6th BIO Life Science Congress, Poznań, Poland

Abstract Book

September 17th–20th 2025

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Session 6 – Nucleic Acids in Disease and Therapy – Agnieszka Fiszer, Institute of Bioorganic Chemistry PAS, Poznan; Jacek Jemielity, Centre of New Technologies, University of Warsaw

PROTEINS & METABOLITES

Session 1 – Protein Chemistry and Structure – Marcin Nowotny, International Institute of Molecular and Cellular Biology Warsaw; Miłosz Ruszkowski, Institute of Bioorganic Chemistry PAS, Poznan
Session 2 – Protein Biology – Adam Szewczyk, Nencki Institute of Experimental Biology PAS Warsaw; Marek Tchórzewski, Faculty of Biology and Biotechnology, Maria Curie-Sklodowska University, Lublin
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Session 5 – Transport & Signalling Pathways – Agnieszka Dobrzyń, Nencki Institute of Experimental Biology PAS Warsaw; Michał Jasiński, Institute of Bioorganic Chemistry PAS, Poznan
Session 6 – Bioactive Compounds and Small Molecules – Marcin Drąg, Wrocław University of Science and Technology Izabela Sadowska-Bartosz, Faculty of Technology and Life Sciences, University of Rzeszów

CELLS & ORGANISMS

Session 1 – Cell Biology – Małgorzata Borowiak, Faculty of Biology, Adam Mickiewicz University, Poznan; Józef Dulak, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow
Session 2 – Developmental Biology – Karolina Archacka, Faculty of Biology, University of Warsaw; Maria Anna Ciemerych-Litwinienko, Faculty of Biology, University of Warsaw
Session 3 – Neurobiology – Aleksandra Pękowska, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw; Dariusz Rakus, Faculty of Biology, University of Wroclaw
Session 4 – Innovative Biomedicine – Natalia Rozwadowska, Institute of Human Genetics PAS, Poznan; Barbara Uszczyńska-Ratajczak, Institute of Bioorganic Chemistry PAS, Poznan
Session 5 – Biotechnology and Microbiology – Katarzyna Hrynkiewicz, the Nicolaus Copernicus University Torun; Grzegorz Węgrzyn, Faculty of Biology, University of Gdańsk
Session 6 – Plant Biology – Paweł Bednarek, Institute of Bioorganic Chemistry PAS, Poznan; Szymon Świeżewski, Institute of Biochemistry and Biophysics PAS, Warsaw

ADDITIONAL SESSIONS

Session of the Molecular Mechanisms of Motility Section of the Polish Biochemical Society – Joanna Moraczewska, Casimir the Great University Bydgoszcz
Session of the Bioenergetic Section and Biological Membranes Section of the Polish Biochemical Society – Wiesława Jarmuszkiewicz, Faculty of Biology, Adam Mickiewicz University, Poznan; Adam Szewczyk, Nencki Institute of Experimental Biology PAS Warsaw; Piotr Koprowski, Nencki Institute of Experimental Biology PAS Warsaw
Career Development in Academia: Fellowships Opportunities – Piotr Laidler Jagiellonian University, Krakow; Bogusz Kulawiak, Nencki Institute of Experimental Biology PAS Warsaw
Applied Biotechnology – Jakub Dalibor Rybka, NanoBioMed Adam Mickiewicz University, Poznan
Acta Biochimica Polonica – news and perspectives – Grzegorz Węgrzyn, Faculty of Biology, University of Gdańsk
Flow Cytometry: Powering Discovery – Lidia Gackowska, Nicolaus Copernicus University, Torun; Department of Laboratory Diagnostics, Jan Biziel University Hospital No. 2 in Bydgoszcz, Bydgoszcz, Poland
Paulina Jackowiak, Institute of Bioorganic Chemistry PAS, Poznan

Invited Speakers

KEYNOTE SPEAKERS

Magdalena Żernicka-Goetz, *University of Cambridge, UK, California Institute of Technology, CA, USA*
Harold Varmus, *Weill Cornell Medicine, New York, USA*
Virginijus Šikšnys, *Vilnius University, Vilnius, Lithuania*
Nikolaus Rajewsky, *Max-Delbrück-Center, Berlin, Germany*
Adrian Krainer, *Cold Spring Harbor Laboratory, New York, USA*

NUCLEIC ACIDS

Session 1 – Nucleic Acids Chemistry and Structure – Jacek Jemielity *CeNT University of Warsaw*
Session 2 – Nucleic Acid Biology – Wojciech Branicki *Jagiellonian University, Kraków*
Session 3 – Nucleic Acid Bioinformatics – Bartosz Wilczyński *University of Warsaw*
Session 4 – Genetics and Epigenetics – Tomasz K. Wojdacz *Pomeranian Medical University in Szczecin*
Session 5 – Genomics and Transcriptomics – Kinga Kamieniarz-Gdula *Faculty of Biology, AMU Poznan*
Session 6 – Nucleic Acids in Disease and Therapy – Andrzej Dziembowski *International Institute of Molecular and Cellular Biology, Warsaw*

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Session 2 – Protein Biology – Marcin Suskiewicz *Centre of Molecular Biology, CNRS, Orleans, France*
Session 3 – Proteomics and Metabolomics – Maciej Łałowski, Faculty of Biology, AMU Poznan
Session 4 – Molecular Machines and Tools – Wojciech Pokrzywa *International Institute of Molecular and Cellular Biology, Warsaw*
Session 5 – Transport & Signalling Pathways – Małgorzata Janicka *University of Wroclaw*
Session 6 – Bioactive Compounds and Small Molecules – Karolina Pierzynowska *Faculty of Biology, University of Gdansk*

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Session 3 – Neurobiology – Aleksandra Pękowska *Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw*
Session 4 – Innovative Biomedicine – Piotr Trzonkowski *Medical University of Gdańsk / PolTREG S.A.*
Session 5 – Biotechnology and Microbiology – Paulina Niedźwiedzka-Rystwej *University of Szczecin*
Session 6 – Plant Biology – Magdalena Krzymowska *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw*

Evaluation Comissions

Comissions for non-PI short talks

Nucleic Acids:
Jolanta Jura
Piotr Ziółkowski
Andrzej Dziembowski
Kinga Kamieniarz-Gdula
Barbara Nawrot

Proteins & Metabolites:
Marek Tchórzewski
Marcin Drąg
Marcin Suskiewicz
Karolina Pierzynowska
Michał Dadlez

Cells & Organisms
Maria Anna Ciemerych-Litwinienko
Paweł Bednarek
Małgorzata Borowiak
Aleksandra Pękowska
Józef Dulak

Additional sessions:
Adam Szewczyk
Grzegorz Węgrzyn
Lidia Gackowska
Joanna Moraczewska
Katarzyna Hrynkiewicz

Best short talk presentations:

Maja Szymańska-Lejman for the short talk entitled „Reprogramming meiotic recombination at hotspots in Arabidopsis through targeted chromatin modification"
Olga Wójcicka (*the FEBS Journal Prize*) for the short talk entitled „The role of Cap2 in the neuromuscular system"
Michał Świrski for the short talk entitled „Massive aggregation of ribosome profiling data reveals hidden translational events and regulatory complexity"
Anna Jarząb for the short talk entitled „Meltome Atlas development and its utility in assessing the impact of fever on bacterial infections"
Marta Białobrzeska for the short talk entitled „Patient-derived induced pluripotent stem cells-based model uncovers cardiomyocyte-specific dystrophin Dp427 preservation and Dp116 expression in Duchenne Muscular Dystrophy caused by atypical splicing of DMD gene"

Commision for posters' presentations

Poster session 1, posters P.1 – P.62
Elżbieta Kierzek
Wojciech Branicki
Miłosz Ruszkowski
Karolina Archacka
Wiesława Jarmuszkiewicz
Piotr Trzonkowski

Poster session 2, posters P.63 – P.124
Marta Szachniuk
Maciej Łałowski
Dominik Strapagiel
Anna Marusiak
Bogusz Kulawiak
Jacek Jemielity

Poster session 3, posters P.125 – P.187
Bartosz Wilczyński
Paulina Niedźwiedzka-Rystwej
Magdalena Łuczak
Szymon Świeżewski
Jakub Dalibor Rybka
Małgorzata Janicka

Poster session 4, posters P.188 – P.248
Paulina Jackowiak
Piotr Koprowski
Barbara Uszczyńska-Ratajczak
Agnieszka Fiszer
Magdalena Krzymowska
Tomasz K. Wojdacz

Best poster presentations:

The Włodzimierz Mozołowski Prize & the FEBS Journal Prize for the best poster presentation:
Natalia Ziojła for the poster entitled „New Insights into V-ATPase Dysfunction: Characterization of a Novel Mutation in Patient Human Fibroblasts"

Distinctions:
Anna Karłowicz for the poster entitled „Pol γB interacts with Pol β in human mitochondria under non-oxidative conditions and regulates its dRP lyase activity to facilitate short-patch DNA repair"
Sara Henry for the poster entitled „Dissecting the role of ETV4 and ETV5 in human pluripotent stem cell state transitions"
Arun Kumar for the poster entitled „Ribosomes as zinc storage modules and their functional role during aging in Caenorhabditis elegans"
Sumita Majhi for the poster entitled „Bridging the missing links in the Nexin-Dynein Regulatory Complex"

Contents

Parnas Lecture

L.PA.1

TENT5-mediated polyadenylation of mRNAs encoding secreted proteins is essential for gametogenesis in mice

Andrzej Dziembowski

Laboratory of RNA Biology, International Institute of Molecular and Cell Biology, Warsaw, Poland
Andrzej Dziembowski <adziembowski@iimcb.gov.pl>

Cytoplasmic polyadenylation plays a vital role in gametogenesis; however, the participating enzymes and substrates in mammals remain unclear. Using knockout and knock-in mouse models, we describe the essential role of four TENT5 poly(A) polymerases in mouse fertility and gametogenesis. TENT5B and TENT5C play crucial yet redundant roles in oogenesis, with the double knockout of both genes leading to oocyte degeneration. Additionally, TENT5B-GFP knock-in females display a gain-of-function infertility effect, with multiple chromosomal aberrations in ovulated oocytes. TENT5C and TENT5D both regulate different stages of spermatogenesis, as shown by the sterility in males following the knockout of either gene. Finally, Tent5a knockout substantially lowers fertility, although the underlying mechanism is not directly related to gametogenesis. Through direct RNA sequencing, we discovered that TENT5s polyadenylate mRNAs encoding endoplasmic reticulum-targeted proteins essential for gametogenesis. Sequence motif analysis and reporter mRNA assays reveal that the presence of an endoplasmic reticulum-leader sequence represents the primary determinant of TENT5-mediated regulation.

Plenary Lectures

L.PL.1

Modeling Human Development Beyond Implantation Using Integrated Stem Cell-Derived Models

Magdalena Żernicka-Goetz

University of Cambridge, UK, California Institute of Technology, CA, USA
Magdalena Żernicka-Goetz <mzg@mole.bio.cam.ac.uk>

Our understanding of human development beyond implantation has been historically limited by the lack of suitable in vitro models. I will present our lab's work on developing advanced stem cell-based systems that model post-implantation stages without the use of intact human embryos. Building on knowledge gained from early developmental biology, we have assembled stem cell populations representing embryonic and extra-embryonic lineages into integrated, embryo-like structures. These stem cell-derived models recapitulate key events of early morphogenesis, including the specification of primordial germ cell precursors. By focusing on these highly controlled models, we are uncovering the cellular and molecular mechanisms that shape early human development, and opening doors to studying processes that were previously out of reach.

L.PL.2

Cancer Genetics: Past, Present, and Future

Harold Varmus

Meyer Cancer Center, Weill Cornell Medicine, New York City, NY, USA
Harold Varmus <varmus@med.cornell.edu>

Earlier this year, the Cold Spring Harbor Laboratory (CSHL) held a remarkable meeting on the history of cancer genetics (https://library.cshl.edu/Meetings/Cancer-Genetics/), coincident with the fiftieth anniversary of the discovery of cellular progenitors of retroviral oncogenes.I will begin my lecture in Poznań with some reflections on the scientific and clinical consequences of this discovery, as summarized at the CSHL meeting; then I will describe some current developments in cancer biology, from my laboratory and others, that are influencing approaches to the control of cancer; and finally I will comment on the factors – biological and political – that will determine the pace at which further progress is likely to occur.More specifically, I will consider several topics in a temporal framework.The Past: how the identification of cancer genes helped to shape our understanding of cell signaling and physiology and created a path to genetically targeted drug therapies.The Present: how mutational and epigenetic changes contribute to drug resistance and to phenotypic transformation during cancer evolution, and how drug combinations, novel immunotherapies, and the toxic effects of oncogenic hyperactivity might reduce resistance.The Future: how the intrinsic complexity of cancer cells and political movements that are undermining science in the United States and elsewhere might slow or subvert the current rate of progress against human diseases, including cancers.

L.PL.3

Arms race in microbial world: from antiphage defense to genome editing and beyond

Virginijus Šikšnys

Vilnius University, Life Science Center, Vilnius, Lithuania
Virginijus Šikšnys <siksnys@ibt.lt>

Bacteria are constantly exposed to viral (bacteriophage) threats. In response, bacteria evolved a wide range of antiphage defense barriers that that can collectively be referred to as a bacterial immune system. It relies on a combination of innate and adaptive mechanisms exemplified by restriction-modification and CRISPR-Cas systems, respectively. CRISPR-Cas immunity is enabled by programmable nucleases that act as DNA scissors that recognize and destroy invading nucleic acids1. Easy programmability of CRISPR-Cas nucleases paved the way for the development of versatile tools for targeted genome engineering. Currently, Cas9 and Cas12 CRISPR-Cas nucleases are rapidly advancing into the clinics for the treatment of different diseases. Despite emerging clinical successes, there are challenges that limit broader therapeutic applications of genome editing technology, therefore, novel tools that are more compact, more precise and safer are highly desirable. Interestingly, CRISPR-Cas nucleases that destroy invading phages evolved from mobile genetic elements (MGEs), like bacterial transposons, illustrating the interplay between antiphage defense and mobile genetic elements. Recently, very compact RNA-directed transposon-related TnpB nucleases have been identified enabling the development of a new class of DNA-scissors2. In last few years, a large number of new antiviral defense systems was identified that do not target invading nucleic acids but kill the host cell or arrest its metabolism, e.g., by depleting key metabolites3. Further exploration and systematic discovery of novel antiviral defense systems is likely to decode novel enzymatic functions that could be repurposed for technological applications.

References:
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Karvelis T, Druteika G, Bigelyte G, Budre K, Zedaveinyte R, Silanskas A, Kazlauskas D, Venclovas Č, Siksnys V. Nature. 2021 599(7886):692-696. doi: 10.1038/s41586-021-04058-1.
Tamulaitiene G, Sabonis D, Sasnauskas G, Ruksenaite A, Silanskas A, Avraham C, Ofir G, Sorek R, Zaremba M, Siksnys V. Nature. 2024 627(8003):431-436. doi: 10.1038/s41586-024-07092-x.

