides, we identified the specific sequences on the C-terminal part of domain 5 (aa 457-518) and the N-terminal part of domain 6 (aa 519-564) that participate in HK binding to enolase. Using a chemical crosslinking method, the specific sequence on enolase molecule (aa 336-349) was found to be directly involved in the interaction with HK.

EFFECT OF TENPN TOXIN ON THE GROWTH OF PATHOGENIC BACTERIA

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A large group of pathogenic bacteria possesses in their genomes operons of toxin-antitoxin systems (TAs). All types of TAs consist of a stable toxin and an unstable antitoxin [1]. Many of the described toxins exhibit endoribonucleolytic activity, including those from type III TAs. One of the characteristic features of the type III TAs genetic module is the presence of a few short tandem repeats upstream of the gene coding for the toxin protein. Upon transcription, these repeats become an RNA substrate trap for the toxin and thus play the role of the antitoxin [2]. Using molecular biology techniques, we cloned a sequence coding for type III TenpN toxin from *Staphylococcus aureus* into pBAD plasmid and transfer the construct to *Escherichia coli*. Subsequently, we induced the expression of the cloned gene and checked what impact it had on the growth of *E. coli* cultures. We observed a decrease in the growth rate of bacteria in the presence of TenpN toxin, and we confirmed overproduction of the recombinant toxin protein. The next step in our research is to examine the effect of TenpN toxin on the growth of *S. aureus*, which will broaden the knowledge about the type III TAs in staphylococci. Moreover, the aim of further research is also the determination of the mechanism of TenpN toxicity both *in vitro* and *in vivo*. That will help to explore possible uses of the toxin as an inhibitor of pathogenic bacteria growth.

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INTERACTION OF *CANDIDA ALBICANS* AND *CANDIDA TROPICALIS* PHOSPHOGLYCERATE MUTASE WITH HUMAN HIGH MOLECULAR WEIGHT KININOGEN

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The cell wall of *Candida* yeast-like fungi contains multiple proteinaceous components. Some of them are considered as atypical because they are loosely, non-covalently associated with the cell surface. These proteins emerge from the cytoplasm via poorly recognized transport mechanisms and at the cell surface acquire new functions. Our present study was focused on an example protein from this group – phosphoglycerate mutase (Gpm1) of *Candida albicans* and *Candida tropicalis* that inside the cell catalyzes the eighth step in the glycolysis pathway but also occurs on the pathogen surface and is probably involved in the adhesion to human host tissues and cells [1,2]. Hereby, we characterized the interaction between candidal Gpm1 and a human plasma protein involved in the blood coagulation and kinin generation cascades – the high-molecular weight kininogen (HMWK).

The recombinant Gpm1 protein was biotinylated and then the saturation binding assay with microplate-immobilized HMWK was performed. The identification of HMWK sequence motifs involved in the interaction with Gpm1 was performed by HMWK-peptide/Gpm1 displacement experiments. The effect of Gpm1 on the release of bradykinin from HMWK by prekallikrein and factor XIIa was analyzed by SDS-PAGE electrophoresis.

Our experiments showed that Gpm1 interact with HMWK. Mapping the interaction sites on HMWK indicated domain 3 and 6 as the major interaction spots for Gpm1 of both species. However, Gpm1 did not affect the kallikrein-dependent bradykinin release on the pathogen surface.

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EFFECTS OF CYANOBACTERIAL TOXIN CYLINDROSPERMOPSIN AND ITS DECOMPOSITION PRODUCTS ON ANTIOXIDANT PROPERTIES OF GLUTATHIONE

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Cylindrospermopsin (CYN) is cytotoxic alkaloid produced by several species of cyanobacteria. Its exposure to animal cells results in many pathological changes but the underlying molecular mechanism(s) of this bioactivity are not fully understood. Some hypotheses are that the harmful effect caused by CYN is related to disruption of glutathione (GSH) metabolism. In this study, we investigated the impact of CYN and its decomposition products on GSH antioxidant properties. The obtained results suggest that both CYN and its byproducts did not affect in vitro GSH activity against free radicals. This study is the first description of the impact of CYN and its decomposition products on GSH in direct reaction, and provides new, valuable information that shines a new light on cylindrospermopsin-glutathione interactions

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SEARCHING FOR MOLECULAR TARGET(S) OF AC27 COMPOUND

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Diabetes is a public health problem that has reached epidemic proportions on a global scale. β-cell dysfunction and insulin resistance are interrelated defects in the pathophysiology of diabetes. Regeneration of β-cell mass is an ultimate goal yet to be achieved. Our previous study of small-molecule inhibitors of DYRK1A kinase provides and validates a proof-of-concept that β-cell proliferation via kinase inhibition is achievable, especially after treatment with our best inhibitor, AC27. Therefore, a comprehensive characterization of its other targets (i.e., are there any other mechanisms of action beyond DYRK1A targeting?) that influence the βcell function is important. Herein, we want to use proteome profiling to deconvolution possible targets of AC27 to fully understand its mechanisms of action. We try to synthesize an innovative, photoaffinity AC27 - based probe consisting of a click chemistry probe skeleton for target binding and enrichment and a photoaffinity group to fix the binding between the probe and the targets. Next, AC27-targets will be fished and identified in cell-based assays: β-cells (MIN6, INS1E, iPSC-derived β-cell-islets) will be incubated with the AC27-based probe to crosslink the probe with the targets covalently. After biotin attachment through a click reaction and the subsequent enrichment with streptavidin beads, the target proteins will be identified with a quantitative proteomics approach with mass spectrometry (MS), based on protein sequence and bioinformatics. We believe that uncovered targets can be used as a proteomic signature resource for further analyses of the effects of AC27 in diabetes and furnish invaluable mechanistic information on AC27. Thus, the proposed approach can facilitate drug discovery efforts in diabetes treatment.

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PANCREATIC DUCTAL ADENOCARCINOMA (PDAC) IN MULTI-MODULE THERAPY BASED ON GEMCITABINE WITH HYPERTHERMIA IN COMBINATION WITH GOLD NANORODS OR CALCITRIOL

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Pancreatic cancer is a highly lethal disease with a fatal prognosis. Current treatment strategies are unsatisfying, and new drugs are not guaranteed success. One of the proposed solutions to this problem is combination therapy. The first of the approaches is the multi-module therapy, based on traditional chemotherapeutic agent gemcitabine with combines mild contact hyperthermia <42°C and calcitriol. The second approach is using gemcitabine in combination with gold nanorods, designed to achieve mild hyperthermia. Nanoconjugate was characterized by DLS, FT-IR, XRF, and HPLC techniques.

The study aimed to compare chemotherapy in combination with contact hyperthermia and active form of vitamin D or hyperthermia with the new theranostics compound on in vitro PDAC model.

In vitro studies on the PANC-1 and PAN 02 pancreatic cancer cells were performed. The cells were heated to 41˚C for 20 minutes (contact hyperthermia) and treated with gemcitabine and calcitriol. The cells were irradiated for 10 minutes using a near-infrared light source in the second approach. MTT tests characterized the cellular response and toxicity. Long-term in vitro observation of cells was performed with the fluorescence microscope.

The results showed that the combination of gemcitabine with calcitriol and hyperthermia reduces cancer cells number and metabolic activity than using the drug alone.
The nanoconjugate and gemcitabine can increase toxicity and reduce the level of metabolic activity of cells. Multimodal treatment can lead to better anti-cancer effects than chemotherapeutic alone, offering new hope for future studies.

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PRELIMINARY BIOCHEMICAL CHARACTERIZATION OF COLTSFOOT (*TUSSILAGO FARFARA* L.) AND TURK'S CAP LILY (*LILIUM MARTAGON* L.) CALLUS CULTURE

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In botany, callus plant cultures are extensively used model systems, because of their regenerative potential, including the ability to organogenesis and somatic embryogenesis. Also, callus cultures offer a wide range of applications in horticulture, agriculture and pharmacy. Both the dicotyledonous plant belonging to the *Asteraceae* family - Coltsfoot (*Tussilago farfara* L.), and monocotyledonous Turk's cap lily (*Lilium martagon* L.), belonging to the *Liliaceae* family are species growing in wild in Eurasia and are known as traditional medicinal plants.

In our study, we analyzed the callus growth kinetics and maximal quantum efficiency of photosystem II (Fv/Fm) in callus cultures raised from coltsfoot and lily. Additionally, an accumulation of photosynthetic pigments, anthocyanins and total proteins were tested. Callus was grown on MS medium supplemented with 3% sucrose and hormones (2 mg/L IAA plus 2mg/L BAP for *T. farfara and* 2 mg/L Picloram plus 2 mg/L BAP for *L. martagon*) up to 5 weeks at 23°C, in the darkness, both on agar and in suspension cultures.

The results demonstrated faster growth of callus *T. farfara* compared with *L. martagon* on solid media. However, suspension cultures resulted in higher biomass accumulation of lily callus. In both callus lines Fv/Fm and chlorophyll concentration were found to be below the detectability level. The observed accumulations of carotenoids and anthocyanins were very low and the total protein level was higher in lily callus line, as compared to coltsfoot callus.

The obtained quantities of tissue are sufficient for identification of active metabolites.