L.PL.4

Predicting disease trajectories

Nikolaus Rajewsky

Max-Delbrück-Center, Berlin, Germany
Nikolaus Rajewsky <rajewsky@mdc-berlin.de>

I will present our recent as well as unpublished work how to quantify RNA expression in tissue slices at sub-cellular resolution. I will discuss how these data enable the systematic discovery of the molecular pathways which are driving phenotypes in these tissues. I will present data where we quantified, in space and in time, gene expression during tumorigenesis in a triple negatigve breast cancer model, starting from the cell of origin and up to the invasive tumor. These data allow unprecedented mechanistic understanding of the molecular "forces" that define the space-time trajectory of the tumors (including the tumor stroma interface). I will also discuss a new computational approach that allows (coupled to large language AI models) to interrogate all sequencing data from the human cell atlas or published spatial omics in real time.

L.PL.5

Antisense Oligonucleotides for Diffuse Midline Glioma

Adrian Krainer

Cold Spring Harbor Laboratory, New York, USA

Nucleic Acid Chemistry and Structure

Lectures

L.1.1

New developments in mRNA technology

Jacek Jemielity

Centre of New Technologies, University of Warsaw, Warsaw, Poland; ExploRNA Therapeutics, Warsaw Poland
Jacek Jemielity <j.jemielity@cent.uw.edu.pl>

Messenger RNA (mRNA) technology has revolutionized modern medicine, with applications ranging from vaccines to personalized therapies. However, its broader use still faces challenges such as limited stability, suboptimal translation efficiency, and immune activation. Recent work from our group addresses these issues through two complementary approaches. First, we have developed AvantCap, a novel class of synthetic cap analogs enabling unprecedented control over mRNA translation and stability (J. Am. Chem. Soc., 2024). AvantCap structures can fine-tune protein expression levels, offering new possibilities for therapeutic mRNA optimization. Second, we have advanced the field of circular RNAs (circRNAs), which exhibit exceptional stability and sustained translation. Our recent study (Nat. Commun., 2025) describes a new strategy for efficient in vitro synthesis of translatable circRNAs, overcoming long-standing technical barriers. Together, these innovations expand the molecular toolbox for RNA-based therapeutics, opening new avenues for next-generation vaccines, protein replacement therapies, and potentially durable gene modulation.

Oral presentations

O.1.1

Post-synthetic RNA modifications in focus: what we gain and what we risk

Paulina Kuwerska, Karolina Podskoczyj, Agnieszka Dziergowska, Grazyna Leszczynska

Institute of Organic Chemistry, Faculty of Chemistry, Lodz University of Technology, Poland
Grażyna Leszczyńska <grazyna.leszczynska@p.lodz.pl>

Currently, more than 180 modified nucleosides have been identified across all classes of cellular RNAs, with transfer RNAs (tRNAs) exhibiting the highest density and diversity of modifications. Studies utilizing chemically synthesized modified nucleosides and site-specifically modified RNAs are providing critical insights into the structural and functional consequences of individual modifications, thereby advancing our understanding of their biological roles.
Herein, we present our contribution to improving chemical access to site-specifically modified oligoribonucleotides *via* post-synthetic strategy of RNA modification. This approach offers several key advantages. It enables the incorporation of labile or reactive groups, that may not tolerate standard synthesis or deprotection conditions (e.g. >C=S, –COOH, –SO□ H, –CHO, fluorescent dyes, photoreactive groups, disulfide crosslinks and nitroxide spin labels). Aditionally, it allows for the efficient generation of homologous RNA sequences bearing diverse modifications. Post-synthetic conversion conditions are however limited to polar solvents, temperatures below 60 °C, and reaction times under 24 hours to preserve RNA integrity and minimize non-specific modifications. All the above-mentioned aspects of the post-synthetic RNA modification strategy will be illustrated in the presentation using several *in-house* developed examples .

O.1.2

First crystal structures of DNA:2′-*O*Me-RNA and RNA:2′-*O*Me-RNA heteroduplexes

Rafał Dolot, Anna Maciaszek, Barbara Mikołajczyk, Barbara Nawrot

Division of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland.
Rafał M. Dolot <rafal.dolot@cbmm.lodz.pl>

2'-*O*-methylation (2'-*O*Me) is a common post-transcriptional RNA modification found in all kingdoms of life, but its distribution between Archaea, eukaryotes and bacteria is not uniform. The 2'-*O*Me decorates the cap moiety of mRNAs as well as non-coding and regulatory RNAs, including tRNAs, rRNAs, snRNAs and miRNAs. While the chemical properties of this modification do not define specific functional roles, it is evident that methylation of the 2'-hydroxyl group, which is often involved in contacts to form higher-order RNA structures, can affect RNA secondary structure, its hydrogen bonding potential, and RNA/nucleic acid and RNA-protein interactions. The use of 2'-OMe RNAs offers several advantages over unmodified models for biopharmaceutical applications, such as a significantly higher binding affinity compared to unmodified duplexes for identical sequences and the delay of oligonucleotide degradation by nucleases. So far, there is only one solved NMR structure of a DNA/2'-*O*Me-RNA duplex. In our studies, we have successfully determined high-resolution crystal structures of DNA:2'-*O*Me-RNA and RNA:2'-*O*Me-RNA duplexes for the first time. Knowledge of the molecular structures of these duplexes could be useful not only to use these models for solving future structures with this motif, such as molecular beacons or complexes with target proteins, but also for the design of a new type of nanostructures containing these types of duplexes.

O.1.3

Naphthyridine carbamate dimer ligand induces formation of Z-RNA-like fold of disease-related RNA and serves as a molecular glue for crystal lattice formation

Martyna Mateja-Pluta[1], Leszek Błaszczyk[1], Magdalena Bejger[1], Kazuhiko Nakatani[2], Agnieszka Kiliszek[1*]

[1]Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego Street 12/14, Poznań; [2]Department of Regulatory Bioorganic Chemistry, SANKEN (The Institute of Scientific and Industrial Research), Osaka University, 8-1 Mihogaoka, Ibaraki, 567-0047, Japan
Agnieszka Kiliszek <kiliszek@ibch.poznan.pl>

RNA emerges as an attractive therapeutic target for neurological disorders, given its involvement in diverse disease mechanisms. Small molecules offer advantages over antisense oligonucleotides, particularly in disorders involving repeat expansions like fragile X syndrome and Huntington's disease.

The Nakatani research team's methodologies, utilizing screening assays, have identified promising small molecules, exemplified by naphthyridine, which exhibits robust affinity for G-rich RNA sequences. This molecule shows potential in addressing conditions such as spinocerebellar ataxia type 31, binding to specific RNA structures with high specificity and inducing structural alterations crucial for disease onset. In this study, we present the structural analysis of the NCD ligand bound to RNA containing the UGGAA/UGGAA motif associated with spinocerebellar ataxia type 31 (SCA31). We characterized two crystal structures of RNA-ligand complexes, along with a previously unreported structure of RNA without ligand binding. Our findings elucidate that the NCD ligand is positioned between RNA molecules related by symmetry, contributing additional interactions within the crystal lattice. This highlights the potential of the NCD ligand as a molecular glue to facilitate crystal formation.

Acknowledgements
Funding: NCN Poland: UMO-2017/26/E/NZ1/00950; UMO-2022/45/B/NZ7/03543.

O.1.4

Single-Particle Cryo-EM of SAH-responsive riboswitch from *Pseudomonas aeruginosa*: a bumpy road to success

Katarzyna Woźniak[1], Agnieszka Ruszkowska[1], Elżbieta Kierzek[1], Jakub Nowak[2], Maciej Antczak[1,3], Joanna Sarzyńska[1,3], Mariusz Popenda[3], Piotr H. Malecki[1], Sebastian Glatt[2], Miłosz Ruszkowski[1], Marta Szachniuk[1,3], Krzysztof Brzezinski[1]

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Infections caused by *Pseudomonas aeruginosa* pose a medical problem worldwide. Thus, there is a need to identify new molecular targets to fight this pathogen. Its genome encodes an operon whose translation is regulated by *S*-adenosyl-□-homocysteine (SAH), a byproduct of *S*-adenosyl-□-methionine (SAM)-dependent methylations and an inhibitor of SAM-dependent methyltransferases. The concentration of SAH controls the expression of the operon *via* the SAH-responsive riboswitch. Binding of SAH activates the expression of five downstream genes encoding (1) SAH hydrolase, (2) an alarmone hydrolase, (3) methylenetetrahydrofolate reductase, (4) a glycosyltransferase and (5) an RNA helicase.

We used single-particle cryo-EM to determine the riboswitch structures in a complex with SAH and SAH-free form. Riboswitches are small and flexible molecules, thus challenging for cryo-EM study. We encountered many challenges throughout the project, analyzing various variants. We performed extensive optimizations to establish ideal cryo-EM sample preparation conditions and acquired data, which allowed us to reconstruct an EM map for the SAH-free aptamer domain with a resolution of ~5.9 Å. Automated atomic modeling in EM maps at this resolution failed. Thus, an initial model was generated with RNAComposer and then refined. This model provides a basis for structure-based design of novel antimicrobials.

Acknowledgements

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Nucleic Acid Biology

Lectures

L.2.1

DNA profiling in the study of human individuality.

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Advanced genomic tools have greatly enriched research into the natural phenotypic diversity of modern humans. GWAS have enabled the identification of genes responsible for important physical traits that show high heritability, such as pigmentation, height and facial features. Current research focuses on understanding the significance of rare DNA variants, which requires studying large cohorts and performing whole-genome analyses. Yet, human individuality arises not only from genetic factors, but also from environmental influences. Until recently, capturing the influence of lifestyle through DNA analysis seemed unattainable but a growing body of research shows that environmental factors can leave distinct signatures in the human methylome. EWAS have not only enabled the development of epigenetic clocks for age estimation and BMI classifiers, but also for making inferences about specific human behaviors. Our research shows that combining genetic and epigenetic data can significantly improve the reconstruction of an individual person's image. The potential use of DNA profiling to determine individual characteristics of a person is being intensively researched for forensic applications, although the resulting tools may also be useful in anthropology and molecular archaeology. Improving the accuracy of predictive models and overcoming technological limitations are crucial for the widespread practical application of these new methods.

Oral presentations

O.2.1

Tightly controlled yet highly mutagenic: exploring the regulation of human DNA polymerase iota

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Translesion synthesis (TLS) polymerases are specialized enzymes that enable DNA replication across damaged templates, playing a critical role in maintaining genomic integrity. However, TLS enzymes act as a double-edged sword; while they promote cell survival under genotoxic stress, they are inherently error-prone and must be tightly regulated. DNA polymerase iota (Polι) is the most mutagenic human DNA polymerase and a very enigmatic TLS enzyme; however, its specific cellular role is still not fully understood.

Our research focuses on dissecting the regulatory mechanisms that govern Polι abundance and activity in human cells. We explore both transcriptional and posttranslational regulation, including the role of protein-protein interactions and posttranslational modifications. Polι is subject to acetylation, as well as mono- and polyubiquitination, with the latter being promoted upon inhibition of the p300 acetyltransferase, further influencing its stability and function.

In this presentation, we will share new data elucidating the molecular mechanisms that control Polι at various levels. Our findings underscore the importance of understanding Polι regulation to prevent its uncontrolled activity, which could lead to mutagenesis and contribute to tumorigenesis. These insights provide a foundation for future strategies aimed at modulating TLS polymerase activity in disease contexts.

O.2.2

Detection of extracellular cancer-derived circRNAs

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O.2.3

Parallel and antagonistic functional roles of MCPIP1 and MCPIP3 ribonucleases in skin homeostasis

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MCPIP1 and MCPIP3 are members of the MCPIP family, also known as the Regnase family. All MCPIP proteins share two conserved domains: a CCCH-type zinc finger domain and a PIN-like domain responsible for RNase activity. The expression of both *ZC3H12A* (encoding MCPIP1) and *ZC3H12C* (encoding MCPIP3) is increased in psoriatic lesions. We further showed that in human keratinocytes the expression of MCPIP1 and MCPIP3 is induced by psoriasis-related cytokines.

Keratinocyte-specific deletion of *ZC3H12A* and *ZC3H12C* genes results in distinct phenotypic outcomes. Silencing of MCPIP1 in keratinocytes promotes the expression of proinflammatory factors, suggesting a modulatory role in cutaneous inflammatory responses. Mice with conditional deletion of MCPIP1 develop progressive skin inflammation and are more susceptible to the development of imiquimod-induced inflammation. In contrast, our recent studies showed that the deletion of MCPIP3 does not exacerbate inflammatory processes in the skin, but results in an increased rate of epidermal proliferation and abnormal differentiation. This process is mediated by the negative regulation of key factors regulating cell division and *via* direct interaction of MCPIP3 with 14-3-3 proteins and modulators of cell polarity.

In conclusion, both MCPIP1 and MCPIP3 RNases are essential for preserving skin homeostasis, partially *via* functionally interconnected regulatory networks.

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O.2.4

Transposons regulate stress response by sequestering HSF-1 in condensates.

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HSF-1 is an essential eukaryotic transcription factor that forms nuclear stress bodies (nSBs) in response to heat. The exact function of these structures remains unknown. Through quantitative microscopy analysis of *C. elegans* embryos, we found that during heat shock, HSF-1 condenses into four major nSBs, in addition to tens of minor ones. Surprisingly for a transcription factor, HSF-1 foci remain prominent even on packed chromatin throughout mitosis when transcription is severely halted. Moreover, formation of these condensates is not disturbed by RNA Polymerase II depletion. The majority of HSF-1 binding sites in the *C. elegans* genome reside within Helitrons, a class of DNA transposons. Using DNA FISH, we show that major nSBs strongly co-localize with Helitron repeats. Analysis of the genome sequence identified several regions with extremely high local enrichment of HSF-1 binding sites. Using CRISPR-Cas9 editing, we deleted one of these regions, which resulted in the disappearance of two major nSBs. Examination of gene expression changes in this transgenic strain revealed increased expression of stress-related genes during heat shock, but also in basal conditions. Our work unveils a surprising role for transposable elements in modulating stress response and opens new avenues for understanding the evolutionary significance of repetitive elements in cellular stress adaptation mechanisms.

Nucleic Acid Bioinformatics

Lectures

L.3.1

Predicting function of non-coding DNA elements – is AI up to this challenge?

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We know that a typical genome of a multicellular organism contains hundreds of thousands of non-coding regulatory elements. Even if we focus on the most well-known two types of these elements: enhancers and promoters, we know that there are more than 200k promoters (according to Ensembl database) and databases like EnhancerAtlas2.0 list more than 6 million data-poitns on cell-type specific enhancer activity.
While such vast amounts of data would suggest that our knowledge of these elements is nearly complete, our understanding on exactly why these exact sequences are active in the tissues they are, and which mutations could diminish or enhance their activity is very limited. And it is an important question, given that the majority of variants associated with diseases in QTL studies are located in non-coding parts of the genome.
Recently, great advances were made not only in documenting the locations of functional enhancers and promoters using ChIP-Seq and ATAC-Seq methods, but also in creating various types of Artificial Neural Network models to predict their function based on the sequence alone, or together with some evolutionary or epigenetic information. We will discuss some of our experiences in generating datasets concening active ehnancers and promoters in human Gliomas as well as our experiences in building ANN models for predicting enhancer and promoter activity. While in some areas these models seem to be very promising, there are still major challenges ahead of us.

Oral presentations

O.3.1

Predicting RNA secondary structure with SQUARNA

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Non-coding RNAs play a diverse range of roles in various cellular processes, with their spatial structure being pivotal to their function. The RNA's secondary structure is a key determinant of its overall fold. Given the scarcity of experimentally determined RNA 3D structures, understanding the secondary structure is vital for discerning the molecule's function. Currently, there is no universally effective solution for de novo RNA secondary structure prediction. Existing methods are becoming increasingly complex without marked improvements in accuracy, and they often overlook critical elements such as pseudoknots. In this work, we introduce SQUARNA, a novel approach to de novo RNA secondary structure prediction. Our benchmarks demonstrate that SQUARNA outperforms leading methods in both single-sequence and alignment-based predictions. Additionally, SQUARNA can predict pseudoknots and incorporate chemical probing data. SQUARNA is available at https://github.com/febos/SQUARNA.

O.3.2

Massive aggregation of ribosome profiling data reveals hidden translational events and regulatory complexity

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Bacterial ribosomes are well-known antibiotic targets, but human ribosome activity is also emerging as a versatile therapeutic entry point. Cancer-specific ribosome-stalling patterns can be pharmacologically exploited, and the "dark proteome" – peptides derived from short, previously overlooked, translated regions (translons), is a rich source of cancer neoantigens.

Ribosome profiling introduced a new paradigm for studying translation, enabling transcriptome-wide interrogation of translational events. Despite its potential, individual studies are limited in their ability to uncover translational phenomena due to a lack of sequencing depth and a narrow focus on specific hypotheses. Here, we processed over half a trillion reads from nearly all public ribosome profiling datasets and its variations, spanning more than 15000 samples in 120 cell lines, 43 tissues, and hundreds of experimental treatments across 81 species.

Through massive data aggregation, we explored genetic decoding diversity including observation of novel phenomena like abortive translation and pervasive intron translation. We annotated thousands of novel translons and through data stratification probed their mutual relationships and revealed tissue-specific translation regulation. We developed an integrated analysis and visualization platform, RiboCrypt, that leverages the power of analyzing thousands of datasets in parallel. Effectively, RiboCrypt provides the most extensive translation atlas to date.

O.3.3

Quantifying interface accuracy in predicted RNA-protein complexes

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Accurate prediction of RNA-protein complexes is essential for understanding their roles in gene regulation, catalysis, and structural organization, yet evaluating the accuracy of computational models remains a challenge in modern structural bioinformatics. Current assessments often rely on global similarity measures that compare a model with a reference structure or experimental map, focusing on overall shape while overlooking the accuracy of intermolecular interfaces – the specific contacts between RNA and protein. Meanwhile, computational experiments on available benchmark sets from CASP and RNA-Puzzles reveal how poorly current predictive systems reproduce these interfaces, underscoring the need for dedicated evaluation methods. In this presentation, we introduce a normalized similarity measure, adapted from RNA structure assessment, to quantitatively evaluate interface accuracy in multi-chain complexes. This scoring function compares residue-residue contacts in predicted and reference structures, yielding an interpretable score from 0 (no correct interactions) to 1 (perfect match), and is complemented by an interface-tailored F1 score that offers a complementary perspective. Applied to RNA-protein docking decoys and other assemblies, the measure shows strong correlation with TM-score in interface-relevant cases and complementary behavior to DockQv2.

O.3.4

Exploring RNA metabolism with custom genome-wide 3′ RACE: from yeast models to human cells

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RNA metabolism and 3'-end modifications are key regulators of gene expression and RNA stability. Among these modifications, uridylation plays a central role by targeting various RNA species for degradation. To investigate this phenomenon globally, we developed a custom genome-wide 3' rapid amplification of cDNA ends (gw3'RACE) method. Originally optimized in our lab for *Schizosaccharomyces pombe*, the protocol includes streamlined library preparation for short-read sequencing and a dedicated bioinformatics pipeline. This enables robust detection of uridylated and other 3'-modified RNAs under diverse physiological conditions, including proliferation, quiescence, and sporulation. Following validation in *S. pombe*, gw3'RACE was successfully adapted for *Saccharomyces cerevisiae*, *Candida albicans*, and human cell lines. In mammalian systems, we employed engineered knockout lines lacking critical RNA decay factors to characterize RNA 3' ends comprehensively. Genome-wide analyses revealed transcript-specific changes in uridylation patterns and degradation signatures, providing detailed insights into RNA stability regulation. The method effectively captures diverse 3'-end structures while addressing technical challenges posed by intronic sequences and poly(A)-rich regions, thus offering a versatile and powerful approach to study RNA decay and post-transcriptional regulation across diverse biological systems.

Genetics and Epigenetics

Lectures

L.4.1

The increasing significance of epigenetic biomarkers in personalized medicine

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Epigenetic biomarkers, especially disease-related changes in DNA methylation patterns, are becoming increasingly central to the advancement of personalized medicine. A growing body of research evidence shows that these alterations occur in the early stages of disease and can drive disease progression. As such, disease-associated methylation changes can be leveraged as diagnostic biomarkers across all stages of disease development from risk assessment and early detection to treatment planning and relapse monitoring.
Advances in high-throughput technologies such as NGS and bioinformatics including the application of machine learning and artificial intelligence to analyse genome-wide epigenetic changes have enabled the identification of disease related highly specific methylation signatures. These signatures allow not only distinguish between different cancer types but also are associated with treatment response and clinical outcomes. Building on this research, a new generation of epigenetic diagnostic tests is emerging, including the first liquid biopsy-based tests for early cancer detection and methylation biomarker-based cancer classifiers. Several of these tests are already in the final stages of clinical evaluation and market clearance.
This talk will focus on the current stage of development of epigenetic biomarkers in personalized medicine and challenges that must be addressed before epigenetic biomarker based diagnostic test become a standard part of clinical practice.

Oral presentations

O.4.1

The role of lamina-associated domains (LADs) and 3D genome architecture in the establishment of epidermal barrier.

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The epidermis is a stratified epithelium formed by keratinocytes differentiating as they move from basal to upper layers. Its proper development is essential for postnatal survival, as a defective barrier leads to fatal dehydration. Most keratinocyte-specific genes cluster in three regions: the epidermal differentiation complex (EDC), and the Keratin I and II loci. We previously showed that during epidermal morphogenesis, the EDC locus relocates from the nuclear periphery to the interior and undergoes compaction to facilitate gene expression. To gain genome-wide insight into 3D chromatin remodelling, we mapped Lamin-Associated Domains (LADs) using pA-DamID in mouse keratinocytes. This revealed keratinocyte-specific LAD patterns critical for cell identity. ATAC-seq and H3K27ac CUT&RUN data showed that active enhancers are largely excluded from LADs, though notable exceptions exist. In basal keratinocytes, most of the EDC detaches from the periphery, except for its 5' and 3' ends. This diverges from earlier FISH studies due to different probe coordinates. In spinous cells, EDC LADs shrink further. As H3K9me3 is linked to LBR-dependent lamina tethering, we profiled its distribution but found no major changes, suggesting other LBR-dependent mechanisms are involved. Our study identifies keratinocyte-specific peripheral chromatin domains and reveals their role in establishing the transcriptional programme for epidermal barrier formation.

O.4.2

Contribution of non-catalytic subunits of the replisome to genetic stability and cell cycle control

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The central role in DNA replication is played by the CMG helicase (CDC45, MCM2-7, and GINS) and DNA polymerase Epsilon (Polε). This polymerase (POLE, POLE2, POLE3, and POLE4 subunits) is the main replicase of the leading DNA strand, while DNA polymerase delta (Polδ) is responsible for DNA synthesis mainly on the lagging strand. Although the role of catalytic elements of the helicase and polymerase is well understood, the involvement of non-catalytic elements requires investigation.
Our studies were focused on non-catalytic, though essential, subunits of GINS and Pol ε. In yeast cells with mutations in the DPB2 and PSF1 genes encoding homologs of POLE2 and GINS1, we show that impaired functioning of these proteins results in various phenotypes of genome instability. Additionally, defective functioning of Dpb2 or Psf1 subunits affects cell cycle progression and its coordination with DNA replication.
Recent findings also show that impaired functioning of POLE2 or GINS1 correlates with human diseases and that these genes are overexpressed in several types of cancer, which often correlates with worse outcomes for patients. Silencing of genes involved in DNA replication, including those encoding proteins with no catalytic function, might reduce proliferation and induce cell apoptosis in cancer cells. A better understanding of these genes' function provides insights translatable into new therapeutic approaches targeting the coordination of DNA replication with the cell cycle.

O.4.3

Reprogramming meiotic recombination at hotspots in *Arabidopsis* through targeted chromatin modification

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The impact of specific chromatin modifications on meiotic crossover frequency is usually inferred from correlative studies, leaving open the question of causality. To address this, we used a dCas9-based system to target histone H3 methylation modifiers to defined genomic loci. Targeting methyltransferases responsible for H3K9me3 and H3K27me3 had little effect on recombination at selected hotspots, whereas targeting H3K4me3- and H3K36me3-associated enzymes often silenced the endogenous genes. The strongest effect was observed with the demethylase JMJ14, which reduced local H3K4me3 levels and decreased crossover frequency within the targeted interval. This was accompanied by reduced transcription of a long non-coding RNA (lncRNA) located at the hotspot and altered crossover topology. Suppressed recombination was also seen at neighbouring, untargeted hotspots. Conversely, directing the transcriptional activator VP64 to the same region elevated lncRNA expression, increased crossover frequency, and raised H3K4me3 levels. Our results reveal a causal relationship between H3K4me3, transcription, and local crossover activity, demonstrating that H3K4me3 levels are tightly associated with both transcriptional output and recombination frequency at specific genomic sites.

O.4.4

Caught in the Act: Natural Hybridization Between *Jacobaea vulgaris* and *J. erucifolia* Revealed by Molecular Markers

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Global climate change and environmental shifts affect biodiversity, species distribution, and population dynamics. Genetic adaptation to these changes relies on inter- and intra-population variability. Habitat modifications, exotic species introduction, and interspecific hybridization can threaten the integrity of rare species and small populations. As hybridization increases, early hybrid detection becomes crucial for conservation. Natural hybridization is common in *Jacobaea*, with many known interspecific hybrids. *Jacobaea erucifolia* and *J. vulgaris* are closely related and often co-occur. Although German botanists previously noted possible hybrids between them, no molecular evidence had confirmed this. Individuals with intermediate morphology from sympatric populations were suspected hybrids. To verify this, two molecular marker systems were used: nuclear and chloroplast DNA sequencing and AFLP analysis. All 25 putative hybrids showed confirmed hybrid origin. AFLP data indicated that most were genetically closer to *J. erucifolia*, suggesting frequent backcrossing. Some individuals previously identified as pure were also likely hybrids. This is the first molecular confirmation of natural hybridization between *J. vulgaris* and *J. erucifolia* in Poland. The process appears bidirectional but asymmetrical, with *J. vulgaris* usually acting as the maternal parent.

Genomics and Transcriptomics

Lectures

L.5.1

Proximity of pre-mRNA 3'end cleavage and transcription termination enhances gene expression and compromises colorectal cancer

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Polyadenylation site (PAS) usage and the cellular machinery responsible is modified in many cancers. In particular, two key factors required for pre-mRNA 3' cleavage and polyadenylation (CPA), CPSF73 and PCF11, have oncogenic properties – their elevated expression is associated with worse patient prognosis. Both proteins also function as transcription termination factors. Termination occurs over a thousand nucleotides downstream of PAS in humans. Here, we report transcription termination shifting proximally in colorectal cancer (CRC) cells. Earlier termination is driven by PCF11 redistribution on chromatin and linked to its protein levels. Notably, compared to cells from primary tumor, which are particularly sensitive to CPA levels, metastatic CRC cells showed partial reversal to termination patterns of normal cells. Surprisingly, changes in PAS usage and transcription termination during CRC are uncoupled. Thus, oncogenic properties of CPA factors are a result of increased proximity of pre-mRNA cleavage and transcription termination rather than alternative polyadenylation.

Oral presentations

O.5.1

Isoform switching as a key mechanism in chemotherapy resistance in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is an aggressive subtype with limited treatment options and variable response to neoadjuvant chemotherapy (NAC). While genomic alterations are well characterized, post-transcriptional mechanisms such as alternative splicing remain poorly understood in this context. To address this, we performed transcriptome-wide isoform analysis in pre-treatment TNBC biopsies from patients stratified by therapy response. Isoform switching events were identified and functionally analyzed for changes in coding potential, domain structure, and pathway association. Non-responders showed significantly more isoform switches, especially at transcription start and termination sites and through intron retention. Functional enrichment of genes with differential isoform usage between complete and non-responders revealed signatures related to immune signaling and DNA repair. Notably, the *XRCC3* gene displayed a shift toward a truncated isoform lacking domain essential for interaction of RAD51 and RAD51C, likely impairing homologous recombination. These findings highlight isoform switching as a relevant regulatory layer in chemoresistance and suggest its value for predictive biomarker discovery in TNBC.

O.5.2

TSC angiofibroma and ungual fibroma have different mutation signatures, with recurrent mutations in *KMT2C*

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Aim: Tuberous Sclerosis Complex (TSC) is an autosomal dominant tumor suppressor syndrome characterized by tumors in multiple tissues, due to inactivating *TSC1/TSC2* variants. Herein, first-ever genome-wide profiling of somatic mutations in a unique collection of angiofibroma (FAF) and ungual fibroma (UF) TSC skin tumors was performed.

Methods: Whole genome sequencing was performed on 9 samples, i.e., 4 TSC FAF and 5 TSC UF, with 6 matched saliva/buccal swab samples from 6 individuals with TSC.

Results: TSC FAF and TSC UF skin tumors have different mutation signatures, with a predominance of UV-related SNV (SBS7a and SBS7b) and DNV (DBS1) signatures in FAF, and aging-related SNV (SBS1 and SBS5) signatures in UF. We also identified a novel DNV signature for TSC-UF, with frequent TG>CA and TT>GG substitutions, not noted previously for any tumor. Further, 3 inactivating somatic mutations in *KMT2C* were also seen in 2 of 4 TSC FAF, and five mutations in other cancer-related genes.

Conclusions: TSC FAF and UF develop through distinct pathogenic mechanisms, i.e., UV-induced mutagenesis in FAF, and aging-related mutation in UF. The mechanism of the novel DNV signature in UFs merits further investigation. The occurrence of *KMT2C* mutations suggests that KMT2C inactivation may contribute to the pathogenesis of TSC FAF, which will be validated further.