PRIMARY CELL WALL HEMICELLULOSES OF *PICEA ABIES* - SYNTHESIS AND STRUCTURE

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Plant primary cell wall is important for plant growth and development and is composed predominantly of cellulose, hemicelluloses and pectins. Hemicelluloses are highly branched plant cell wall polysaccharydes, with a main chain linked by β-1,4-glycosidic bonds. The detailed structure of hemicelluloses varies between different plant species, tissues and cell types. Hemicelluloses strengthen plant cell walls by cross-linking cellulose microfibrils. Despite their significant role in plant cell walls, their biochemical structure remains not fully understood. Here I investigated the presence of hemicellulose biosynthetic pathways in a genome of a model gymnosperm species *Picea abies* and analysed the structure of hemicelluloses in *P. abies* and *Arabidopsis thaliana* cell walls. Using molecular phylogenetics, I was able to identify putative synthases of two hemicelluloses: galactoglucomannan (GGM) and xyloglucan in the genome of *P. abies*. Moreover, I performed acidic and enzymatic hydrolysis of spruce callus primary cell walls and then analysed the products using High Performance Liquid Chromatography (HPLC) and Polysaccharide Analysis through Carbohydrate gel Electrophoresis (PACE). My results indicate that primary walls of *P. abies* contain more xyloglucan than GGM. Additionally, a greater amount of GGM was observed in the secondary rather than in the primary cell walls of spruce. Further analysis of the gymnosperm cell wall structure will enable optimization of wood processing, and will significantly faciliate further research in conifer developmental biology.

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EXAMINING BIOLOGICAL PROPERTIES OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM/ STROMAL CELLS OBTAINED FROM HEALTHY AND DIABETIC DONORS

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Mesenchymal stem/ stromal cells (MSCs) represent one of the most extensively investigated adult stem cell population due to their pro-regenerative activity observed following their application into injured tissues. However, it has been shown that physiological and clinical status of human donor or a type of source material for MSC isolation could affect their biological properties.

Human adipose tissue- derived MSCs (AT-MSCs) obtained from healthy donors and patients suffering with diabetes type 2 (T2D) underwent comparable biological characterisation including assessment of: morphology, viability in various conditions, proliferation rate, antigenic profile as well as trilineage differentiation potential *in vitro*. In our study, three types of cell culture media (CM) were used: i) control - standard CM, ii) standard CM containing high glucose concentration (diabetic conditions), or iii) standard CM containing high glucose concentration and addition of insulin (insulin- treated diabetic conditions).

In our preliminary study, we observed no significant differences in morphology, viability, proliferation rate as well as expression of positive (CD90, CD105) and negative (CD19, CD45) MSC markers between AT-MSCs derived from healthy and T2D donors in the tested media. In case of chondrogenic and osteogenic differentiation, we also did not observe any significant differences in expression of genes related to each differentiation pathway at diffrent time points (3, 7, 14, or 21 days of differentiation). However, AT-MSCs derived from T2D donors exhibited significant decrease in adipogenic genes expression along with similar aggregation of lipid droplets observed after histochemical staining, when compared to healthy donors.

To summarise, AT-MSCs obtained from healthy and T2D donors exhibited similar biological properties including differentiation capacity and as such may be considered for future use in autologous applications in human patients.

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BREAST CANCER RADIOSENSITIZATION USING OXYGEN MICROBUBBLES AND METABOLIC EFFECTS

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Hypoxia is a major determinant of human tumor sensitivity to radiation therapy. Ultrasound sensitive microbubbles filled with oxygen (OMB) act as a tissue radiosensitizer. Oxygen from microbubbles can be released locally by an ultrasound impulse, leading to a controlled, local increase in pO_2. The aim of this study was to investigate synergistic action of metformin and OMB and the impact of this drug combination on radiotherapy effectiveness.

Anionic pegylated microbubbles composed of DSPC and DSPE-PEG2000 were used. Cavitation of microbubbles was performed with 2W/cm^2 ultrasound impulse (Vevo SoniGene). In vivo EPR oximetry was conducted with L-band CW spectrometer (Bruker Elexsys-II E540) in a 4T1 tumors growing in a mammary fat pad of Balb/c mice (N = 120). Mice were divided into four groups: control, treated with OMB (iv, twice a week) and metformin (ip, 350 mg/kg, daily) alone and treated with both OMB and metformin. Temporal changes in tumor oxygenation were measured twice a week with OxyChip as an oxygen sensor. Tumor growth and vasculature were monitored twice a week with the use of ultrasonography (Vevo 2100). Radiotherapy (12 Gy, gamma rays) was carried out in the therapeutic window of combined therapy.

Two weeks of combined therapy resulted in therapeutic window with higher oxygenation and normalized vasculature. We observed the slowdown of tumor growth after radiotherapy in all treated groups, however the biggest impact was seen in group pretreated with combined therapy. Treatment with metformin and oxygen microbubbles effectively increase the oxygenation of tumors and sensitize them to radiotherapy.

THE ROLE OF MONOCYTE CHEMOATTRACTANT PROTEIN-INDUCED PROTEIN 1 IN LIVER FIBROSIS AND ACTIVATION OF HEPATIC STELLATE CELLS

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Monocyte chemoattractant protein-induced protein 1 (MCPIP1) negatively regulates inflammation by, inter alia, cleaving transcripts coding for proinflammatory cytokines. Overexpression of Mcpip1 reduces liver injury in septic mice, by inhibition of inflammatory reaction. Moreover, mice $Mcpip1^{fl/fl}Alb^{Cre}$ lacking Mcpip1 in epithelial liver cells develop liver fibrosis. In this study, we analyzed Mcpip1 level in hepatic cells isolated from CCl_4-treated mice and in human fibrotic livers. We also examined MCPIP1 impact on activation of LX-2 human hepatic stellate cell line.

We analyzed liver biopsies isolated from 30 patients with fibrosis stage ranging from 0 to 4. Moreover, we induced liver fibrosis in mice during *in vivo* experiments, by injections with CCl_4 dissolved in corn oil (0,2 mg/g), 3 times a week for 4 weeks. To examine process of liver fibrosis *in vitro*, we treated LX-2 cells with TGF-β (5 or 10 ng/ml).

Analysis of liver biopsies revealed that MCPIP1 level in liver tissue correlates with the severity of fibrosis. Treatment with CCl_4 induced Mcpip1 expression in hepatocytes and hepatic stellate cells. Stimulation of LX-2 cell line with TGF-β resulted in increase of MCPIP1 level. Additionally, overexpression of MCPIP1 in LX-2 cells led to decreased mRNA level of HSCs activation markers. Contrary, MCPIP1 silencing in LX-2 cells resulted in their increased activation status.

Our results indicate a potent MCPIP1 role in liver fibrosis and activation of HSCs. MCPIP1 presence in HSCs can be essential to prevent their excessive activation.

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THE ROLE OF RETINOIDS IN PANCREATIC STELLATE CELLS

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Pancreatic fibrosis, one of the most severe disorders of the pancreas, is a main feature of pancreatic cancer or chronic pancreatitis (CP). Persistent damage of the pancreas associated with CP leads to the autonomous repair reaction, in which activated pancreatic stellate cells (PSCs) play a central role. Transition of PSCs from a fibroblast-like to myofibroblast-like phenotype and excessive deposition of extracellular matrix components is the main cellular mechanism underlying pancreatic fibrosis. While quiescent PSCs are well known for their ability to store retinol and its metabolites, activation of these cells is corelated with loss of retinoids and increased expression of α-smooth muscle actin (α-SMA). Consequently, treatment with retinol and all-trans retinoic acid (ATRA) was shown to be associated with decreased activation of rat PSCs. The aim of this study was to investigate the effect of retinol and ATRA on the phenotypic transition of human PSCs (hPSCs) and determine whether these retinoids influence cell signalling, particularly Ca^{2+} homeostasis. The presence of retinoids was demonstrated in hPSCs by fluorescence imaging and quantitative measurements by high-performance liquid chromatography (HPLC). Incubation of hPSCs with retinol or ATRA substantially reduced TGF-β-induced activation of hPSCs evidenced by decreased expression of α-SMA. In order to assess the effects of retinoids on Ca^{2+} homeostasis, Ca^{2+} signals in control and retinoid-treated hPSCs were investigated by fluorescence real-time imaging, and the expression of selected Ca^{2+}-handling proteins was assessed in hPSCs. Our findings shed a new light on the mechanisms by which retinoids regulate PSC physiology, which involve intracellular Ca^{2+} signalling.

THE LACK OF RNase ACTIVITY OF MCPIP1 INCREASES MIGRATION OF CLEAR CELL RENAL CELL CARCINOMA CELLS BY INFLUENCING ON Rho GTPase, IL1β AND FOCAL ADHESION KINASE

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The MCPIP1 protein encoded by the *ZC3H12a* gene is distinguished by the presence of an RNase domain and a single zinc finger of the CCCH type. MCPIP1 acts mainly as an endonuclease that degrades the mRNA of pro-inflammatory cytokines. Activity MCPIP1 plays a role during the process of tumorigenesis by regulating the viability, proliferation, and apoptosis of tumor cells. Our recent study revealed that MCPIP1 may act as a tumor suppressor that prevents EMT by stabilizing Wnt inhibitors and decreasing the levels of active β-catenin and EMT inducers.
In this study, we investigated how MCPIP1 affects ccRCC cell migration. We showed that MCPIP1 prevents morphological transformation and drastically reduces the migration of ccRCC cells. MCPIP1 decreases the levels of Rho GTPases and reduces the phosphorylation of FAK and Src and an increase in migration activity. Moreover, we observed increased expression of IL1β in ccRCC cells and tumors lacking MCPIP1 RNase activity. Increased levels of IL1β may explain the FAK and Rho GTPase activation and consequent increased migration observed for cells expressing the mutant form of MCPIP1. Additionally, microarray analysis of tissues from patients with ccRCC revealed changes in the expression of several genes correlated with migration as tumor progression occurred.
This study indicates an important role of MCPIP1 as a regulator of migratory potential and invasiveness in ccRCC.

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PHLDA1 SILENCING AFFECTS TYROSINE KINASES PATHWAYS IN HUMAN NEUROBLASTOMA

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Neuroblastoma, derived from neural crest cells, is the most common solid extracranial tumor in children. One of the treatment methods of minimal residual disease of high-risk neuroblastoma patients is an immunotherapy based on chimeric anti-GD2 ganglioside antibodies (ch14.18/CHO). Previously performed studies demonstrated that PHLDA1 protein (pleckstrin homology-like domain family A member 1) was strongly increased in IMR-32 cells treated with anti-GD2 ganglioside antibody (14G2a), which was correlated with decreasing viability of the cells [1].

shRNA interference (obtained via plasmid transfection) was performed by us to better understand PHLDA1 function in neuroblastoma. *PHLDA1*-silenced cells displayed changes in morphology and differentiation-like phenotype. Moreover, western blot analysis revealed that downregulation of PHLDA1 led to increasing EGFR level and decreasing CaMKII, MCPIP1, IR, pro-IGF-1R and IGF-1R levels, while the level of pro-IR was constant in control and *PHLDA1*-silenced cells. Additionally, in this study combination treatments with anti-GD2 antibody (ch14.18/CHO) and EGFR inhibitors (gefitinib and lapatinib) were performed. Ch14.18/CHO, gefitinib and lapatinib separately affect IMR-32 cells in a dose dependent manner, however, combined treatment enhanced the cytotoxic effect of both agents (gefitinib and ch14.18/CHO) on IMR-32 cells *in vitro*.

Obtained results will contribute to better understanding the role of PHLDA1 and tyrosine kinases in neuroblastoma tumor and may help with future therapy development.

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THE FUNCTIONAL PROPERTIES OF EXTRACELLULAR VESICLES OF TWO DIFFERENT *CANDIDA ALBICANS* STRAINS

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Candida albicans is one of the microorganisms belonging to the physiological microbiota in healthy humans. Impairment of the human immune system may lead to the excessive growth of this yeast-like fungus, in consequence resulting in initiation of both, relatively mild superficial fungal infections or life-threatening systemic candidiases. In the process of candidal pathogenesis various mechanisms are involved, including biofilm formation, production of adhesins and hydrolytic enzymes or formation and release of the extracellular vesicles. The last ones are a nanometer-sized membranous spherical structures transferring different virulence factors, including proteins and nucleic acids. Due to their diversified cargo, they can have a significant influence on the microbial community as well as on the host organism during infection.
In the present work, the response of cells THP-1 from human leukemia monocytic cell line was investigated after their stimulation with extracellular vesicles released by biofilm structures formed by two strains of *C. albicans* – *C. albicans* strain ATCC ® 10231 ™ and *C. albicans* strain SC5413. Therefore, the production of proinflammatory cytokine tumor necrosis factor α (TNF-α), chemoattractant cytokine IL-8 and anti-inflammatory cytokine IL-10 by human cells was tested at different time points. The level of cytokines was increasing in time, TNF-α and IL-10 levels were approximate for EVs from both *C. albicans* strains. The release of IL-8 was higher after stimulating THP-1 cells with EVs from *C. albicans* strain ATCC ® 10231™.
Moreover, the presence of biofilm-derived EVs during first stage of biofilm formation by *C. albicans* affects the biofilm thickness and dispersal ability and was found to be advantageous for fungi in the presence of the antifungal drug caspofungin.

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A METABOLIC SHIFT IN NEUTROPHILS LACKING DGAT1 INHIBITS THEIR PROINFLAMMATORY ACTIVITY

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Immune cells have high functional plasticity since they can reprogram diverse metabolic pathways according to the different immunological environments. Neutrophils participate in the immune response and are the first responders during inflammation. Earlier studies suggested that neutrophils were committed to simple metabolic pathways, however, recent advances revealed that neutrophils use diverse metabolic pathways [1].

Among leukocytes, mouse neutrophils have the highest expression of the acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) gene. DGAT enzymes catalyze the synthesis of triglycerides - the major form of stored energy and a source of glycerol, which can be used in glycolysis. Additionally, DGAT1 participates in the conversion of retinoic acid - a potent signaling molecule, involved in the regulation of a wide range of physiological processes.

Since DGAT1 regulates the homeostasis of lipids and retinoids, we hypothesized that lack of DGAT1 may lead to changes in standard metabolic pathways and inhibition of proinflammatory activity of neutrophils. To verify this hypothesis, we analyzed the glycolytic rate and mitochondrial respiration of neutrophils isolated from WT and Dgat1KO. Neutrophils lacking DGAT1 showed significantly higher glycolytic capacity, but no differences in basal mitochondrial respiration. In chemotaxis assay and a mouse model of psoriasis, we observed a significantly lower number of Dgat1KO neutrophils. Since it is known that the energy necessary for chemotaxis comes mainly from mitochondrial respiration, we activated neutrophils and measured oxygen consumption during an oxidative burst. In this test, we showed that neutrophils from Dgat1KO mice consume even 2-fold less oxygen to produce ROS, than neutrophils from WT mice.

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THE ROLE OF PROTON PUMPS IN PHOTOTROPIN SIGNALING LEADING TO CHLOROPLAST MOVEMENT RESPONSES

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Concentration of protons inside plant cells is controlled by plasma membrane proton pumps (H^+-ATPases). Transport of protons out of the cell creates an electrochemical gradient used in basic physiological processes such as pH regulation or transport of ions and metabolites. Proton gradients are also important during response to stress conditions e.g. salt stress. *Arabidopsis thaliana* has 11 genes encoding H^+-ATPases - AHA1-11. H^+-ATPases are involved in signal transduction pathways in blue light-regulated processes: stomatal opening, leaf movement and phototropism [1]. Proton pumps are likely to be involved in other processes controlled by phototropins. Among processes, that may be affected by the activity of proton pumps, are blue light-induced chloroplast movements. This hypothesis is supported by the fact that H^+-ATPase inhibitors have been shown to inhibit the blue light controlled chloroplast relocation in the mesophyll of two higher plant species [2].
The *A. thaliana* T-DNA insertion mutant of the *aha1* gene was used to determine the involvement of proton pumps in light signals in chloroplast movements. Transient responses of chloroplasts to light pulses were measured using a photometric assay [3]. The results may suggest AHA1 H^+-ATPase involvement in in blue light signaling associated with chloroplast movements. Chloroplast movement responses are slightly reduced but not completely stopped in the absence of AHA1 H^+-ATPase. This indicates that the pumps encoded by the *AHA* gene family are likely to be functionally redundant.

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DIFFERENT PLACEMENT AND ORIENTATION OF LUTEIN AND ZEAXANTHIN IN LIPID MEMBRANES – IN SILICO EXPLANATION

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Lutein and zeaxanthin are two carotenoid pigments synthesized mainly by plants and bacteria. Although animals are unable to synthesize carotenoids, they are present in large quantities in animal tissues. Protecting the cell from light radiation, free radicals and oxidative stress, carotenoids are essential components of a lipid bilayer. Depending on the chemical structure, carotenoids locate at various positions and in different orientations in the hydrophobic environment of biomembrane. Because of the additional hydroxyl groups, lutein and zeaxanthin are members of the xanthophyll subgroup of carotenoids. Hydroxyl-substituted ionone rings anchor themselves in the polar environment of the interphase of lipid bilayers. Thanks to this anchoring, it was suggested that lutein can take both transmembrane and parallel orientation within the lipid bilayer. On the other hand parallel orientation was never observed for zeaxanthin.
To find an explanation for the different behaviour of lutein and zeaxanthin we've used classic molecular dynamic simulations with atomic resolution. We started with carotenoids intercalation experiments, in which lutein molecule, having two various but representative ionone rings intercalated inside the lipid bilayer. We concluded that depending on the terminal ring, carotenoid intercalation is not symmetrical [1]. Next, we checked the behaviour and interactions of lutein and zeaxanthin molecules placed within the bilayer. We observe the all-transmembrane orientation of zeaxanthin and both trans, and parallel orientation of lutein. We provide a detailed description of intermolecular interactions, stabilizing such orientations of both lutein and zeaxanthin [2].

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METALOME OF SLIME MOLDS - MANGANESE ACCUMULATION IN PLASMODIUM AND OTHER STRUCTURES OF THEIR LIFE CYCLE

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True slime molds (Mycetozoa) are simple eukaryotic organisms that have been widely used in the life sciences as model organisms. Many slime molds reveal features common with much more complex organisms [1]. In addition, the slime molds possess the ability to accumulate certain metals in amounts much higher than in typical eukaryotic cells. Various stages of development have been investigated for the presence of such elements as manganese, iron, or zinc [2]. EPR is the method that has been found of a great use in slime molds studies, e.g., to demonstrate the presence of some paramagnetic compounds in their plasmodia [3].

Our work has been focused on verification if accumulation of manganese(II) of different slime mold species is characteristic for these organisms. Material used for EPR spectroscopy was collected from *Didymium* sp. and two other species (*Physarum polycephalum* Schwein., *Fuligo septica* (L.) F.H. Wigg), which have been previously demonstrated to accumulate large amounts of manganese(II). Based on EPR spectra, it can be stated that colorless plasmodia of *Didymium* sp. do not display the ability to accumulate such large amounts of manganese(II) as pigmented plasmodia of *Physarum polycephalum* or *Fuligo septica*. In contrast, the sporangia of *Didymium* sp. exhibit strong signal derived from manganese(II) ions. Manganese(II) is also accumulated in peridia and sclerotes of *F. septica*.

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DEVELOPMENT OF DIAGNOSTIC APTAMERS FOR PERIODONTOPATHOGENS

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Periodontal disease is caused by chronic multi-bacterial infection localized in periodontal pockets. The disease develops over a long period of time and involves gradual changes in microbial community leading to anaerobes predominance with three major species- *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*.
Presented work aimed to developed novel periodontal diagnostic tools based on short single stranded oligonucleotides (aptamers). Our work focused on *P. gingivalis* and *T. forsythia* cells. With 10 selective rounds we were able to select and multiply two pools of strongly target binding aptamers by the cell-SELEX procedure. NGS revealed prevalence of two highly abundant clusters in *T. forsythia* binding pool and only one scarce for *P. gingivalis*. Further analysis of individual aptamers proved particularly good binding properties (with Kd<than 10 nM) of three independent anti-Tf aptamers. In addition, we showed that the cell surface binding partners are not one of *T. forsythia* semicrystalline layer proteins.
In further research will plan to test our compounds against clinical isolates of *T. forsythia* obtained from periodontal pockets of periodontal patients.