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O.5.3

Whole-miRNome Sequencing

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Interest in the genetic variation of noncoding genomic elements, including miRNAs, is growing, and several mutations in miRNA genes implicated in human diseases, including cancer, have already been detected. However, the lack of dedicated analytical tools severely hampers progress in this area. In this study, we developed the first whole-miRNome sequencing (WMS), which enables the targeted sequencing of all human miRNA genes (n~2000) and 28 miRNA biogenesis genes. By sequencing various types of DNA samples, including ~300 tumor/normal pairs, from lung, colorectal, ovarian, renal, and basal cell carcinomas, we identified ~2,000 mutations, including 879 in miRNA genes. These mutations were located in all parts of the genes, including seed or cleavage sites essential for the functioning of miRNA genes. The high reliability of the mutations was confirmed through various approaches, including different sequencing methods. The analysis identified several miRNA genes with functional enrichment of cancer mutations, including *MIR3928*, which was specifically mutated in basal cell carcinoma, suggesting its potential role in this cancer. WMS also allowed the identification of multiple copy number alterations, which often encompassed miRNA genes. WMS provides highly effective, low-cost sequencing of all miRNA genes in different types of samples, including highly degraded ones.

O.5.4

Unexplored RNA biology of non-model organisms as a source of putative drug targets – polyadenylation signals in Giardia

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Cleavage and polyadenylation are the final steps of eukaryotic mRNA 3' end formation. The most critical element in model organisms is the poly(A) signal, an AAUAAA hexamer. We recently discovered that the deeply branching eukaryote – Giardia lamblia uses a different but well-defined poly(A) signal, AGURAA. Then we performed direct RNA sequencing on four protists within the Metamonada supergroup and two outgroup protists. Both outgroup protists and the non-Giardia Metamonada species use the AAUAAA poly(A) signal, indicating it is the ancestral signal. In contrast, all Giardia species use the WGURAA poly(A) signal, indicating it is a derived feature within Giardia or Fornicata. The change in this ubiquitous regulatory element raises questions about the sequence features that specify genuine poly(A) sites and avoidance of premature cleavage in the coding sequence. We used a machine learning model that was able to nearly perfectly discriminate between WGURAA sites in 3'UTRs and those in the CDS. We found that G. lamblia uses nucleotides directly flanking the poly(A) signal for its recognition, with identity of the nucleotide just downstream of the the poly(A) signal being the most important. Poly(A) signals are flanked by pyrimidines, whereas WGURAA hexamers in coding sequences by purines. These unique regulatory features of the Giardia pathogens can be exploited for target therapy.

Lectures

L.6.1

Complex metabolic pathways of therapeutic mRNAs *in vivo*

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Mammalian mRNA turnover pathways were typically studied using model cell lines and rarely in vivo. In my talk, I will summarize our recent efforts to investigate the stability regulation of therapeutic mRNA in more physiological setups. The analysis, which utilized transgenic mouse models and transcriptional analysis with Direct RNA Sequencing, revealed high variability among cell types and tissues in pathways that either stabilize or lead to rapid degradation of mRNA. Additionally, the localization of the protein product can affect the metabolism of mRNA. For example, mRNA vaccines, which after intramuscular administration are mainly taken up by macrophages, are stabilized by TENT5-mediated cytoplasmic polyadenylation. This process is not active in model cell lines or in the liver.

Oral presentations

O.6.1

Small activating RNA-based therapeutic modulation of *MBNL1* transcription in myotonic dystrophy.

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Sequestration of Muscleblind-like (MBNL) proteins on toxic RNA with CUG-repeat expansion (CUG^{exp}) is a hallmark of myotonic dystrophy (DM), a rare genetic multisystem disorder. Depletion of MBNLs is the key driver of DM pathology and underlies a massive shift in the alternative splicing (AS) pattern from adult-type towards fetal-like isoforms, giving rise to proteins inapt to perform in adult, differentiated tissues. To overcome MBNL insufficiency in DM cell models, we harnessed a conserved mechanism of RNA activation (RNAa) *via* rationally designed small activating RNA duplexes (saRNA) targeted to the most active promoter of *MBNL1*, the major *MBNL* gene paralog. We identified saRNAs that enhanced *MBNL1* transcription *via* an on-site mechanism involving AGO2-mediated loading of the antisense strand of saRNA duplex onto cognate promoter sequence, followed by recruitment of RNAPII and auxiliary canonical RNAa pathway components. Using chemically modified saRNAs, CUT&RUN scanning across their target sites and gene reporter assays, we dissect the underlying molecular mechanism. Moreover, we show that RNAa upregulates MBNL1 protein content in DM cells and corrects the AS of multiple MBNL1-regulated biomarker exons. This is first report that site-specific augmentation of *MBNL1* transcription mitigates AS defects and as such, it expands possible points of therapeutic interventions against DM.

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O.6.2

Functional Insights and Validation of Blood-Based miRNA Biomarkers in Alzheimer's Disease Using the 3xTg-AD Model

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that develops silently over years before clinical symptoms appear. The search for early, non-invasive biomarkers has highlighted the diagnostic potential of circulating microRNAs (miRNAs) due to their blood stability and regulatory roles in AD pathology. Our lab previously identified and patented six plasma miRNAs (EP3449009, 2021) as early AD biomarker candidates. However, their validation in animal models remains limited. Here, we analyzed plasma levels of these miRNAs by RT-qPCR in 3xTg-AD and wild-type (WT) mice (n = 17/group) aged 16–20 months. The 3xTg-AD model replicates key features of AD, including Aβ plaques and tau pathology. miR-39-3p (spike-in) and miR-192-5p (endogenous) were used for normalization. miR-29b-3p and miR-486-5p were significantly upregulated, while miR-483-5p was downregulated in 3xTg-AD mice, consistent with changes seen in human AD plasma. Functionally, miR-29b-3p regulates BACE1 and Aβ production, miR-483-5p is linked to tau phosphorylation, and miR-486-5p to neuroinflammation and synaptic function. These results highlight the translational relevance of these miRNAs and support the 3xTg-AD model as a valuable tool for miRNA biomarker validation in AD.

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O.6.3

Dissecting the dual role of LINC00116 in B-cell lymphoma: functional contribution of the lncRNA and its encoded micropeptide Mitoregulin

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Long non-coding RNAs (lncRNAs) are defined by their lack of protein-coding potential; however, certain lncRNAs can be translated into functional micropeptides. LINC00116 is one such lncRNAs, as it encodes Mitoregulin (MTLN) micropeptide. LINC00116 is overexpressed in B-cell lymphoma compared to normal B cells, but the functions of LINC00116 and MTLN in lymphoma remain unknown. The aim of this study is to determine if LINC00116 and MTLN have distinct roles in B-cell lymphoma cells. We showed that MTLN was higher expressed in Hodgkin lymphoma (HL) cells compared to normal B cells and was a target gene for the miR-17 family. We silenced LINC00116/MTLN in HL cells with two shRNAs in lentiviral vectors. In GFP competition assay, we showed significant decrease in the percentage of GFP+ HL cells with silenced LINC00116/MTLN compared to co-cultured wild-type cells over 21 days. This decrease was not due to apoptosis, as Annexin V-APC staining showed no change in apoptotic cell percentage following MTLN/LINC00116 silencing. Next, we selectively overexpressed LINC00116 or MTLN using lentiviral vectors. In GFP competition assay, the percentage of GFP+ L428 cells with MTLN overexpression was significantly decreased over 21 days, whereas no change was observed after overexpression of LINC00116 without MTLN-encoding potential. In conclusion, we showed that MTLN is expressed in HL cells and silencing MTLN/LINC00116 and overexpressing MTLN, but not LINC00116, suppressed HL cell growth.

O.6.4

Cancer mutations turn key miRNAs into broken regulators

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of many genes. Although their role is well established, the impact of mutations in miRNA genes remains poorly understood. We investigated this by analyzing mutations in two cancer-associated miRNA genes, MIR142 and MIR205. We assessed the effects of 51 mutations using computational predictions and found that the majority (42/51) of them decrease the stability of miRNA precursors. To experimentally validate these results, we generated 33 cell lines expressing mutated precursors and used them for the first-ever in-cell SHAPE-MaP analysis of miRNA precursors, revealing that many mutations significantly deform the structure. Small RNA sequencing of the 39 cell lines expressing mutated precursors showed that 35 altered miRNA levels, 15 changed DROSHA/DICER1 cleavage sites, and 11 reversed 5p/3p strand balance. Dual-luciferase assays showed that several mutations reduced the silencing efficiency of native targets and may create new targets. Our systematic analysis revealed that all mutations within the sequence encoding hairpin-shaped miRNA precursors were detrimental to miRNA gene functions. Our results show that miRNA gene mutations can disrupt miRNA biogenesis and function, suggesting their role in human diseases, including cancer, and their value as biomarkers or therapeutic targets.

Protein Chemistry and Structure

Lectures

L.7.1

Cardiolipin-Mediated Regulation of Nuclease Activity in the Mitochondrial Repairosome

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Our research focuses on elucidating the mechanisms that govern the activity of the mitochondrial repairosome, a multiprotein complex dedicated to the repair of mitochondrial DNA. By employing a range of biochemical, biophysical, and structural techniques, including reconstituted membrane systems, confocal microscopy, molecular dynamics simulations, as well as nuclease activity and binding assays, we have uncovered important insights into the regulation of the human mitochondrial nuclease EXOG. Our findings have revealed that EXOG selectively binds to membranes containing cardiolipin, the signature phospholipid of the inner mitochondrial membrane. Furthermore, we have demonstrated that this interaction inhibits EXOG nuclease activity, suggesting that cardiolipin functions as a negative regulator that anchors EXOG to the membrane. We have also shown that membrane-restrained EXOG acts as a beacon for the coordinated assembly of the repairosome with DNA polymerase γ and APE1. Finally, we demonstrate that oxidation of cardiolipin causes the release of EXOG from the membrane, yielding the active enzyme and providing a direct molecular link between oxidative stress, lipid damage, and deregulated DNA repair. In this talk, I will provide an update on our progress in understanding the complex regulation of mitochondrial DNA repair, highlighting our latest discoveries on lipid-mediated control of the mitochondrial repairosome.

Oral presentations

O.7.1

Halfway to hypusine. Structural biology of (deoxy)hypusination

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Hypusination is a unique PTM that occurs exclusively on a single lysine residue of eukaryotic translation initiation factor 5A (eIF5A), formed in a two-step reaction initiated by deoxyhypusine synthase (DHS). This modification is essential for translation elongation and cell *via*bility, and its disruption has been associated with various pathological conditions, including rare neurodevelopmental disorders.
A cryo-electron microscopy (cryoEM) structure of the human DHS–eIF5A complex at 2.8 Å resolution provides detailed insight into substrate recognition and the molecular architecture of the active enzyme-substrate assembly. Complementary high-resolution crystallography of DHS in a trapped reaction intermediate state further elucidates key catalytic steps and supports a mechanistic model for deoxyhypusine formation.
Additionally, a crystallographic fragment screening campaign against human DHS identified chemically diverse fragment clusters targeting multiple functional regions, including the active site entrance, the regulatory ball-and-chain motif, and allosteric surfaces. Notably, a covalent fragment was discovered that selectively modifies the catalytic lysine in an oxidoreductase-specific manner, demonstrating the potential of this approach for modulating enzyme activity.
These findings provide a comprehensive framework for understanding the molecular basis of hypusination, its dysregulation in human disease, and the potential for targeted therapeutic intervention.

O.7.2

Phosphorylation of ribosomal P-stalk proteins governs auxiliary factor binding to the ribosome

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Protein synthesis is a highly regulated process, with the ribosome playing a central role in shaping the cellular proteome. This regulatory capacity relies on trans-acting factors and ribosomal elements, which frequently undergo post-translational modifications. In this context, the GTPase-associated center (GAC), particularly its primary functional element on the 60S subunit, the P-stalk, emerges as a key regulatory component. The GAC has dual functions: it engages with translational GTPases to support translation and interacts with the Gcn2 kinase, thereby linking ribosomes to the integrated stress response pathway. The P-stalk consists of three proteins, uL10, P1, and P2, which form a pentameric complex sharing a common C-terminal domain (CTD). We investigated the role of phosphorylation within the CTD of these proteins and found, that P-stalk proteins exist exclusively in a phosphorylated state. This ensures optimal translation during decoding and supports an effective Gcn2-dependent stress response. Molecular dynamics simulations suggest that adding a phosphate group induces a transition from a collapsed globule to a coil-like structure, enhancing the P-stalk's ability to interact with various factors. Notably, unlike most ribosomal proteins that are phosphorylated in an on/off manner, P-stalk proteins remain constitutively phosphorylated, maximizing their interactions with auxiliary factors.

O.7.3

Mapping the nucleophilic attack in three Classes of L-asparaginases by structural correlations

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The principle of structural correlations (PSC) can be used to map a reaction path if sufficiently many crystal structures are available with the reactants experiencing different crystal environments ('fields'). The PSC has an interesting application in structural enzymology, for instance to explain the mechanism of amide hydrolysis, which proceeds *via* a nucleophilic attack with the formation of an acyl-enzyme intermediate. An example of such a reaction is the hydrolysis of the side-chain amide of L-Asn by L-asparaginases. There are three unrelated Classes of L-asparaginases, represented by the EcAII, EcAIII, and ReAV archetypes. The nucleophile (Nuc) in each case is either a Thr or Ser, but in each Class there are two potential residues considered for this role. Lubkowski & Wlodawer used PSC analysis for Class 1 asparaginases, arriving at a clear resolution of the ambiguity (and identifying Thr12 as the nucleophile in EcAII), despite an error in the definition of one of the parameters. Our comprehensive PSC analysis, based on all available crystal structures in the PDB, of all three Classes of asparaginases allows us to establish the primary nucleophile in each Class (T179 in EcAIII and S48 in ReAV) and to propose a set of optimal stereochemical parameters for future PSC analyses of the nucleophilic attack on C=O containing substrates (Nuc…C distance *d*, the Bürgi-Dunitz angle Nuc…C=O, and the Herschlag dihedral Nuc…O=C-Cβ).

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O.7.4

Cargo-Pex5-Pex14 ternary complex for peroxisomal import

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Peroxisomes are membrane-bound organelles. Lacking the machinery to synthesize proteins, peroxisomes rely on importing necessary enzymes *via* the peroxin (Pex) system. Proteins tagged with a peroxisome targeting sequence 1 (PTS1) are recognized in the cytosol by the receptor Pex5. The complex then docks at the peroxisomal membrane translocon composed of Pex14 and Pex13, allowing the cargo to be transported into the peroxisomal matrix. The structural details of this import mechanism remain only partially resolved.

We analyze the ternary complex of cargo, Pex5, and the N-terminal domain of Pex14 (Pex14NTD) from *Trypanosoma cruzi*. Using cryo-EM, we constructed a model of Pex5 bound to malate dehydrogenase (MDH) and Pex14NTD. This model reveals insights into conformational heterogeneity and highlights secondary interaction sites. We identify orientations of the Pex5hTPR domain relative MDH, covering a range of 17°. We detect interaction surfaces – independent of both the PTS1 motif and the Wxxx(F/Y) motif – at the MDH–Pex5hTPR and Pex5hTPR–Pex14NTD interfaces.

Mutational studies suggest that non-PTS1 interface between MDH and Pex5hTPR contributes minimally to overall binding affinity, emphasizing the dynamic but structurally constrained nature of the interaction. The Pex5hTPR–Pex14NTD interface represents an extended binding region where Pex14NTD engages a broader surface of Pex5hTPR. These findings offer new perspectives on the molecular mechanics of peroxisomal protein import.

Protein Biology

Lectures

L.8.1

Of protein filaments and condensates: the underappreciated world of protein homomultimerisation

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Abstract: Filament formation is generally regarded as a relatively rare protein property, most often associated with specialised functions such as those of the cytoskeleton. In this talk, I will suggest that the ability to self-assemble into filaments is in fact far more common than generally appreciated. From a biological perspective, it arises easily during evolution and can confer numerous functional advantages, yet it also poses technical challenges when we attempt to study these proteins, as filaments often become insoluble – perhaps explaining why their occurrence has been underestimated. I will illustrate these ideas by discussing our recent accidental discovery of filament formation in a large family of vertebrate transcription factors known as ZBTB [ref. 1], alongside other examples from our recent and ongoing work. The presentation will bring together structural and cell biology with insights from bioinformatics and computational structure prediction.

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Oral presentations

O.8.1

OCIAD1, prohibitins and regulation of the TIM23 translocase

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Mitochondrial proteins are transported and sorted to the matrix or inner mitochondrial membrane by the presequence translocase, TIM23. In yeast, this essential and highly conserved machinery is composed of the core subunits Tim23 and Tim17. The human genome encodes two paralogs of Tim17, TIMM17A and TIMM17B, which assemble into separate pools of the TIM23 translocase. Contrary to TIMM17B, the TIMM17A paralog is short-lived and undergoes degradation by the YME1L protease following various cellular stresses. The mechanism of this regulation, as well as the assembly of these two forms of the human TIM23 complex, is poorly characterized.

We present an unexpected role of the ovarian cancer immunoreactive antigen domain containing 1 (OCIAD1) protein and the prohibitin complex in the biogenesis of human TIM23. Prohibitins stabilize both the TIMM17A- and TIMM17B-containing variants of the translocase. Interestingly, OCIAD1 assembles with the prohibitin complex to protect the TIMM17A variant from degradation by the YME1L protease. We postulate that OCIAD1, together with prohibitins, constitutes a regulatory axis that regulates variants of human TIM23.

O.8.2

Unexpected ATP hydrolysis activity of human ribonuclease Dicer

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The ATP hydrolysis activity associated with the helicase domains of Dicer proteins has been well-documented for *Drosophila melanogaster, Caenorhabditis elegans*, plants, and fission yeast systems, where it contributes to substrate processing and regulatory functions. In contrast, despite structural conservation of the helicase domain, there have been no prior reports conclusively demonstrating ATPase activity in vertebrate Dicers. This apparent absence has led to the prevailing assumption that vertebrate Dicers lack this enzymatic function.
In this study, we challenge this view by providing evidence that human Dicer (hDicer), through its helicase domain, is capable of ATP hydrolysis. To the best of our knowledge, this is the first time this activity has been reported for vertebrate Dicers. We also show that the hDicer helicase domain binds single- but not double-stranded nucleic acids, which is in contrast to the helicase domains of invertebrate and plant Dicers interacting with RNA duplexes. Moreover, the hDicer helicase domain might influence the structure of the bound RNA. Our findings open new avenues for future studies aimed at defining the cellular activities of hDicer that may be associated with these newly described biochemical properties.

O.8.3

The *modus operandi* of ribosome inactivating proteins at the molecular and cellular levels

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The most potent toxic proteins known in nature are Ribosome Inactivating Proteins (RIPs), which are found in plants, bacteria and other organisms. They are present in human food and are also used in traditional medicine. Furthermore, RIP proteins pose a significant social risk as they can be utilised as biological weapons. These toxins threaten human life and health, and also attract attention due to their wide range of potential biotechnological applications in medicine. However, the molecular mechanism of action of these toxins remains unclear from the point of view of their biology. The currently adopted model assumes that RIP proteins depurinate the large ribosomal subunit by removing an adenine base from the Sarcin Ricin Loop (SRL), thereby inhibiting protein biosynthesis and is considered the main trigger of apoptotic cell death. However, it should be noted that there is no clear functional link between depurination and cell death, highlighting the gaps in our current knowledge of the molecular aspects of RIP toxicity.
Our current research shows that the main binding platform for RIPs on the ribosome are the P proteins, with the P1-P2 heterodimer structure representing an optimal docking site. We also suggest that ribosome depurination may induce the ribotoxic stress response (RSR), resulting in the activation of signalling pathways that lead to apoptosis. Consequently, we challenge the widely held concept that inhibition of protein biosynthesis lies at the heart of RIP toxicity.

O.8.4

Filling the gap: molecular identification and biochemical characterization of mammalian L- fucose dehydrogenase

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Fucose is a unique monosaccharide, since it is the only endogenous L-sugar present in vertebrates. It plays an important role in many biological processes, including the determination of *AB0* blood groups or cancer metastasis. In vertebrate cells, the biosynthesis and degradation pathways of L- fucose coexist. The pathways for the formation of GDP-L-fucose, an active form of the sugar, are relatively well described, while very little is known about the intracellular degradation of the monosaccharide. The enzyme L-fucose dehydrogenase initializes the breakdown of L-fucose. However, the molecular identity of this protein remained unknown until recently.

The enzyme was purified from rabbit liver in a 6-step purification procedure. Mass spectrometry analysis of the purified protein preparation identified mammalian hydroxysteroid 17-β-dehydrogenase 14 (HSD17B14) as the best candidate for L-fucose dehydrogenase. Rabbit HSD17B14 was expressed in HEK293T, purified, and shown to catalyze the oxidation of L-fucose to L-fucono-1,5-lactone, which was then confirmed by mass spectrometry and NMR analysis. Substrate specificity studies revealed that L-fucose, not β-estradiol, as previously reported, is the preferred substrate for HSD17B14.

These findings open the way to unraveling the physiological role of the mammalian L-fucose degradation pathway, which remains unknown.

Proteomics and Metabolomics

Lectures

L.9.1

Multi-omics analyses in rare neurodegenerative diseases: lessons to be learned.

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CLN5 disease, a form of juvenile dementia within the neuronal ceroid lipofuscinosis (NCL), is associated with mutations in the *CLN5* gene. CLN5 is highly expressed in the cerebellar Purkinje cells, cortical neurons, hippocampal pyramidal cells and interneurons, areas known to be degenerated in CLN5 human brains. Hypomorphic gene variants in CLN5 also occur in other common neurodegenerative disorders, including Alzheimer's and Parkinson's Disease. A recent outbreak in the field demonstrated that CLN5 encodes the lysosomal bis(monoacylglycero)phosphate synthase (BMPS), the enzyme responsible for the production of bis(monoacylglycero)phosphate (BMP). We developed and characterized a new cln5 knock-out zebrafish model that replicates key features and molecular signatures of the human disease. Loss of Cln5 function in vivo altered axonal growth of retinal ON-bipolar cells and disrupted calcium homeostasis in the cerebellum, revealing new disease features. Multi-omic **analyses** at different developmental stages revealed an impaired glucose metabolism as an original finding in NCL. A novel biomarker, PHGDH**,** was validated in zebrafish and human skin fibroblasts harboring pathogenic variants in CLN5, and in CLN7. We also tested *metformin*, the main first-line medication for the *treatment of type 2 diabetes*, which elevated the expression of PHGDH in patient-derived cells, and rescued zebrafish behavior. This work offers a promising avenue toward targeted therapies for juvenile dementia.

Oral presentations

O.9.1

Meltome Atlas development and its utility in assessing the impact of fever on bacterial infections

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Meltome Atlas is globally recognized proteomics resource, which delivers data on thermal denaturation of thousands of protein from different organisms from different branches of the phylogenetic tree, from archaea, through bacteria, yeast, plants, worms, flies, fish, rodents, to humans, and covers a wide temperature range from 30 up to 90°C. It is used by many scientists all over the world and opens completely new branch of proteomics – Meltomics. In our research, we utilize Thermal Proteome Profiling (TPP) and variety of biochemical, immunoenzymatic, proteomics and bioinformatics techniques to focus on a very important biological question that has never been fully addressed – whether fever, which increases body temperature caused by bacterial infections, can potentially lead to the denaturation of bacterial proteins and accelerate the healing of infectious diseases. In our current research, we focus on thermal denaturation of bacterial proteins to assess their thermal stability and identify a group of bacterial proteins that may be responsible for bacterial death during the development of fever. This issue is particularly important because in modern society fever is considered a negative symptom of infection and is often treated with antipyretics and antibiotics, which may not be the optimal method of treating infections.

O.9.2

Biphenyl hydrolase-like protein (BPHL) depletion affects neurofilament cytoskeleton organization and mitochondrial function in mice brain

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Introduction: Alzheimer's disease (AD) is the most prevalent cause of dementia in the elderly that affects millions of people worldwide. Altered mitochondrial homeostasis is considered an early event in AD development, which precedes the symptomatic stage of disease. Elevated plasma total homocysteine (tHcy), hyperhomocysteinemia (HHcy) and low Hcy-thiolactonase (HTLase) activity are emerging risk factors for AD.

Aim: We have studied the effect of BPHL, mitochondrial HTLase depletion on mouse cerebral cortex proteome.

Methods: To identify proteome changes between cerebral cortex of 8-month old *Bphl*$^{-/-}$ (n=6) and *Bphl*$^{+/+}$ (n=5) male mice TMT labelling and proteomic quantification were performed.. Bioinformatic analysis were performed using STRING version 12.0 software.

Results: In total 6966 proteins were quantified of which 201 had significantly altered expression in *Bphl*$^{-/-}$ *vs.* *Bphl*$^{+/+}$ mice. 88 proteins were upregulated and 113 proteins were downregulated in *Bphl*$^{-/-}$ mice compared to controls. STRING functional enrichment analysis indicated that the dysregulated proteins were involved in the following top 5 biological processes: neurofilament cytoskeleton organization, carboxylic acid metabolic process, 2-oxoglutarate metabolic processes, tricarboxylic acid cycle and axon development.

Conclusion: Depletion of BPHL causes neurodegeneration related proteome changes including dysregulated neurofilament cytoskeleton organization and mitochondrial homeostasis.

O.9.3

Presence of HPV peptides in small extracellular vesicles released by tumor cells

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HPV-dependent head and neck squamous cell carcinoma exhibits reduced resistance to therapy, resulting in more favorable prognoses. The molecular mechanisms responsible for these differences remain under investigation. Current research supports the hypothesis that small extracellular vesicles (sEV) derived from HPV-positive cancer cells convey HPV-specific antigens, subsequently inducing a heightened immune response through antigen presentation.

The study employed size exclusion chromatography to isolate sEVs from the supernatant of both HPV-positive (SCC-2 and SCC-47) and HPV-negative (PCI-13 and PCI-30) cell lines and from serum samples of patients with HPV(+) and HPV(-) HNSCC. Characterization of these sEV populations adhered to the guidelines established by MISEV. The experimental methodology encompassed sample preparation *via* a modified FASP protocol [1]. Proteomic analysis was subsequently conducted using an nano-LC system conntected to QExactive Plus Orbitrap mass spectrometer. Additional confirmation of the presence of viral peptides in sEV was performed by high-resolution fluorescence microscopy with co-localization of 3 proteins: CD63, HPV16 E7 and HPV16 E2.

Data analysis revealed substantial differences in the proteomes of HPV(+) and HPV(-) sEVs [2]. Three viral peptides were identified in sEVs produced by HPV(+) cell lines, corresponding to three distinct HPV proteins: Replication protein E1, Minor capsid protein L2, and Probable protein E5. This represents the first documented instance of HPV proteins detected within sEVs originating from HPV(+) tumor cells.

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O.9.4

The Relationship Between Fatty Acid and Bacterial Metabolite Levels in Crohn's Disease: Potential Diagnostic and Prognostic Biomarkers

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Crohn's Disease (CD) is a chronic inflammatory bowel disease whose pathogenesis is linked to gut microbiota dysbiosis and associated with metabolic changes. The presented studies highlight the significance of these metabolic alterations, analyzing levels of 16 fatty acids (FA) and 14 bacterial metabolites (SCFA, TMA, and TMAO) in the blood serum of 30 CD patients and 30 healthy controls using ultrasensitive mass spectrometry methods.

In these LC-MS studies, we have detected significant changes in the levels of seven fatty acids, including arachidic acid ($p.adj=0.0281$), arachidonic acid ($p.adj=0.0088$), and nervonic acid ($p.adj<0.0001$). Notably, nervonic acid showed the greatest difference in concentration, showing significantly lower levels in CD patients (67.03 μg/mL) compared to healthy controls (146.53 μg/mL), making it a promising diagnostic biomarker with a high AUC of 0.880. Other significant correlations were found between CD and the levels of three out of eight investigated short-chain fatty acids (SCFAs). A reduction in acetic acid (AA, $FC=1.696$, $p=0.004$) and an increase in butyric acid (BA, $FC=0.682$, $p=0.004$) were observed, along with changes in indoxyl sulphate ($FC=0.624$, $p=0.023$). These disturbances strongly correlate with the severity of inflammation in CD patients, and acetic acid ($AUC=0.714$) and butyric acid ($AUC=0.717$) also may be promising biomarkers.

These studies indicate a complex interplay between gut dysbiosis, the production of bacterial metabolites, and fatty acid profiles, reflecting the inflammatory state and physiological disturbances in CD. Monitoring these metabolites can significantly contribute to a quicker and more precise diagnosis, assessment of disease progression, and optimization of personalized therapeutic strategies, ultimately leading to better disease control and an improved quality of life for patients.

Molecular Machines and Tools

Lectures

L.10.1

PROTAC-based approach for selective MLK3 degradation in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is associated with poor prognosis due to the absence of effective targeted therapies. Mixed-lineage protein kinase 3 (MLK3, MAP3K11), a member of the MLK family of serine/threonine kinases within the MAP3K subfamily, is frequently upregulated in TNBC and contributes to its tumorigenic potential. Here, we describe a novel and selective MLK3 degrader, CEP1347-VHL-02, generated by conjugating the pan-MLK inhibitor CEP1347 with a von Hippel–Lindau (VHL) E3 ligase ligand using proteolysis-targeting chimera (PROTAC) technology. CEP1347-VHL-02 efficiently induced MLK3 degradation via the ubiquitin–proteasome system across multiple cell line models while sparing other MLK family members. In TNBC cells, CEP1347-VHL-02 robustly degraded MLK3 and suppressed its oncogenic functions, as evidenced by reduced clonogenic growth and migration, cell cycle arrest, and apoptosis induction in MDA-MB-468 cells.
In summary, CEP1347-VHL-02 selectively degrades MLK3 in TNBC by harnessing the ubiquitin-proteasome system, highlighting PROTACs as powerful molecular tools to redirect cellular machines for therapeutic benefit.