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RESVERATROL AND ASCORBIC ACID PROTECT HUMAN KERATINOCYTES AGAINST $PM_{2.5}$ PHOTOTOXICITY

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The human skin is exposed to various environmental factors including solar radiation and ambient air pollutants. Although, due to its physical and biological properties, the skin efficiently protects the body against the harm of environmental factors, their excessive levels, and possible synergistic action may lead to harmful effects. Among particulate matter present in ambient air pollutants, $PM_{2.5}$ is of particular importance for it can penetrate both disrupted and intact skin, causing adverse effects to skin tissue [1]. Our previous study has demonstrated that light interacting with particulate matter increases the damage of human skin cells *in vitro* in both season-dependent and light-dependent ways [2]. The synergistic effect of the two was found to trigger the photoproduction of free radicals and singlet oxygen that resulted in decreased cell viability, elevated levels of lipid peroxides, and changes in mitochondrial membrane potential. In the current study, we have demonstrated the protective effect of resveratrol, ascorbic acid, and the combination of the two against $PM_{2.5}$-induced phototoxicity in cultured human keratinocytes (HaCaT).

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IMMUNOCOMPETENT AND RPRODUCTABLE MOUSE MODEL OF ORTHOTOPIC PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most deadly human malignancies. Despite studies conducted in recent years, there are still gaps in understanding the progression and poor therapy response of PDAC. Therefore, it is necessary to develop reliable, reproducible, and inexpensive animal models in an orthotopic position to better understand the processes of occurrence, development, invasion, and metastasis of this disease. Furthermore, the application of this model can be used to study the mechanism of action of drugs and prompt the discovery of new therapeutic agents. [1]

The aim was to optimize an orthotopic, immunocompetent murine model of pancreatic cancer in mice and study the effectiveness of the applied chemotherapy with gemcitabine.

Spheroids embedded in Matrigel® were inoculated into mouse pancreas during surgical procedures. Tumor progression was imaged by a non-invasive ultrasound method (US). When tumors appeared (20-45 days after inoculation), the animals were scheduled to receive gemcitabine chemotherapy. The therapy lasted 16 days, during which 6 doses of gemcitabine were administered (72 h time interval) at a concentration of 45 mg/kg of the animal's body weight.

The new model showed >80% acceptability and presented the response to gemcitabine treatment.

Due to its orthotopic nature, the new mouse model of pancreatic cancer will reproduce not only the neoplastic process itself but also the organism's responses, including the immune system, which will significantly improve our knowledge of pancreatic cancer, as well as enable research into new therapies and screening drugs that may prove to be significant importance in clinical treatment.

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THE EFFECT OF HYDROGEN SULFIDE ON THE DYSTROPHIC MUSCLES – *IN VIVO* STUDY ON THE *MDX* MODEL OF DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is caused by the lack of dystrophin, the muscle functional protein, due to the mutations in encoding it one of the largest human genes (*DMD)*. Dystrophin absence leads to progressive muscle weakness, degeneration, and premature death of patients. For now, there is no effective drug treatment for DMD. Hydrogen sulfide (H_2S), the pleiotropic agent with anti-oxidant, anti-inflammatory, and proangiogenic activities might be considered as a promising therapeutic factor for DMD.

In this study, we analyzed the effect of intraperitoneal administration of an H_2S donor, sodium hydrosulfide (NaHS) on the state of skeletal muscles in dystrophin-deficient *mdx* mice. We found that everyday treatment with NaHS (100 µmol/kg/day) for 5 weeks increased Akt kinase activity, upregulated cytoprotective heme oxygenase-1 (HO-1) level, decreased creatine kinase activity, transforming growth factor-β (TGF-β) and osteopontin level. It also led to the regulation of vascular endothelial growth factor (*Vegf*) and its receptor (*Kdr*) expression but did not affect the protein level of angiogenic markers. The histological analysis showed no effect of H_2S donor on inflammation, fibrosis, and regeneration. Finally, treatment with NaHS did not improve muscle function in dystrophic animals as assessed by grip strength measurement.

Overall, treatment with an H_2S donor regulates the gene and protein expression of the molecules associated with DMD pathophysiology. However, it did not attenuate significantly the dystrophic conditions in *mdx* mice, and its impact on muscle pathology in other schemes of experiments warrants further investigation.

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LIPID PEROXIDATION AND GLUTATHIONE PRODUCTION IN RELATION TO INTRACELLULAR CONTENT OF HEAVY METALS IN EPIGEIC LICHEN *CLADONIA REI*

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Heavy metals may generate reactive oxygen species (ROS) that react with membrane lipids leading to their peroxidation. To avoid cell damage, organisms have various mechanisms that deactivate ROS. One of the most important ROS scavenger in lichens is reduced glutathione (GSH). We analysed the relationships between intracellular concentrations of Zn, Pb, Cd (determined by atomic absorption spectrometry; AAS), contents of secondary metabolites, glutathione and malonyldialdehyde (MDA) in *Cladonia rei* Schaer. collected from highly contaminated post-industrial dumps. The degree of lipid peroxidation, expressed as MDA content, was determined spectrophotometrically; GSH and secondary metabolites were analysed using high-performance liquid chromatography (HPLC with PDA detector).
The relationships were verified using Pearson's correlation coefficient; if a significant result was returned, then the relation was tested with various linear and nonlinear regression models to find the best fitted oneaccording to the coefficient of determination (R^2). Increased intracellular accumulation of Cd and Pb caused an increase in content of MDA and decrease in content of GSH; various nonlinear relationships turned out to be the best-fitted. The quantity of secondary metabolites did not depend significantly on any of the examined elements.
It seems that depletion of the pool of reduced form of glutathione impairs the antioxidant potential of cells, which in turn increases peroxidation of membrane lipids. This is probably due to an excessive oxidation of GSH to glutathione disulfide (GSSG) and/or a disturbance in the reduction of GSSG to GSH.

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DEVELOPING OF HUMAN iPS-DERIVED CARDIAC CELL LINE *IN VITRO* MODELS FOR STUDYING AN IMPACT OF EXTRACELLULAR VESICLES IN HEART REPAIR - PRELIMINARY REPORT

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Growing data demonstrates that extracellular vesicles (EVs) may serve as potential new-generation cell-free therapeutic agents. Moreover, it has been shown that human induced pluripotent stem cell (hiPSC)-derived EVs (hiPSC-EVs) may transfer e.g. miRNA molecules as bioactive cargo promoting regeneration of cardiac tissue after the injury. However, since a detailed mechanism of this phenomenon remains unknown, in the current study we aimed at developing an *in vitro* models to investigate the role of selected miRNA clusters in pro-regenerative activity of hiPSC-EVs.

Based on pluripotent capacity of hiPSCs, we differentiated them into three cardiac cell lines: cardiomyocytes (CM), cardiac fibroblasts (CF) and cardiac endothelium (CE), that would serve as target cells for hiPSC-EVs in further functional *in vitro* assays. Due to the lack of well-established differentiation protocols, we compared several approaches and experimental conditions on two different hiPSC lines. The differentiation efficacy was assessed by microscopy as well as flow cytometry and gene expression analyses.

Our preliminary results demonstrated the successful differentiation of hiPSCs into three desired cardiac cell lines, as indicated by changes in the morphology and expression of specific lineage markers. However, we observed different efficacy between tested hiPSC lines and experimental conditions, which indicates the complexity of the differentiation process.

In conclusion, we successfully established target cardiac cell lines as an *in vitro* model, which may be suitable for the investigation of miRNA role in heart regeneration mediated by hiPSC-EVs. However, due to the observed variations between selected experimental conditions, further studies are required to select the most optimal differentiation approach.

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NRF2 TRANSCRIPTIONAL ACTIVITY INFLUENCES COLONIC ESTROGEN RECEPTORS

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The proper functioning of gastrointestinal system relies on an intricate crosstalk between a plethora of cell types and signaling pathways. Recent studies clearly indicate that both estrogen signalling and stress activated transcription factor Nrf2 are pivotal, and interrelated players in the maintenance of colon homeostasis. We aimed to elucidate how lack of Nrf2 transcriptional activity impacts estrogen signaling in female mice and to establish the role in the prevention of Nrf2 activity-deficiency-related impairment of colon function.

The lack of Nrf2 transcriptional activity (Nrf2 tKO) leads to a significant decrease in 17β-estradiol level in serum in comparison to 3 m.o. female wild-type (WT) counterparts. Moreover, the immunofluorescent stainings revealed a remarkable deregulation of estrogen receptors in the colon of Nrf2 tKO mice: increased protein level of ER-alpha and downregulated level of GPR30. Of note, there is no change in ER-beta level.

To address the role of estrogens further, we inserted the implants releasing 17beta-estradiol (0.7-1.3 µg/day) or placebo. Importantly, there was a significant increase in serum 17β-estradiol after 28-day treatment. Functional tests of gastrointestinal track activity showed that estradiol treatment normalized colon motility in the Nrf2 tKO mice. Additionally, estradiol treatment influenced colon estrogen receptors level and increased the expression of cytoplasmic GPR30 receptors.

In conclusion, the transcriptional activity of Nrf2 influences colon functionality and estrogen receptors expression in the colon. Targeting estrogen signaling seems a promising therapeutic strategy to counteract which may be partially reversed by the estrogen treatment.

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THE ROLE OF REGNASE-1 IN THE PROGRESSION OF INFLAMMATORY BOWEL DISEASE

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Ulcerative colitis (UC) and Crohn's disease (CD) are referred to as Inflammatory Bowel Disease (IBD). The incidence of IBD has increased significantly throughout the world over the last 50 years, especially in developing countries, including Poland, so it is important to investigate the causes and mechanisms of the disease progression. IBD is characterized by chronic and periodic inflammation of the intestines, the predominant symptoms of which are diarrhea, abdominal pain and anemia. IBD is strongly associated with the development of neoplastic changes in the gut [1]. An important role in the pathogenesis of IBD are disorders of the immune response against the intestinal commensal microflora. It is postulated that negative regulators of signaling pathways from TLRs, including A20, SIGIRR and CYLD, play an extensive role in maintaining the immune balance that prevents the development of IBD. The role of other negative regulators in exacerbation or remission of IBD is still poorly understood. One of the negative regulators called Regnase-1 (MCPIP-1) was selected for the presented studies. This protein acts as an RNase that destabilizes mRNA transcripts for pro-inflammatory factors, such as: IL-1beta, IL-6, IL-8, IL-12p40, responsible for the exacerbation of IBD [2,3].
The presented results confirm the hypothesis that MCPIP-1 plays an important role in the regulation of qualitative and quantitative composition of the intestinal microbiome, leading to dysbiosis. The obtained results could influence the development of new therapeutic strategies for the treatment of inflammatory bowel diseases by influencing the regulatory functions of the described protein.

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COOPERATION OF ABC EFFLUX SYSTEMS ACCOUNTS FOR THE DOXORUBICIN-RESISTANCE OF T98G GLIOBLASTOMA MULTIFORME CELLS

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Glioblastoma multiforme (GBM) is regarded as the most aggressive subtype of brain neoplasms (IV-grade glioma; WHO). Consequently, it represents the greatest challenge for the current neuro-oncology. Doxorubicin (DOX) has been proposed for the 1st line of GBM chemotherapy. However, the long-term consequences of GBM adaptation to DOX-induced stress for GBM malignancy are unknown. GBM chemoresistance is strongly related to the activity of ABC transporters (ATP-binding cassette transporters). They act as membrane-associated and/or intracellular pumps, which effectively remove various xenobiotics from intracellular spaces. Relatively high DOX-resistance of T98G cells, accompanied by relationships between their mesenchymal phenotype and drug-efflux efficiency, prompted us to focus on the mechanistics of T98G DOX-resistance.

Microscopic analyses of the calcein-efflux intensity confirmed the involvement of at least 3 efflux pumps in the drug-resistance of T98G cells (ABCB1, ABCC and ABCG2). This was illustrated by reduced intensity of calcein efflux in the presence of DOX (showing the potential competition between the drugs) and by enhanced activity of these pumps in T98G cells after DOX-treatment. It correlated with enhanced ATP synthesis in DOX-treated cells. Functional studies revealed increased ABCB1 protein levels in DOX-treated T98G cells and the considerably higher calcein efflux efficiency from mesenchymal cells than from their "epithelioid" counterparts. Ectopic down-regulation of individual pumps by esiRNAs revealed their cooperation in securing DOX-resistance of T98G cells. Actually, only negligible inhibition of calcein efflux intensity and DOX-resistance could be seen after the application of esiRNAs against ABC-encoding mRNAs.

Our data show that phenotypic plasticity of GBM cells and cooperative ABC-dependent DOX efflux is a prerequisite for short-term adaptation of invasive GBM lineages to DOX-induced stress.

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ANTICANCER POTENTIAL OF THE MANGANESE PORPHYRIN AND SODIUM ASCORBATE IN CELL CULTURE *IN VITRO*

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Porphyrins are used medicinally in the treatment of many diseases. They are best known for photodynamic reactions in which electromagnetic radiation causes the production of free radicals in cancer cells, which in turn leads to damage of these cells. Currently work leads to create system to generate reactive form of oxygen and do not need light. For this purpose, create new porphin coordinating manganese ion and consist sulfo and sulfonamide group. The toxicity of the new MnTPPS and MnF2Met molecules and the influence of the new porphyrins on the rate of migration of cancer cells were investigated. High concentration of sodium ascorbate in culture medium leads to cell death. Cell line differ in distractive concentration of sodium ascorbate. The presence of MnTPPS and MnF2Met increases the toxicity of ASCs and leads to uncontrolled cell death. As a result of the interaction of ASC with manganese porphyrins, the redox environment of the cell is disturbed, which in turn leads to an increase in the production of reactive oxygen species and damage to the cell membrane structures.

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FACTORS OF SKELETAL MUSCLE FATIGUE AND EXCESSIVE VULNERABILITY TO FATIGUE IN MITOCHONDRIAL DISEASES IN THE LIGHT OF SYSTEMS BIOLOGY

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In recent years increase of acidity and accumulation of the phosphate ions have been described as the crucial metabolic fatigue factors disturbing the proper action of muscle fibers. This fact is especially important in the context of mitochondrial diseases in which excessive fatigue is one of the possible symptoms. However, little is known about the precise fatigue inducing thresholds of work intensity in mitochondrial diseases of various types and in various stages of their severity. Possible interactions of further factors such as disturbances in concentrations of electrolytes (i.e. magnesium ions) were also not defined. One of the best suited tools for this kind of problems is system biology which enables modeling of metabolic pathways. In this research computer model of working skeletal muscle [1,2] was adapted. Changes of stationary metabolite concentrations to different interruptions of oxidative phosphorylation were analyzed. Curves of dependence between activity of mitochondrial complexes and threshold work intensities were determined. Additionally, relevance of changes of magnesium ion concentrations in models of these pathologies was also assessed.

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PATHOGENIC ORAL BACTERIA MODULATE GINGIVAL FIBROBLAST RESPONSES TO INTERFERONS

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Periodontitis is a chronic inflammatory disease caused by dysbiosis in the periodontal tissues. The disease is caused by pathogenic oral bacteria, such as *Porphyromonas gingivalis (Pg)*, *Fusobacterium nucleatum (Fn)*, and *Filifactor alocis (Fa)*, which stimulate excessive activation of the immune system while evading elimination by the host inflammatory response. Gingival fibroblasts (GFs) participate in the immune response by secreting a broad range of inflammatory mediators in response to bacterial cells, products, and virulence factors, as well as cytokines produced by other cells. In periodontitis patients, the levels of interferons IFN-α and IFN-γ are increased in gingival crevicular fluid and prolonged production of IFN-α promotes alveolar bone destruction. Here, we investigated how the presence of bacteria influences inflammatory profile of GFs stimulated with IFN-α or IFN-γ. While GF infection with oral bacteria failed to elicit significant induction of IFN-responsive genes, infection with *Fn* or *Fa* in the presence of IFN-α or IFN-γ led to synergistic increases in expression and/or production of the chemokines CXCL9, CXCL10, CXCL11 and CCL20. Surprisingly, *Fn* and *Fa* did not affect STAT1 activation by IFNs, suggesting that modulation of other signaling pathways is responsible for synergistic action of bacteria and IFNs. Our preliminary data also revealed that during simultaneous infection, *Pg* strongly reduces the synergistic effect of *Fn* on IFN-γ-induced CXCL10 production by GFs, possibly due to proteolytic activity. Collectively, these results show that bacterial infection strongly modulates GF response to IFN stimulation, either amplifying or diminishing cell activation depending on the composition of bacterial community.

INTERACTION OF *C. ALBICANS* WITH NEUTORPHILS - YEAST DOMINANCE ON THE BATTELFIELD OR NEUTROPHILS ADAPTATION?

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Advances in therapies based on the use of a wide range of antibiotics and immunosuppressive drugs have contributed to the prevalence of fungal infections. The most common pathogen responsible for such diseases is *Candida albicans*, a commensal yeast colonizing mainly the skin and mucous membranes. The imbalance between commensals and the immune system caused by immunosuppression or depletion of the microflora drives *Candida* domination and leads to the development from mild superficial to systemic infections [1]. The invasiveness of *C. albicans* is correlated with the formation of biofilms, three-dimensional structures surrounded by extracellular matrix, characterized by unique protective properties and excellent adhesion ability. Studies conducted on bacterial models indicated that biofilms may dysregulate the response of the host's immune system by suppression or overstimulation of cells response, especially phagocytes [2].
In recent studies, we attempted to evaluate the response of neutrophils upon contact with *C. albicans* biofilms at various stages of fungal infections. Our *in vitro* research focused on analyzing neutrophils' morphology, localization, viability, and release of the neutrophil extracellular traps (NETs). The results showed significant differences in neutrophil response to biofilm compared to the response to planktonic cells and isolated virulence factors. In particular, a gradual inhibition of NETs release was observed as biofilm density increased, with a concomitant prolongation of neutrophil viability. A better understanding of neutrophil responses during the development of *C. albicans* infection may contribute to the efficacy of antifungal therapies in the future.

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ANALYSIS OF THE EFFECT OF PIGMENTATION OF MELANOMA CELLS ON SPHEROID FORMATION

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Spheroids are increasingly being used in cancer research. They allow for the study of tumors in a microenvironment that is more closer to the in vivo situation. The 3D model has many important elements of physiological spatial growth and intercellular interactions. Cancer cells in spheroids are subjected to heterogeneous exposure to oxygen and nutrients, as well as cell-cell and cell-matrix interactions [1, 2].
Melanoma is a malignant tumor in which cell-cell interactions play a key role in the pathobiology of the tumor as well as in response to therapeutic measures. One of the characteristics of melanoma is the ability to synthesize melanin. In in vitro cultures (2D model), the melanin synthesis process by melanoma cells seems to be disrupted [3].
Our aim is to present how melanoma cell pigmentation influences the basic parameters of spheroids. The growth rate of spheroids in the medium stimulating and not stimulating pigmentation was compared. Then the spheroids were subjected to histological examination. Markers of proliferation were analyzed. HIF1a as, a marker of hypoxia, were estimated immunohistologically. The level of adhesive proteins was also tested. The presented results will allow for a better selection of the 3D model in research on melanoma biology and anti-cancer therapies.