Oral presentations

O.10.1

CRISPRi screen revealed new genes involved in CAG repeat shortening process following endonuclease-induced DNA break

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Polyglutamine disorders (polyQ) are rare neurodegenerative genetic diseases caused by the expansion of CAG repeats in associated genes. These include Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), spinal-bulbar muscular atrophy (SBMA), and a number of spinocerebellar ataxias (SCAs). PolyQ diseases are incurable and characterized by progressive neurodegeneration and severe motor and cognitive impairments. One of the proposed therapeutic strategies is to shorten CAG repeats to a non-pathological length using genome editing tools, such as TALENs, CRISPR-Cas9, and Cas9 nickase. Studies in yeast models, human cells, and mice have shown that the induction of double-strand breaks (DSBs) or several single-stranded DNA nicks within the repeats leads to their shortening. However, the DNA repair mechanisms involved in this process are poorly understood. Here we used a dedicated CRISPR interference (CRISPRi) screen to identify DNA repair pathways and candidate genes involved in CAG repeat shortening following endonuclease-induced DNA breaks in human cells. We discovered factors responsible for the formation of the specific editing outcomes. Finally, we harnessed discovered genes to control the CAG repeats contraction process and enrich in-frame shortening. Our studies indicate that CAG repeat contraction is a complex process that involves multiple DNA repair proteins at different repair stages.

O.10.2

Beyond icosahedral symmetry: Insights into the self-assembly of recombinant virus-like particles derived from icosahedral plant viruses

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RNA viruses assemble within infected cells, where their nascent RNAs are encapsidated into the protein capsids. Viral capsids or virus-like particles (VLPs) can be also formed *in vitro* by mixing the RNA, polyanions or other core particles with native or recombinant capsid protein (CP) under appropriate buffer conditions. This process, known as virus self-assembly, occurs due to inherent interactions among the proteins and RNA, without the need for external energy input. Recently, a growing number of studies have explored the application of VLPs as protein-based drug carriers, nanoreactors, or nanoscaffolds. Our research focuses on the self-assembly process and its products using recombinant CPs derived from plant viruses. In addition to icosahedral VLPs that resemblie native capsids, we observe intermediate forms and non-icosahedral VLPs, whose morphology is influenced by the encapsidated core particle. Our findings support a dynamic model of VLP self-assembly that is governed by the interplay of subunit-subunit and subunit–core interactions, mechanical forces, and the system's overall chemical potential. Notably, despite lacking icosahedral symmetry, the resulting VLPs are effective in delivering siRNA in cell cultures.

O.10.3

From Genotype to Function: Tools for Analysis of *LDLR* (Low-Density Lipoprotein Receptor) Variants

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Familial hypercholesterolemia (FH) is a genetic disorder primarily caused by pathogenic variants of the *LDLR* gene, which encodes the low-density lipoprotein receptor (LDLR). These variants lead to elevated levels of low-density lipoprotein (LDL) in the bloodstream, significantly increasing the risk of coronary artery disease. Substitutions in *LDLR* can affect various aspects of receptor function, including gene expression, protein maturation, membrane localization, ligand binding, internalization, and receptor recycling. Therefore, functional characterization of *LDLR* variants is essential for their accurate classification.
For the functional study, an LDLR-defective HEK293T-*ldlr*G1 cell line was established *via* a CRISPR/Cas9-mediated luciferase–puromycin knock-in. An expression vector, pTetRedLDLR, with the *LDLR* gene under the control of a regulated promoter and with a reporter gene has been constructed to overproduce LDLR variants in the host cell. A cellular model based on confocal techniques allows us to measure the activity of receptor variants in a single cell in real-time. Additionally, the luciferase knock-in at the *LDLR* promoter provides a system for studying the transcriptional regulation of the *LDLR*. This reporter system offers a convenient tool for screening novel HMG-CoA reductase inhibitors, such as statins and other therapeutics targeting atherosclerosis.

O.10.4

Mitoxantrone binds the ribosomal exit tunnel: structural and functional explanation for translation inhibition, stress granule formation, and therapeutic relevance in multiple sclerosis

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Mitoxantrone (MIT), a clinically used anthracenedione anticancer agent, has been identified as a modulator of protein synthesis and cellular stress responses. Using a combined structural approach—X-ray crystallography of bacterial ribosomes and cryo-electron microscopy of human ribosomes—we demonstrated that MIT binds within the nascent polypeptide exit tunnel (NPET). Three MIT molecules occupy the tunnel in a configuration that likely obstructs the passage of elongating nascent chains, indicating that the ribosome may serve as a direct and functionally relevant target of MIT.

Notably, MIT induces stress granule (SG) formation through a mechanism independent of eIF2α phosphorylation and mTOR signaling. SG assembly in this context appears to be driven by ribosome aggregation, even under conditions of global translation repression. MIT-induced SGs are enriched in 80S ribosomes and large ribosomal subunits, in contrast to the composition of SGs formed in response to classical stressors like sodium arsenite.

The inhibition of translation by MIT, along with its ability to induce SG formation, may help to explain its mechanism of action in the treatment of multiple sclerosis (MS). By blocking the synthesis of immunoreactive proteins, MIT likely reduces immune system activity, which is beneficial for patients and contributes to the alle*via*tion of disease symptoms.

Transport and Signalling Pathways

Lectures

L.11.1

Plant plasma membrane proton pump, its role in transport and signalling.

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Plants are constantly exposed to adverse conditions. Therefore, they have evolved systems that detect and respond to stimuli. This requires the generation and transmission of the stress signals. In addition to the signalling molecules used to transmit signals, there are evidences supporting the existence of an information system based on changes in ion flow and electrical signals (ESs). ESs represent a category of stress signals affecting a wide range of physiological processes and increasing tolerance to adverse factors in plants. The generation of ESs is accompanied by the changes in activity of the plasma membrane (PM) proton pump (PM H^+-ATPase). The PM H^+-ATPase exports cytoplasmic protons to the apoplast using the energy from ATP hydrolysis. In addition to generating a transmembrane chemical gradient of H^+, it also establishes an electrical gradient. Almost all other transport proteins in the plant PM are energized by this electrochemical gradient of H^+. The PM H^+-ATPase is involved in many physiological processes in plants. Due to its multifunctionality and sensitivity to various environmental factors, the PM H^+-ATPase plays a crucial role in plant adaptation to biotic and abiotic stress. This enzyme is an important player in plant signalling that helps survive adverse conditions. On the one hand, PM H^+-ATPase is involved in the formation of ESs, and on the other hand, its activity is modified by various signalling molecules such as plant hormones, H_2O_2, NO, and H_2S.

Oral presentations

O.11.1

Localization and functioning of the bHLH family of transcription factors

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bHLH (basic helix-loop-helix) transcription factors (TFs) are a diverse family of proteins found in all eukaryotes, acting as activators or repressors of transcription and playing key roles in numerous biological processes, including neuronal cell specification and differentiation in vertebrates. Crucial to their function is the ability to translocate between the cytoplasm and the nucleus in response to cellular signals. Nuclear localization enables DNA binding and recruitment of cofactors, while export to the cytoplasm serves as a regulatory off-switch. Additionally, some TFs exert non-genomic roles in the cytoplasm. Transport across the nuclear pore complex (NPC) requires specific localization signals – NLS for import (*via* importins α/β) and NES for export (*via* Exportin 1/XPO1). The balance between these signals determines protein localization. Dysregulation can disrupt signaling pathways, cell cycle control, and apoptosis, contributing to cancer development. Restoring proper localization can reactivate tumor suppressors or inhibit oncoproteins, making nucleocytoplasmic transport a promising therapeutic target. Although earlier studies (1999–2006) identified individual NLS/NES motifs, systematic analysis of their distribution – especially in the bHLH family – remains limited. The presentation will highlights the significance of nuclear transport signals using selected bHLH proteins (TCF4, NeuroD, NPAS4) as case studies.

O.11.2

Highly polyanionic, intrinsically disordered proteins regulate biomineralization through liquid-liquid phase separation

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Biomineralization plays a critical role in many organisms, yet the molecular mechanisms controlling mineral formation remain incompletely understood. Non-classical crystallization pathways are proposed to involve transient liquid phases of calcium carbonate stabilized by polymers, such as acid-rich proteins secreted into the skeletal organic matrix. However, direct evidence for protein-containing liquid phases has been lacking. Here, we demonstrate that highly charged acid-rich proteins regulate calcium carbonate nucleation and growth through liquid–liquid phase separation (LLPS). Using AGARP, the first acid-rich protein cloned from Acropora millepora, as a model, we show that LLPS occurs under physiologically relevant, crowded conditions forming liquid protein-calcium condensates (LPCC) that act as crystallization precursors. AGARP remains intrinsically disordered upon counter-ion binding, highlighting charge mediated interactions as key drivers. These findings introduce LPCCs as biologically relevant intermediates preceding and putative effective calcium export vesicles. This mechanism offers a new molecular-level conceptual framework, bridging the fields of phase separation and biomineralization, and suggests strategies for bioinspired materials design leveraging protein phase behavior.

O.11.3

Wnt signaling pathway inhibition as the foundation for combined molecular targeted therapy of head and neck squamous cell carcinoma

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Head and neck squamous cell carcinoma (HNSCC) is a common tumor type. Human papillomavirus-positive (HPV-positive) cases show better response to therapy and longer patient survival time. HPV-negative cases present more complex molecular changes, which contribute to patients' worse prognosis. Thus, improved concepts of molecular targeted therapy of HNSCC are needed.

Our previous research showed the importance of the Wnt signaling pathway in HNSCC cells. Inhibition of this pathway has anticancer effects, which, unfortunately, may be insufficient in monotherapy. Therefore, effective combinations of molecular targeted therapies which included Wnt signaling inhibition were searched for.

The research was conducted on HPV-negative HNSCC cell lines, using small-molecule inhibitors of Wnt signaling (PRI-724, IWP-O1), Hedgehog signaling (Vismodegib), EGFR signaling (Erlotinib), PI3K signaling (HS-173, Akt inh.), and glycolysis (2-deoxyglucose, Lonidamine).

All combinations presented beneficial effects against HNSCC cells. Inhibition of the Wnt pathway and EGFR or PI3K signaling reduced *via*bility and induced apoptosis synergistically. PRI-724 and Vismodegib synergistically reduced cell migration. In turn, Wnt pathway inhibitors mixed with anti-glycolytic agents decreased glucose metabolism.

The combined inhibition of Wnt signaling with other molecular targets crucial for HNSCC is promising for more efficient HNSCC therapy.

O.11.4

Stroma-driven horizontal transfer of TCA proteins mediates metabolic plasticity and imatinib resistance in leukemia

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Alterations in cancer cell metabolism have gained attention as a cause of adaptation and resistance to therapy. However, the molecular mechanisms, especially in leukemia resistance occurring in the bone marrow microenvironment, remain elusive. Here, we investigated the role of direct stroma-leukemia interactions as a mechanism of stroma-driven protection. Leukemia cells were cultured alone or co-cultured with stromal HS-5 stromal cells to mimic the bone marrow conditions. Interactions with stromal cells enhanced metabolic plasticity and oxidative capacity in leukemia, thereby protecting against loss of metabolic homeostasis in response to imatinib. The physical cell-cell contact was necessary for these effects. We found that stromal cells efficiently transferred membrane vesicles along with TCA-related proteins to leukemic cells. Metabolome profiling assessed that co-cultured leukemic cells treated with imatinib exhibit increased levels of TCA-related metabolites. Expression levels of genes encoding transferred TCA-related proteins strongly correlated with clinical resistance. Together, we describe a novel mechanism of direct bone marrow-mediated protection of leukemic cells, related to metabolic adaptation. These findings indicate that the presence of stromal cells triggers leukemic cells towards the protection from therapy-induced metabolic drop. Elements involved in the TCA-related metabolic plasticity may be targeted to achieve therapeutic efficacy and overcome bone marrow-mediated resistance.

Bioactive Compounds and Small Molecules

Lectures

L.12.1

The role of peroxisomes in the regulation of autophagy and ferroptosis: a novel mechanism in NBIA pathogenesis

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Neurodegeneration with Brain Iron Accumulation (NBIA) is a heterogeneous group of rare neurodegenerative disorders characterized by pathological iron deposition, primarily in the central nervous system, progressive neuronal loss, and motor and cognitive impairment. Despite advances in research, the molecular mechanisms underlying neurodegeneration in NBIA remain incompletely understood, which hinders the development of effective therapeutic strategies. Increasing evidence over the past years suggests that intracellular iron accumulation may be a secondary phenomenon resulting from other metabolic disturbances. In this study, we investigated the impact of peroxisomal dysfunction on the regulation of autophagy and ferroptosis in patient-derived cellular models of various NBIA subtypes. Immunocytochemical analyses and quantitative protein assessments revealed a marked reduction in peroxisome number and in the levels of key peroxisomal proteins, indicating a significant loss of these organelles and their function. This was accompanied by pronounced dysregulation of pathways leading to autophagy and ferroptosis, manifested by elevated ROS levels, enhanced lipid peroxidation, and altered levels of proteins involved in iron homeostasis, autophagy, and ferroptotic signaling. Our findings suggest that peroxisomes play a pivotal role in maintaining the balance between lipid metabolism, ROS regulation and iron homeostasis and open new therapeutic opportunities.

Oral presentations

O.12.1

Therapeutic targeting of hydrogen sulfide in Duchenne muscular dystrophy: functional and molecular benefits of H_2S donors in dystrophic mouse models

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Duchenne muscular dystrophy (DMD) is a severe, incurable genetic disorder caused by mutations in the *DMD* gene, leading to a deficiency of the muscle structural protein, dystrophin, which results in damage to skeletal and cardiac muscles. We have revealed altered expression levels of hydrogen sulfide (H_2S)-producing enzymes in dystrophic muscles, indicating a potential role of this gasotransmitter in DMD pathogenesis [PMID: 38002330].

To explore the impact of H_2S signaling in dystrophic muscles, we employed two mouse models of DMD, the *mdx* strain, which exhibits a milder phenotype, and the D2.*mdx* mice, known for more severe disease progression. We evaluated the effects of slow (GYY4137) and rapid (NaHS) H_2S-releasing donors. Our findings demonstrate that GYY4137 significantly improved exercise capacity and reduced markers of inflammation, fibrosis, oxidative stress, apoptosis, and necrosis in the dystrophic skeletal muscles [PMID: 40476490]. In contrast, although NaHS modulated the expression of genes and proteins associated with DMD pathophysiology, it failed to improve muscle function, as assessed by grip strength measurements [PMID: 37507045], suggesting a lower therapeutic potential compared to the slow-releasing donor.

These results underscore the therapeutic potential of H_2S donors in mitigating DMD progression, with GYY4137 demonstrating superior efficacy over NaHS.

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O.12.2

Ten years of targeting PD-L1 with small molecules: improving the bioactivity and deciphering the mechanism of action

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Started with early compounds released in 2015, the struggle for small-molecule drug candidates targeting PD-L1, an alternative to monoclonal antibodies, continues. This effort not only led to the discovery of highly potent small molecules but also to the development of cell co-culture models and the identification of new mechanisms of action (MOA).

In this report, chemical, biochemical, and biological aspects of the 10 years of the development of compounds targeting PD-L1 will be presented. The progress will be exemplified by the structure-activity relationship (SAR) analysis of terphenyl-based PD-L1-targeting molecules of our authorship. This SAR was possible thanks to the introduced experimental setups, including multiparametric flow cytometry analysis of activation of primary lymphocytes and death of cancer cells, kept in co-culture. In addition, some new biochemical observations were also made, like the formation of a PD-L1 dimer upon the binding of a small molecule or unexpected PD-L1 localization upon binding of a covalent PD-L1 ligand. Also, surprisingly, the increased expression of PD-1 on T cells turned out to reflect the efficacy of a successful PD-L1 blockade *in vitro* and *in vivo*. Altogether, the introduction of new generations of improved cell-based models allowed for progressing towards promising PD-L1-targeting small-molecule drug candidates and deciphering their MOA.