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ANALYSIS OF TUMOR NECROSIS AS A PARANEOPLASTIC FACTOR

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Necrosis in tumors can be caused by many factors, including hypoxia, immune response, irradiation or chemotherapy. Hemorrhagic necrosis is characterized by the progressive extravasation and ectopic accumulation of hemoglobin. Tumor necrosis is also a stimulator of the immune response of the host, i.a. with the participation of nitric oxide (NO). The aim of this study was to analyze the EPR signals of NO complexes with endogenous (hemoglobin) and exogenous (DETC) spin traps in hemorrhagic necrosis of tumors.

As a model DBA/2 mice were used with advanced solid tumors Cloudman S91 melanoma, which form a central hemorrhagic necrosis and alive, vascularized cortical layer. The type and extent of tumor necrosis were determined using H&E staining. EPR CW X band measurements of NO complexes in tumors were performed at 77 K.

EPR signal amplitude of HbNO and $DETC(Fe)_2NO$ complexes in the tumors varied considerably from barely detectable to very intensive. The strongest signals of both types complexes were observed in the necrotic core while the accompanied signals in the surface part of the tumor were generally lower or in some cases even hardly detectable. Furthermore, when very intensive signals were observed in the cortical layer, signals in the necrosis were strong. It demonstrates that the necrotic areas are the primary sites of the intense stimulation of nitric oxide production in the S91 tumors. The requirements for a strong NO production in the cortical layer of tumor is a strong production of NO in the necrotic part, and not vice versa. Very intensive signals of $DETC(Fe)_2NO$ in necrosis were detected in tumors weighing from 0.5-1.5 g and were associated with the presence of relatively weak HbNO signals, and the necrosis was characterized by low extravasation. If necrosis revealed strong HbNO signal, then $DETC(Fe)_2NO$ signal was weak, and the degree of extravasation high. The few cases of simultaneous occurrence of strong HbNO signal and strong $DETC(Fe)_2NO$ signal were observed in the surface part in advanced tumors (>1.5 g), and accompanied by an even stronger HbNO signal in the necrosis. It indicates that the cortical layer of the tumor is more heterogeneous than the central necrosis, and can be a place of both a strong hemorrhagic foci of necrosis, beside sites of a weak extravasation at the same time.

EFFECT OF DIFFERENT CONCENTRATIONS OF SEA SALT ON GROWTH AND PHOTOSYNTHESIS QUANTUM YIELD IN *PHAEODACTYLUM TRICORNUTUM* (PRELIMINARY RESULTS)

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One of the consequences of the greenhouse effect is the increase of water levels, which is caused by the melting of the glaciers. This leads to the reduction of salt concentration in marine waters, and consequently it impacts the physiology of phytoplankton, of which diatoms are the main component. In one of the previous reports, researchers focused on the short exposure of salinity stress [1]. In the present study, the long-term effects of changing the sea salt concentration on a model diatom species, *Phaeodactylum tricornutum* were investigated. The purpose of this study was to analyze the influence of the salinity level on the growth and photosynthesis quantum yield of diatoms. The photosynthetic pigment contents and protein concentration were also determined. The results showed correlations between salt concentration and growth kinetics of the diatoms. The optical density and cell density achieved a maximum level on the 32nd day, and then both parameters decreased for cultures grown in the absence of salt. In other cultures, the value of these parameters increased with time. In the case of photosynthesis quantum yield, for all salt concentrations and in all samples the maximum values of this parameter were obtained on the 32nd day of the experiment, and ratio began to decrease. The photosynthetic pigments, as well as protein concentration, reached the highest levels on the 32nd or 40th day of cultivation. For the culture without salt after 40^{th} day decrease of these parameters was observed but the slow increasement in the remaining samples.

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LOSS OF MYELOID MCPIP1 PROMOTES A PROALLERGIC PHENOTYPE IN THE SKIN

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Skin, the body's largest organ, is our first and best defense against harmful external stimuli. While it is mainly composed of keratinocytes, it also contains other types of cells, such as immune cells and melanocytes, which together provide skin homeostasis. Dysregulation of this unique interaction may lead to the development of chronic inflammatory skin diseases, like psoriasis, atopic dermatosis or cancer.

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 and transcription factors, thereby acting as essential regulator of inflammation. Importantly, MCPIP1 expression is elevated in human psoriatic epidermis. Our recent study found that keratinocyte-specific Mcpip1-deficient ($Mcpip1^{EKO}$) mice develop spontaneous skin pathology and systemic inflammation. Moreover, keratinocyte Mcpip1 has been shown to be involved in the pathology of psoriatic disease.

Here, we analyzed skin phenotype of mice with myeloid-specific ablation of Mcpip1 ($Mcpip1^{MKO}$), especially in macrophages, granulocytes and dendritic cells. We observed proallergic phenotype, that was manifested by the development of lesions localized to the ears and neck in adult $Mcpip1^{MKO}$ mice. At the histological level, we observed infiltration of mast cells and eosinophils into the skin, which was correlated with increased expression levels of Th2-type cytokines and chemokines. Next, we utilized mouse skin disorder models: Delayed-Type Hypersensitivity reaction to allergen and IMQ-induced psoriasis, which showed enhanced sensitivity to stimulation in $Mcpip1^{MKO}$ mice.

Overall, our work improves the current understanding of the role of myeloid MCPIP1 in modulating skin allergic inflammation.

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ANALYSIS OF PRIMARY BILIARY CHOLANGITIS IN Mcpip1 KNOCK OUT MICE AFTER PHARMACOLOGICAL TREATMENT

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Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease that results from slow, progressive destruction of the intrahepatic bile ducts. PBC progression leads to the development of fibrosis, cholestasis, liver cirrhosis. There is only one FDA-approved drug for PBC treatment, ursodeoxycholic acid (UDCA). Obeticholic acid (OCA) can be used in combination with UDCA in patients that don't respond to UDCA treatment.

The newest murine model of PBC is Mcpip1fl/flAlbCre mice which are characterized by deletion of the *Zc3h12a* gene (encoding Mcpip1 protein) in liver epithelial cells. Mcpip1fl/flAlbCre mice in the age of 6 and 24 weeks were analyzed for typical PBC symptoms. Subsequently, mentioned knock out mice and control mice at the age of 6 weeks were randomly divided into five groups to be pharmacologically treated: i) solvent, ii) UDCA, iii) UDCA+Lakcid (*L. rhamnosus*), iv) UDCA + OCA, v) Lakcid (*L. rhamnosus*).

Mcpip1fl/flAlbCre mice developed a number of typical PBC symptoms, such as presence of antimitochondrial and antinuclear antibodies, total bile acids elevated, increased activity of alkaline phosphatase, liver fibrosis, bile duct injury and hyperplasia, inflammation and granuloma formation.

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ROLE OF LIGHT SIGNALLING IN PLANT CELL WALL BIOSYNTHESIS

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Plant cell wall is an important global carbon sink and has great influence over plant growth and development. The plant primary cell wall is composed of polysaccharides such as cellulose, hemicelluloses and pectins. Despite our extensive knowledge of the cell wall composition and structure we know very little about the molecular processes that control its biosynthesis. Since light is one of the main stimuli perceived by plants it is very likely that it influences primary cell wall biosynthesis. One of the approaches to understand the importance of light for the cell wall biosynthesis is to analyse the cell wall polysaccharides present in photoreceptor mutants [1] in which signalling from specific parts of the light spectrum is impaired. Here we present preliminary analysis of such plant cell wall structure in Arabidopsis mutants of phytochrome, cryptochrome and phototropin photoreceptors. Phenotyping of mutants reveals major changes in their morphology, when compared to wild type plants, what suggests potential alterations in the cell wall structure. To investigate these potential changes, we performed biochemical characterisation of hemicelluloses and cellulose in photoreceptor mutants using colorimetric assays, High Performance Liquid Chromatography (HPLC) and Polysaccharide Analysis using Carbohydrate Gel Electrophoresis (PACE). Our results provide some insight into the cell wall structure in these mutants and open new avenues for future exploration of the importance of light signalling for plant polysaccharide biosynthesis.

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EFFECT OF YEAST-BACTERIAL BIOFILM ON THE CONDITION OF EPITHELIAL CELLS (BEAS-2B) IN A TWO- AND THREE-DIMENSIONAL LUNG EPITHELIAL MODELS

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Aspiration pneumonia is a lung condition caused by uncontrolled aspiration of stomach or mouth contents. It has been suggested that excessive colonization of the oral cavity by pathogens may contribute also to the colonization of the human respiratory tract during aspiration pneumonia.
Many infections caused by microorganisms are multi-species. The involved pathogens can create the biofilm structure which plays a protective function towards them. Recently, it has been proved, that the yeast colonizer of the oral cavity - *Candida albicans* is able to reduce the oxygen content in the mixed biofilm, creating appropriate conditions for the development of anaerobic bacteria like *P. gingivalis* [1].
In the studies, we present the comparison of the cellular responses of human lung epithelial cells (BEAS-2B), grown in the 2D and 3D air-liquid interface models, and contact the supernatant from the yeast-bacterial biofilm or the whole biofilm. The examination of mitochondria condition and interleukin-8 productions showed a significant difference in the cell responses, pointing to the need for the use of a more physiologically relevant and predictive 3D model with a higher degree of structural complexity to study the progress of mixed-species infection of the host lung cells.

References:
[1] Bartnicka D *et al.* (2019) Adhesive protein-mediated cross-talk between *Candida albicans* and *Porphyromonas gingivalis* in dual-species biofilm protects the anaerobic bacterium in unfavorable oxic environment. *Sci Rep* ***9***:4376.

Acknowledgements:
The work was supported in part by the National Science Centre of Poland (grant no. 2019/33/B/NZ6/02284 awarded to M.R.-K.).

CRISPR/CAS9-MEDIATED KNOCKOUT OF RIPK4 IMPARTS REMARKABLE ANTIPROLIFERATIVE RESPONSE IN HUMAN MELANOMA CELLS IN 3D *IN VITRO* AND *IN VIVO*

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Melanoma is one of the most aggressive, potentially fatal forms of skin cancer. However, despite significant advances in targeted and immune therapies, melanoma cells develop resistance causing relapse. Therefore, additional approaches based on novel mechanisms are needed to effectively treat melanoma. Receptor-interacting protein kinase (RIPK4), which targets multiple signaling pathways such as Wnt/b-catenin, NFKB or MAPK also important for melanoma cell survival, appears to be a potential target. This is supported by the fact that RIPK4 kinase levels are elevated in melanoma cells compared to normal melanocytes [1]. In this study, we determined the effects of genetic manipulation of RIPK4 in human malignant melanoma: A375 and WM266.4 cells on tumor growth and spheroid formation.

Human malignant melanoma cells A375 and WM266 were transfected using lentiviral vectors for the DNA endonuclease protein, Cas9, and a nucleic acid fragment, gRNA, complementary to the gene fragment encoding the RIPK4 protein (InvitrogenTM, Thermo Fisher Scientific) or a nucleic acid fragment whose sequence shows no homology with any region in the human genome; in addition, the construct encoded a GFP protein. The downregulation of RIPK4 in the derived cell clones was verified by Western blot. The modified cells were injected subcutaneously into NOD/SCID mice to monitor the tumor growth or culture in three-dimensional culture as spheroids [2].

Our data showed that CRISPR/Cas9-mediated knockout (KO) of RIPK4 in both melanoma cells resulted in significantly reduced tumor growth in NOD/SCID mice as well as steroid formation *in vitro*.

References:

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Acknowledgements:

This research was funded by the National Science Centre, Poland, grant number UMO-2018/31/B/NZ5/01423.

THE YEAST-NEUTROPHIL WAR - MECHANISMS OF INTERACTION BETWEEN *CANDIDA ALBICANS* AND NEUTROPHILS

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Candida albicans is a natural flora of the skin and mucous membranes of humans. In most cases, these yeasts act as commensal and do not pose a threat but upon host immunosuppression, they acquire pathogenic features and break down further barriers, causing life-threatening and difficult to treat systemic infections called candidiasis.

The pathogenic form of *C. albicans* contacting host tissues induces an immediate defense response, involving neutrophils as the primary rapid responding cells. Among a number of neutrophil defensive strategies, the most noteworthy mechanism is the release of Neutrophil Extracellular Traps (NETs) - structures composed of DNA and incorporated proteins with biocidal properties. Due to their activity, NETs can damage the yeast cells and limit the spread of *C. albicans* infection.

In our work, we focused on the identification of yeast factors involved in mechanisms of netosis activation and NET release, as well as yeast strategies used by them to avoid neutrophil responses. We have shown that pathogen cell wall components, such as glucans and mannans, as well as selected aspartyl proteases (Sap), which are virulence factors released by *C. albicans* during infection, can activate both the classical ROS-dependent and ROS-independent netosis pathways. We identified receptors and messengers of the signaling pathway involved in the induction of netosis. On the other hand, our results documented also that this pathogen can effectively inactivate neutrophils by a "Trojan horse mechanism", which involved Sap. This mechanism leads to damage of intracellular oxidative processes, blocks ROS-dependent netosis, and in consequence, induces neutrophil death.

THE ROLE OF Mcpip-1 IN GINGIVAL KERATINOCYTES DURING ALVEOLAR BONE REMODELING

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The maintenance of bone homeostasis requires an orchestrated activity of osteoblasts, osteoclasts and osteocytes. Recently, the role of the epithelium in the regulation of that triad was discovered [1,2]. This novel concept suggests keratinized oral mucosa as a crucial component in the remodeling of alveolar bone. Gingival keratinocytes significantly shape the immune response of oral mucosa producing a plethora of mediators of inflammation, including cytokines. Their secretion is strictly modulated by negative regulators of inflammatory response. Among them, MCPIP-1 which is abundantly expressed in gingival keratinocytes seems to play a pivotal role [3]. Therefore, in our study, we aimed to examine if tissue-specific MCPIP-1 depletion in gingival keratinocytes would affect the structure of alveolar bone in mice. Using the micro-computed tomography method (micro-CT), we demonstrated that mice lacking MCPIP-1 expression in gingiva have increased alveolar bone loss compared to the control group. Interestingly this phenomenon is not observed in the femurs, indicating that the MCPIP-1 deletion affects bone structure locally, but not systemically. Intriguingly, in our *in vivo* model bone impairment was significantly reduced in mice on a soft diet, indicating that mastication of hard chow triggers bone destruction. Molecular analysis of the above phenomenon revealed that the appearance of pro-osteolytic phenotype in mice with MCPIP-1 depletion in gingival keratinocytes is caused by an exaggerated local inflammation, intensified by mastication of hard irradiated diet. Taken together, our data exploring epithelium as a novel field in osteoimmunology, announce the MCPIP-1 as an important player in the pathophysiology of the disease.

References:

[1] Dutzan N, *et al.* (2017) On-going Mechanical Damage from Mastication Drives Homeostatic Th17 Cell Responses at the Oral Barrier. *Immunity* **46(1)**:133-147.

[2] Iwamoto Y, *et al.* (2016) Intercellular Communication between Keratinocytes and Fibroblasts Induces Local Osteoclast Differentiation: a Mechanism Underlying Cholesteatoma-Induced Bone Destruction. *Mol Cell Biol* **36(11):**1610-1620.

[3] Gasiorek A, *et al.* (2021) Subversion of Lipopolysaccharide Signaling in Gingival Keratinocytes via MCPIP-1 Degradation as a Novel Pathogenic Strategy of Inflammophilic Pathobionts. *mBio* **12(3)**:e0050221.

Acknowlegdements:

Our study was financed by the National Science Centre, Poland UMO-2018/29/B/NZ6/01622

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Tandemowe spektrometry mas zapewniają bezpieczeństwo światowych dostaw żywności, która jest przetwarzana w różnych środowiskach zgodnie z przepisami i normami (m.in. Sante, BfR).

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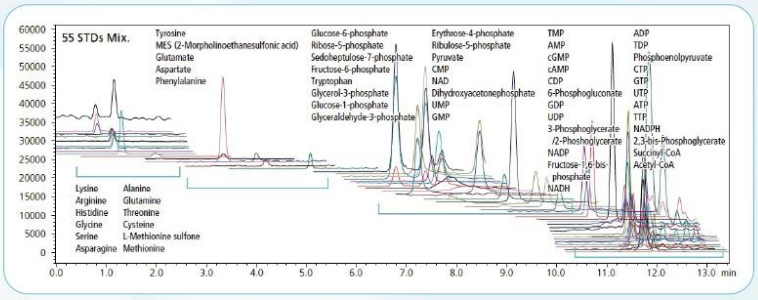
Podejście DDA/DIA oraz możliwość sprzęgania z licznymi bazami danych umożliwia realizowanie ambitnych strategii „unknown workflow". Szeroka gama technik jonizacji w LCMS-9030 umożliwia prowadzenie badań wielokierunkowych. Z trybu analizy PESI-HRMS (brak konieczności wstępnego przygotowania próbki), można przejść w zaledwie 10 sekund w tryb ESI-HRMS wykorzystując przy tym moc całkowicie inertnego systemu UHPLC Nexera. Spektrometr łączy w sobie niezrównaną szybkość, jakże nietypową dla analizatorów TOF oraz doskonałą czułość i wysoką rozdzielczość.



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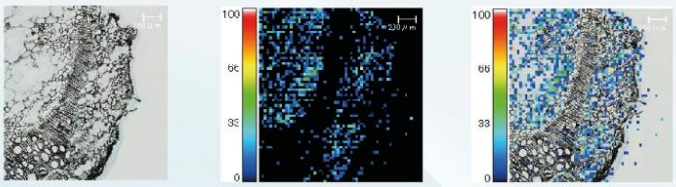

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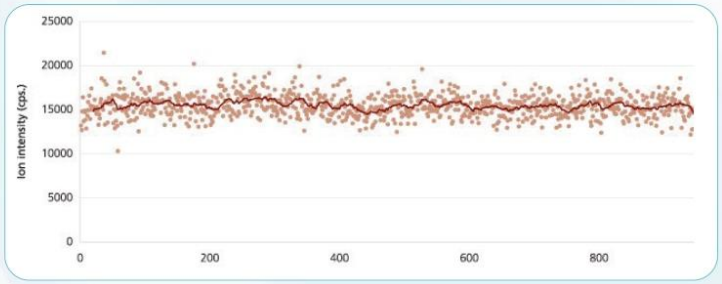

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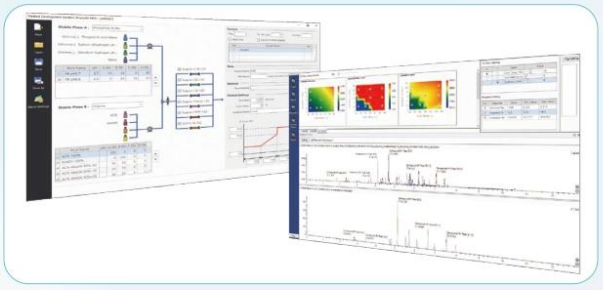

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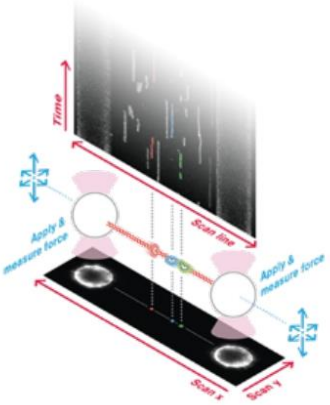

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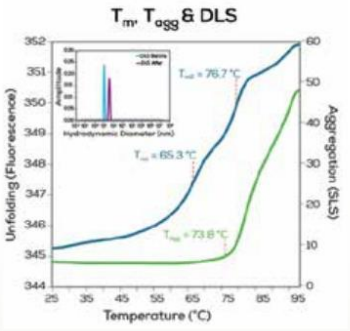