Acknowledgements
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O.12.3

Bis-(di-4-phenyl-benzylaminethiocarbonyl) disulfide Reverses Cisplatin Resistance of ABCC2 and ALDH3A1 Overexpressing Non-Small Cell Lung Cancer Cells

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Background: The best treatment modality of lung cancer is surgical resection. However, it becomes ineffective in the advanced metastatic stage. Thus, cisplatin-based chemotherapy, though restricted by intrinsic and/or acquired chemo-resistant phenotype, remains the first-line therapy for advanced non-small cell cancer (NSCLC).

Aim: Evaluation of the anticancer properties of bis-(di-4-phenyl-benzylaminothiocarbonyl)disulfide in NSCLC.

Methods: mRNA expression and protein levels of ABC and ALDH in patient-derived NSCLC samples (GSE102287, GSE43580) and cell lines A549 and NCI-H158 (and respective cisplatin-resistant variants), were assessed to identify their role as potential markers of cisplatin resistance. New ABCC2 and ALDH inhibitor - bis-(di-4-phenyl-benzylaminethiocarbonyl)disulfide was synthesized and its cisplatin-sensitizing abilities were tested *in vitro via* WST-1 assay.

Results: We identified significant subgroups of ABCC2 and ALDH3A1 overexpressing NSCLC patients, which *in vitro* (A549) are involved in the acquisition of cisplatin resistance. Bis-(di-4-phenyl-benzylaminethiocarbonyl)disulfide reverses cisplatin resistance in a cisplatin-resistant variant of A549 cells (*via* ALDH and ABCC2 inhibition), but not in NCI-H158.

Conclusions: Molecular categorization of NSCLC is essential for predicting therapy outcomes, enabling the use of bis-(di-4-phenyl-benzylaminethiocarbonyl)disulfide as a cisplatin therapy enhancer for ABCC2 and ALDH3A1 overexpressing patients.

O.12.4

Sonodynamic therapy with violacein encapsulated in nanoliposomes for melanoma treatment

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The incidence of melanoma has been increasing in the last decades, urging the need for improved treatment strategies. Violacein, a bisindole dye derived from *Chromobacterium violaceum*, has been found to exert interesting anti-cancer properties, but its limited solubility in aqueous solutions restricts its broader application in medicine. The aim of this study was to evaluate the anti-melanoma properties of violacein encapsulated in nanoliposomes as compared to violacein dissolved in PBS, with and without the use of ultrasounds. Two human melanoma cell lines, i.e. MICH-2 (primary melanoma), and HTB-140 (metastatic melanoma), and normal cell line derived from lung fibroblasts (MRC-5) were used in this study. The cytotoxicity of the evaluated therapeutic approaches was analyzed using MTT and LDH release assay after 36 and 48 hours following treatment, while the impact on reactive oxygen species generation and apoptosis induction was analyzed using flow cytometry. Overall, our study provides evidence that violacein, and in particular its nanoliposomal form, when used in combination with ultrasounds, exerts strong anti-melanoma effects, which deserve further investigation.

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Cell Biology

Lectures

L.13.1

Mechanosignaling in human pancreatic beta cell development and function

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Pancreatic beta cells are critical for maintaining glucose homeostasis, and their dysfunction leads directly to diabetes. A deeper understanding of the molecular mechanisms governing their formation and function is therefore crucial for developing new therapeutic strategies. Our research, leveraging human pluripotent stem cells and multi-omics analysis, reveals a critical and robust role for mechanosignaling in both the derivation and function of human beta cells. In this presentation, we will detail how various mechanosignaling pathways regulate beta cell fate, replication, and insulin secretion. We will also explore how these pathways operate at different cellular levels and compartments, highlighting their intricate crosstalk with metabolic processes. Our findings establish mechanosignaling as a fundamental determinant of human beta cell biology, opening new avenues for therapeutic intervention in diabetes.

Oral presentations

O.13.1

MCPIP Proteins as Key Modulators of Skin Cancer Fate

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The MCPIP (Monocyte Chemoattractant Protein-Induced Protein) family, also known as Regnases, comprises endonucleases with PIN and CCCH-type zinc finger domains. It includes four members (MCPIP1–4) with tissue- and cell-specific expression. In skin, MCPIP1 is found in differentiated epidermal cells, while MCPIP3 is present in basal layer cells. MCPIP proteins help resolve inflammation and maintain tissue homeostasis.

We used the DMBA/TPA model to induce skin carcinogenesis in mice lacking MCPIP1 or MCPIP3 specifically in keratinocytes (MCPIP1-eKO, MCPIP3-eKO). Tumors were analyzed by histology and RNA-seq.

MCPIP1 deficiency accelerated squamous cell carcinoma (SCC) development, resulting in larger, more aggressive tumors with elevated markers of proliferation and angiogenesis, alongside increased IL-6, IL-33, and TGF-β. The RNase activity of MCPIP1 was crucial for suppressing genes encoding SCC antigens and MMP-9.

In contrast, MCPIP3-eKO mice developed papillomas similar in number and onset to controls, but with distinct morphology. Unlike SCC-like papillomas in controls, MCPIP3-eKO tumors lacked keratin pearls and showed sebaceous hyperplasia and keratin-filled cysts. RNA-seq revealed strong activation of the type I interferon response.

In summary, MCPIP1 and MCPIP3 loss in keratinocytes leads to divergent effects on tumor differentiation and aggressiveness in chemically induced skin cancer.

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O.13.2

Mutations in the *EMD* gene disrupt myogenesis of EDMD1 patients' cells.

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Emery-Dreifuss muscular dystrophy type 1 (EDMD1) is a rare genetic disease caused by mutations in the *EMD* gene encoding emerin. Emerin, a nuclear envelope protein, is involved, among others, in the cell nucleus structure maintenance, chromatin organization, DNA-damage repair, regulation of gene expression, and stress response. The main symptoms of EDMD1 are manifested in the skeletal muscles and in the heart, including weakness and muscle wasting, tendon contractures, and cardiac dysfunction. Mechanisms underlying the development of EDMD1 phenotype remain poorly understood.

In our studies, using our novel unique model of iPSCs-derived muscle cells from EDMD1 patients, we observed the wide-ranging aberrations during myogenesis. iPSCs from three EDMD1 patients together with cells from healthy donors were differentiated *in vitro* to satellite cells (SCs), myoblasts (MBs) and myotubes (MTs). Our RNA-seq analysis revealed extensive differences in the gene expression between patients and control SCs. Moreover, we indicated increased apoptosis level and decreased migration rate in SCs from emerin-null patients. We also observed a delay in expression of the MBs marker – *MYOD*, and delay in the timeline of MTs formation. Furthermore, an increased level of DNA double-strand breaks was observed.

Our results indicated that mutations in the *EMD* gene cause serious aberrations during myogenesis in EDMD1 patients' cells and affect many aspects of this process.

O.13.3

Context-Dependent Role of miR-378a in Diabetic and Ischemic Heart Disease: Evidence from Multi-Omics and hiPSC-CM Applications

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Diabetic cardiomyopathy and myocardial infarction share mechanisms like impaired metabolism, inflammation, and cardiomyocyte death. The muscle-enriched microRNA-378a, regulating IGF1R, may influence cardiomyocyte survival and remodeling.

Diabetes was induced by streptozotocin (STZ) in male C57BL/6 wild-type (miR-378a+/+) and knockout (miR-378a−/−) mice, in parallel with a human model where hiPSC-derived cardiomyocytes (hiPSC-CMs) of both genotypes were exposed to high glucose (HG). AAV-miR-378–modified hiPSC-CMs were injected into NOD-SCID mouse hearts post-myocardial infarction (MI).

Loss of miR-378a promoted hiPSC-CM hypertrophy, calcium signaling defects, and in vivo cardiac fibrosis. Multi-omics analyses revealed disrupted metabolic and signaling pathways linked to diabetic cardiomyopathy. Interestingly, HG elevated miR-378a levels and activated pro-hypertrophic pathways in both hiPSC-CMs and hyperglycemic miR-378a+/+ mouse hearts. However, hyperglycemia-induced dysfunction, marked by reduced output despite preserved contractility, occurred regardless of miR-378a presence. Post-MI, miR-378a overexpression did not enhance hiPSC-CM therapy benefit.

These findings suggest miR-378a is vital for cardiomyocyte metabolic balance and integrity, however, its post-infarction overexpression offers limited benefit. Similarly, elevated miR-378a levels in diabetic hearts fail to prevent hyperglycemia-induced dysfunction, indicating limited therapeutic value in this context.

O.13.4

The role of Cap2 in the neuromuscular system

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The neuromuscular system controls voluntary movement through signals from motoneurons to muscles, mediated by acetylcholine. This process relies not only on synaptic integrity but also on proper cytoskeletal organization. Actin dynamics and its regulators, such as cyclase-associated protein 2 (Cap2), play a key role in maintaining neuromuscular function. However, the precise function of Cap2 in this system remains unclear. Systemin Cap2 KO mice have abnormal contractile machinery organization in the form of rings perpendicular to the muscle fiber, which is a hallmark of several muscular dystrophies. Mutant mice exhibit also increased number of neuromuscular junctions (NMJs) with atypical sizes, postsynaptic machinery fragmentation, and presynaptic axonal swelling. While motoneuron-specific Cap2 knockouts have only presynaptic changes, muscle-specific deletion causes extensive postsynaptic alterations in contractile machinery and NMJ, emphasizing the importance of Cap2 in maintaining neuromuscular integrity.

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Developmental Biology

Lectures

L.14.1

Uncovering the role of the noncoding genome in heart development

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The heart plays a vital role in circulating oxygen-carrying blood and nutrients throughout the body. The core genetic program and stepwise morphogenesis governing its development are largely conserved across metazoans, and aberrations to this process could lead to congenital heart disease (CHD). Transcriptomics and epigenomic analyses in the zebrafish model revealed non-coding elements with putative enhancer activity across heart developmental stages. One such element, designated *-6.8got2b*, drove gene expression in the cardiac trabeculae, a structure essential for cardiac contraction. The *-6.8got2b*-driven EGFP+ expression domain overlapped cardiomyocytes but not endocardium. Treatment with Erbb2 inhibitor which blocks trabeculae development significantly reduced the EGFP+ domain, supporting its identity as cardiac trabeculae. Motif analysis identified binding sites for several transcription factors, suggesting potential upstream regulators of trabeculae development. Ongoing functional validation and cross-species comparisons continue to highlight the significance of this novel enhancer in cardiac trabeculation. In parallel, we developed a computational pipeline to systematically identify and analyse human single nucleotide polymorphisms (SNPs) associated with CHD, revealing additional candidate enhancers driving heart expression. I will present ongoing work from both zebrafish and human studies, aiming to uncover regulatory mechanisms in heart development and disease.

Oral presentations

O.14.1

The role of mitochondria in shaping of bovine embryonic developmental competence – from oocyte maturation to the first cleavage division

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Early embryonic development relies on maternal factors stored in the ooplasm. Oocytes grow and mature surrounded by the cumulus cells (CC). CC come in direct contact with zona pellucuda and *via* the trans zonal projections (TZP) allow for movement of growth factors and signalling molecules necessary for oocyte maturation. At this time, high requirement for energy is met by mitochondria (Mt). Mt associate with microtubules for quick movement towards certain cellular compartments. Mt may stabilise the karyokinetic spindle and secure proper chromosome segregation during cell division. Organelle malfunction and aneuploidies are the leading causes of embryo developmental arrest. We have evaluated changes in Mt distribution during oocyte maturation, followed Mt movement from the CC towards the oocyte during maturation, investigated Mt-karyokinetic spindle (KS) associations during meiotic progression, zygote formation and at first cleavage divisions. The results suggest that during oocyte maturation Mt migrate from the CC (*via* TZP) in a vesicle like structures to be deposited at the oolemma, plausibly complementing oocyte's Mt pool. Our results indicate that during oocyte maturation, concomitantly with ER, Mt relocate to form cluster-like structures that surround the KS. Furthermore, we have observed that a certain pool of mitochondria is retained within the polar body and associated with the transient structure recognised as the midbody.

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O.14.2

Diet-induced hyperhomocysteinemia impairs mouse oocyte quality and reveals distinct transcriptional alterations at single-cell level

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Hyperhomocysteinemia (HHcy), a condition of elevated homocysteine (Hcy), negatively affects reproduction. Although its detrimental impact on fertility is documented, the mechanisms by which HHcy affects oocyte condition remain poorly understood. As oocyte quality relies on mitochondrial function and lipid storage, this study investigates how diet-induced HHcy alters oocyte competence and gene expression at the single-cell level.

HHcy was induced in C57BL/6 females by methionine Met (1%) or Hcy (0,1%) diet for 5 weeks. Urinary total Hcy confirmed successful HHcy induction. Oocytes were collected post-mortem for assessment of mitochondrial DNA copy number, mitochondria, lipid droplets (LD), reactive oxygen species (ROS), and glutathione by fluorescence imaging and qPCR. Single-cell transcriptomic profiling was performed using 10x Genomics Chromium on ovarian nuclei.

Both diets impaired oocyte mitochondrial content, redox balance, and promoted lipid accumulation. Transcriptomic profiling revealed distinct, diet-specific molecular signatures. The Met diet activated stress-related pathways and compensatory metabolic responses, while the Hcy diet evoked a stronger mitochondrial stress reaction and disrupted transcriptional programs essential for oocyte maturation.

These alterations likely underlie the reduced oocyte developmental competence and quality observed under HHcy, highlighting novel mechanisms that warrant further study.

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O.14.3

GPX2 and oxidative stress as gatekeepers of pancreatic cell fate

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Cell fate decisions in human endoderm development are tightly regulated with extensive transcriptional networks described, yet the mechanistic role of metabolic products in this process remains elusive. Endodermal posterior foregut (PFG) gives rise to multiple organs, like pancreatic, liver and intestine. Here, we identify Glutathione Peroxidase 2 (GPX2) as a critical regulator of human PFG differentiation, revealing oxidative stress as a key determinant of pancreatic versus non-pancreatic cell fate. We demonstrate that GPX2-deficient hPSCs under pancreas-promoting conditions differentiate also into hepatic progenitors. Through bulk and single-cell RNA-sequencing, ATAC-sequencing, and functional studies, we reveal that GPX2 orchestrates lineage commitment by regulating PDX1 and other key transcription factors, shaping PFG patterning and leading to the emergence of multilineage pro-liver and pro-intestinal progenitors. Mechanistically, GPX2 deficiency triggers extracellular matrix (ECM) remodeling, activating BMP signaling, and skewing differentiation away from the pancreatic lineage. Manipulating oxidative stress - either by inducing or alle*via*ting ROS levels - recapitulates or rescues the effects of GPX2 loss, establishing oxidative stress as a gatekeeper of pancreatic fate. These findings establish GPX2 as a critical redox-sensitive determinant of human pancreatic differentiation and underscore the impact of oxidative stress on human endoderm lineage specification.