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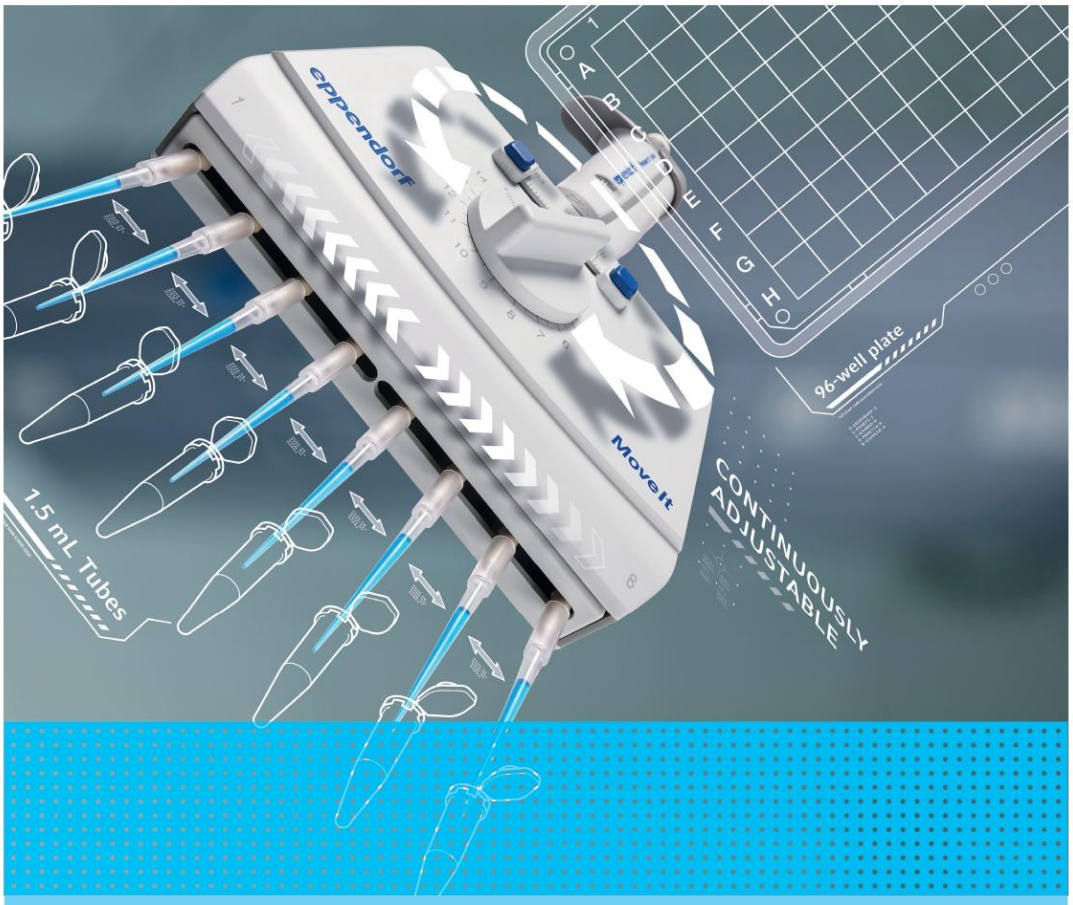

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Fig. 1. Porównywalna wydajność reakcji PCR z wykorzystaniem Color Taq PCR Master Mix (2x) nr kat. E2525 na matrycach DNA uzyskanych zestawami z kuleczkami magnetycznymi i kolumienkowym. MM - Perfect 100-1000 bp DNA Ladder (nr kat. E3141). K+ DNA z cisu, K- kontrola negatywna.

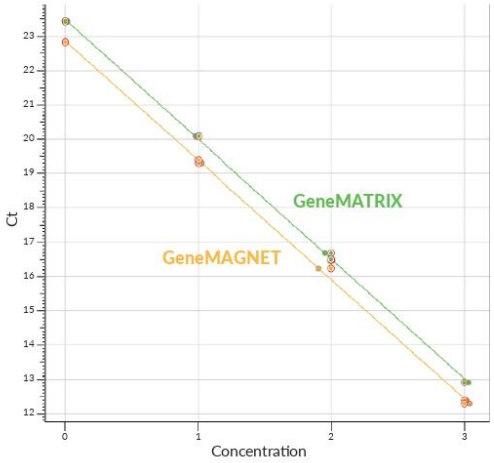

Fig. 2. Bardzo dobra wydajność reakcji qPCR Fast SG qPCR Master Mix (2x) nr kat. E0411 na matrycy DNA uzyskanej zestawem z kuleczkami magnetycznymi GeneMAGNET Food DNA Purification Kit vs. zestaw kolumienkowy GeneMATRIX Food-Extract DNA Purification Kit (nr kat. E3525) ze śruty sojowej.

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GeneMAGNET Wydajność reakcji: 93.97%,
Współczynnik nachylenia krzywej: -3.48,
Błąd: 0.008

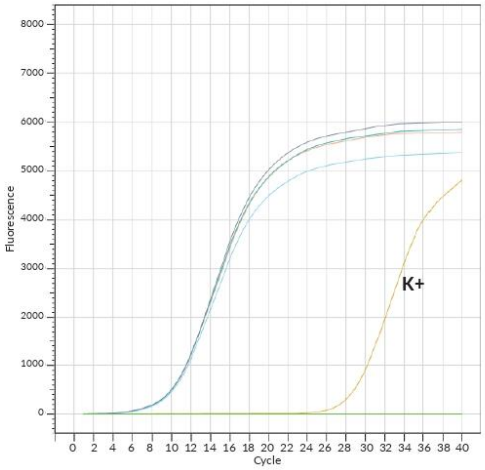

Fig. 3. Detekcja ssaczego genu kodującego MT-CYB z qPCR Fast SG qPCR Master Mix (2x) nr kat. E0411 na matrycy DNA oczyszczonej z parówki zestawem GeneMAGNET Food DNA Purification Kit. K+: kontrola pozytywna.

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Autoryzowany dystrybutor

Od lat skupiamy się na współpracy z jednostkami naukowymi i dokładamy wszelkich starań, aby podążać za wymaganiami Klientów. Oferujemy szeroki wybór najwyższej jakości odczynników oraz innowacyjnych rozwiązań do badań naukowych. Zapewniamy profesjonalną obsługę, szybką realizację zamówień, a także wsparcie techniczne i wszelką pomoc merytoryczną.

Jesteśmy przedstawicielem czołowych firm działających na globalnym rynku Life Science - Cell Signaling Technology, New England BioLabs oraz Norgen Biotek.

- Przeciwciała pierwszo i drugorzędowe oraz odczynniki do analiz WB, IP, IF, IHC, ChIP.
- Zestawy ELISA.
- Testy komórkowe i biochemiczne.
- Zestawy do izolacji i oczyszczania DNA.
- Zestawy do izolacji i oczyszczania RNA.
- Zestawy do pobierania i przechowywania materiału biologicznego.
- Odczynniki do przygotowania bibliotek NGS.
- Produkty do Real-Time PCR.
- Polimerazy DNA i RNA.
- Odwrotne transkryptazy.
- Zestawy do PCR i RT-PCR.
- Enzymy restrykcyjne.
- Ligazy.
- Enzymy modyfikujące RNA/DNA.
- Zestawy odczynników do klonowania, mutagenezy i transformacji.
- Komórki kompetentne.
- Systemy do edycji genów CRISPR/Cas-9 oraz odczynniki do transfekcji.
- Drabinki i markery wielkości DNA, RNA i białek.
- Odczynniki do badań epigenetycznych i glikobiologii.
- Inhibitory, aktywatory, cytokiny, czynniki wzrostu, hormony, białka rekombinowane.
- Bufory, roztwory barwiące i inne odczynniki pomocnicze.
- Wiele innych odczynników niezbędnych w biologii molekularnej.

SARSTEDT

Life Science Products

Badania PCR i biologia molekularna

Certyfikowane produkty wysokiej jakości do badań (q)PCR

W razie pytań: Chętnie służymy pomocą.

Znajdź poszukiwany wariant płytki PCR na naszej stronie internetowej.

Skontaktuj się bezpośrednio z naszym Account Managerem:

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LKB Biotech

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www.lkb-biotech.pl

- ✓ Zestawy do fluorescencyjnego znakowania białek w elektroforezie 1D i Western Blotting
- ✓ Zestawy do multipleksowego fluorescencyjnego znakowania białek – elektroforeza 2D i technika DIGE
- ✓ Zestawy do analizy białek różniących się potencjałem REDOX
- ✓ Oprogramowanie do zaawansowanej analizy obrazów żeli 2D
- ✓ Substraty chemiluminescencyjne (ECL) do Western Blotting i ELISA oraz zestawy do oznaczania białek metodą BCA
- ✓ Membrany do western blottingu
- ✓ Gotowe kolumny i złoża do chromatograficznego rozdzielania białek (chromatografia jonowymienna, białka rekombinowane - złoża niklowe, złoża z białkiem A i inne)
- ✓ Kolumienki typu „spin", kolumny grawitacyjne oraz gotowe mikropłytki do oczyszczania próbek
- ✓ Kolumienki/koncentratory do zagęszczania, separowania, odsalania roztworów białkowych, kwasów nukleinowych, peptydów
- ✓ Płytki filtracyjne do „high throughput analysis" z różnorodną gamą membran i mediów do prefiltrowania, sterylizowania, zagęszczania, frakcjonowania, wymian buforowych
- ✓ Kapsuły filtracyjne oraz filtry strzykawkowe do prefiltrowania, oczyszczania i sterylizowania mediów, buforów, hodowli komórkowych
- ✓ Filtracja styczna (tangencjalna- TFF) do zagęszczania, frakcjonowania, odsalania roztworów białkowych, kwasów nukleinowych, peptydów
- ✓ Filtry oddechowe do hodowli komórkowych
- ✓ Odczynniki do syntezy oligonukleotydów