O.14.4

Dystrophin isoforms DP71 and DP427 determine cell *via*bility during proliferation and myofiber differentiation

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Duchenne muscular dystrophy (DMD) is an X-linked disease caused by mutations in the dystrophin gene that affects 1 in 5000 boys. DMD manifests with progressive skeletal muscle wasting, respiratory difficulties, cardiac dysfunction, and cognitive and neuropsychiatric symptoms. As the disease progresses, affected boys lose their ambulation and die prematurely. All DMD patients lack the full-length DP427 dystrophin, while approximately 10% lose all dystrophins, including DP71, synthesized ubiquitously in various cell types, including myoblasts. There is evidence that the cognitive symptoms of DMD patients vary depending on the location of the mutation site and the number of missing dystrophins, and there is some indication that this may also be the case in skeletal muscle. We therefore asked about the specific roles of DP427 and DP71 during cell proliferation and fiber differentiation. We showed that despite different expression patterns, the absence of either protein resulted in a similar phenotype, including increased membrane permeability, mitochondrial aggregation, increased reactive oxygen species, and increased cyto- and genotoxicity. These results explain the observed differences in disease severity in patients with different levels of DP71 and suggest that patients deficient in both DP71 and DP427 may require a different therapeutic approach than patients deficient in only DP427.

Neurobiology

Lectures

L.15.1

Evolution of foetal astrocyte functions in primates

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Astrocytes play a crucial role in the establishment and regulation of the brain's higher-level functions. Evolutionary changes in astrocyte activity during development and adulthood likely contribute to the establishment of the unique cognitive capacities of the human brain. However, while the transcriptional differences between human and non-human primate (NHP) adult astrocytes are increasingly better defined, the molecular signature of fetal astrocyte evolution is unknown. Likewise, the functional contribution of evolutionary changes in astrocyte gene activity in brain biology remains uncharted.

We used human, chimpanzee, and macaque induced pluripotent stem cell-derived astrocytes (iAstrocytes) as a robust source of foetal astrocytes. Human iAstrocytes are bigger and more complex than NHP iAstrocytes. We found new loci and cellular pathways related to the interspecies differences in astrocyte size and complexity. Strikingly, genes related to intercellular communication are a frequent target of evolutionary changes in expression in human compared to NHP iAstrocytes. Our most recent data show the implication of downregulation of apolipoprotein E (APOE) expression in the human lineage in the regulation of neuronal functions.

Evolution is largely fuelled by changes in gene activity, which in turn arise as a corollary to genetic modification of distal DNA regulatory elements, including enhancers. Enhancers evolve fast. Yet, whether there are general and broadly applicable sequence changes that lead to functional activation of enhancers in evolution remains enigmatic. Our multilevel regulome analysis and machine learning revealed that functional activation of astrocytic enhancers coincides with a previously unappreciated, pervasive gain of binding sites of 'stripe' transcription factors, which are general transcriptional regulators. Altogether, we uncover genes and pathways linked to fetal astrocyte evolution and shed new light on a mechanism driving the acquisition of the regulatory potential of enhancers.

Oral presentations

O.15.1

Virus-like system of neuronal communication: deciphering the mammalian Arc – RNA interactions.

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The human genome contains almost 100 genes encoding Gag-like proteins derived from domesticated Ty3/Gypsy retrotransposons. One of these proteins is Arc (activity-regulated cytoskeleton-associated) protein, which serves as a central hub for synaptic interactions. Arc is essential for synaptic plasticity, memory formation, and postnatal cortical development, and its dysregulation is linked to multiple neurological disorders.

Arc mediates a unique mode of intercellular communication reminiscent of viral infection: Arc proteins assemble into virus-like capsids that encapsulate Arc mRNA, exit the parent neuron *via* exocytosis, and spread to recipient cells, where the mRNA is released and translated. We used a multifaceted approach, including quantitative binding measurements, structural RNA mapping, 3D modeling, and nucleic acid chaperone activity assays, to elucidate how Arc mRNA is selected and packaged into virus-like capsids. We demonstrate that Arc binds its own mRNA in a highly specific manner, targeting conserved sequences within the 5' region of its coding sequence. This binding is guided by RNA structure, occurring near highly stable, solvent-exposed helices. Furthermore, both the matrix-like and capsid-like domains of Arc contribute to RNA binding. Finally, we provide evidence that Arc possesses nucleic acid chaperone activity.

Acknowledgements

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O.15.2

Transcriptional and nuclear pathology underlying Purkinje cell dysfunction in spinocerebellar ataxia type 7

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Spinocerebellar ataxia type 7 (SCA7) is a neurodegenerative disorder characterized by the progressive loss of coordination, which can be traced to the dysfunction and degeneration of Purkinje cells (PCs) - inhibitory neurons located in the cerebellar cortex. Despite decades of research, the disproportionate vulnerability of PCs in SCA7 and other forms of ataxia remains poorly understood. Using single-cell analysis of SCA7 mouse models, we identified a progressive accumulation of gene expression changes in PCs, culminating in the transcriptional collapse of genes that define zebrin-II identity. We also observed a global reduction in mRNA levels within these neurons. Our findings suggest that these pathological changes are associated with alterations in chromatin architecture. Specifically, we observed a reduction in nuclear size in PCs from both SCA7 mice and human patients. This nuclear shrinkage correlates with the formation of liquid–liquid phase separation (LLPS)-like condensates containing ubiquitinated histone H2B, RNF20, RNF40, and poly-ADP-ribose. The composition of these structures suggests a disruption of transcriptional dynamics mediated by RNA polymerase II, potentially explaining the transcriptional failure observed in ataxic PCs. Our findings underscore the importance of nuclear integrity in maintaining PC identity and resilience in the context of neurodegeneration.

O.15.3

Pathogen threat proximity shapes host extracellular vesicle production in pre-infection response

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Extracellular vesicles (EVs) play a crucial role in immune responses, yet it remains unclear whether pathogen metabolites alone can stimulate EV production prior to infection. Using *Caenorhabditis elegans*, we investigate this question through the lens of exophers—large, evolutionarily conserved EVs known to enhance proteostasis and improve reproductive fitness. Our study uncovers regulatory mechanisms driving exopher production in response to pathogen-derived volatile and non-volatile metabolites, providing insights into host–pathogen signalling before physical interaction. We reveal a sophisticated network that adjusts EV production based on pathogen proximity: non-volatile metabolites, signalling an immediate threat, activate immune-dependent EV pathways, while volatile metabolites, forewarning potential danger, initiate immunity-independent exopher production. Both responses are regulated by different neuronal circuits compose of multiple sensory neurons and interneurons. Moreover, transcriptomic profiling revealed four GPCRs essential for the non-volatile response, and untargeted metabolomics pinpointed the pathogen-derived tripeptide as a direct inducer of EV production. Notably, volatile-induced exopher biogenesis enhances offspring survival against pathogens. Thus, *C. elegans* discriminates pathogen proximity through metabolite type, engaging EV-dependent physiological programs tailored to immediate or anticipated threats, and fine-tuning survival strategies.

O.15.4

Infrared Spectroscopy Identifies Biomarkers for Non-Invasive Diagnosis of Neurodegeneration in ALS Mouse Models

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Background: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease with a challenging diagnosis. Fourier Transform Infrared (FTIR) spectroscopy enables detailed molecular profiling of biological samples and shows promise in distinguishing diseased from healthy tissues and fluids, though its use in ALS biomarker analysis remains limited. **Objective:** To evaluate (1) the potential of FTIR spectroscopy to distinguish diseased from healthy controls, (2) the extent of protein aggregation, and (3) changes in total homocysteine (tHcy) levels in a mouse model of ALS. **Methods:** Plasma, brain, liver, and spleen from transgenic SOD1^{G93A} mice (n=5–12) and WT controls (n=8–9) were analyzed using FTIR spectroscopy, Thioflavin T (ThT) assay, and HPLC. **Results:** FTIR spectra of plasma showed a peak at 1587 cm^{-1}, indicating increased β-sheet-rich proteins, likely fibrinogen. Additional shifts at 1177, 1744, and 3050–2800 cm^{-1} suggested altered lipid composition and fatty acid saturation. The CH□ /Amide I ratio was higher in the SOD1 group, indicating disrupted lipid-to-protein balance. ThT assay showed no significant differences in protein aggregation. tHcy levels were elevated in the spleen and liver of SOD1 mice. **Conclusion:** FTIR-detected molecular alterations that may serve as promising biomarkers and support its use as a non-invasive ALS diagnostic tool.

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Lectures

L.16.1

From bench to bedside – T regulatory cells in the treatment of autoimmunity

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T regulatory cells (Tregs) are considered a *via*ble option in immunosuppressive treatment in the clinic. First promising clinical experiments and trials with clinical-grade Tregs cultured as advanced therapy medicinal product (ATMP) are completed already. In our centre, the drug has been tested in graft versus host disease, type 1 diabetes and multiple sclerosis. We will present the path from preclinical studies to the results of clinical trials and future perspectives on the application of this cellular drug in the treatment and prophylaxis of autoimmune diseases.

Oral presentations

O.16.1

Modelling PACS2 syndrome in the nematode *Caenorhabditis elegans* for drug repurposing screening

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Rare diseases may be rare individually, sometimes only one case in the world, but rare disease patients are numerous. Rare diseases affect almost 10% of the human population. There are approximately 10000 rare diseases, For most, there is no cure. Tackling rare and ultra-rare diseases requires a radically different approach than traditional drug discovery. This approach has to be extremely fast and cost-effective. Here, we present an approach for modelling rare diseases in the model organism *C. elegans*.

We developed a *C. elegans* model of PACS2 syndrome, a developmental and epileptic encephalopathy. PACS2 syndrome is an ultra-rare disease, with only 70 cases diagnosed worldwide. PACS2 syndrome is a developmental and epileptic encephalopathy characterised by the onset in the first days or weeks of life of seizures, global developmental delay, hypotonia, behavioural abnormalities, and dysmorphic features or ophthalmologic defects. The disease is caused by a point mutation E209K in phosphofurin acidic cluster sorting protein 2 (PACS2). We have introduced the homologous gene into the *C. elegans* genome. The *C. elegans* mutant is *via*ble and superficially wild-type, but mitochondria have an altered morphology. The mutant animals are not sensitive to aldicarb, an insecticide that is an acetylcholinesterase inhibitor. The behavioural abnormalities can be utilised in a high-throughput drug repurposing screen, allowing for the distinction between the "cured" and "sick" animals.

O.16.2

Ductular reaction in Mcpip1-deficient mice is alle*via*ted by *Lactobacillus rhamnosus* treatment *via* targeting gut-liver axis.

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Background and Aims: Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease characterized by progressive cholestasis and biliary destruction. It remains incompletely understood in its pathogenesis and current therapies, including ursodeoxycholic acid (UDCA), exhibit limited efficacy in advanced disease. Here, we investigate the therapeutic potential of *Lactobacillus rhamnosus* (Lbr) in a murine model of PBC using Mcpip1fl/flAlbCre mice.
Methods: Six-week-old male Mcpip1fl/fl and Mcpip1fl/flAlbCre (knockout) mice were randomized into five treatment groups for six weeks: (1) corn oil (control), (2) Lbr, (3) UDCA, (4) UDCA + Lbr, and (5) UDCA + OCA. Liver and gut pathology, serum biomarkers, transcriptomic profiles, and microbiome composition were analyzed post-treatment.
Results: Control knockout mice exhibited severe cholangiocyte proliferation, fibrosis, elevated serum total IgM, bile acids, and anti-PDC-E2 autoantibodies, alongside gut pathology marked by intraepithelial lymphocyte infiltration and mucosal hypertrophy. NGS of liver tissue revealed enrichment of humoral immune responses and T cell activation pathways in knockouts, all of which were significantly attenuated by Lbr monotherapy. Lbr treatment also restored gut architecture, reduced inflammation, and modulated the microbiome.
Conclusion: PBC symptoms in 6-week-old Mcpip1fl/flAlbCre mice were significantly alle*via*ted by targeting gut-liver axis by *Lactobacillus rhamnosus* probiotic.

O.16.3

From first clinical-stage CAR-T product developed in Poland to advancements on international biotech landscape – summary of FamiCordTx clinical trials and ongoing research.

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CAR-T cell therapy has transformed the treatment of hematologic malignancies such as B-cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma (DLBCL), and mantle cell lymphoma (MCL), yet access in Poland remains limited due to high costs and dependence on imported products. To address this unmet need, in collaboration with Warsaw Medical University and Gdańsk Medical University, we initiated the first Polish clinical trial (FCTX-CL19-1) evaluating an autologous CD19-directed CAR-T product (Tarcidomgen Kimleucel) manufactured entirely in Poland under GMP conditions. We have developed full protocols for both: vector and final product manufacturing that have been used in clinical trial.
Despite a limited number of treated patients and relatively low doses, all participants exhibited remission in peripheral blood markers by day 30, with one maintaining a complete response a year post-treatment. The entire manufacturing process was developed and conducted in Poland, establishing strategic independence in CAR-T therapy development. Currently, FamiCordTx is advancing allogeneic, off-the-shelf therapies by integrating novel molecular designs and construct technologies with the immunological advantages of cord blood-derived cells, aiming to enhance safety and efficacy while minimizing mutagenesis risk. Additionally, the company is expanding into solid tumor research, with new programs planned for the coming years.

O.16.4

3D cardiac microtissue model reveals vascular, contractile and transcriptomic dysregulation in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a progressive X-linked disease caused by mutations in the dystrophin gene, leading to skeletal muscle wasting and cardiomyopathy – the primary cause of death. Due to the limitations of animal models, patient-specific human systems are needed to study cardiac pathophysiology in DMD. Hence, we generated 3D cardiac microtissues (cMTs) from three isogenic pairs (DMD and control, CTRL) of hiPSC-derived cardiomyocytes (CMs), endothelial cells (ECs) and cardiac fibroblasts (CFs) to investigate pathophysiological processes underlying DMD-related cardiomyopathy. CMs (75%), ECs (15%), and CFs (15%) self-assembled into spontaneously beating tissues with confirmed expression of cell specific markers: cardiac troponin, CD31/vWF and DDR2/vimentin. DMD cMTs were smaller, displayed reduced vessel-like network formation and enhanced contraction rate with delayed relaxation as compared to CTRL cMTs. DMD and CTRL cMTs revealed low G2/S/M cell cycle fractions in each cardiac cell types suggesting a mature, low-proliferative phenotype. A slight increase in G1-phase cells in DMD cMTs may reflect impaired cell cycle re-entry. Transcriptomic analysis indicated metabolic stress adaptation (*ENO3, ATP5ME* upregulation) and downregulation of genes linked to regeneration, structural organization, and vascular interaction (*LBH, ITGA1, NCAM1, NRG3, NRXN3*). These findings demonstrate that DMD cMTs capture key pathological features and offer a model for therapeutic studies.

Biotechnology and Microbiology

Lectures

L.17.1

Interplay Between Autophagy and Apoptosis in Viral Infections: Insights from *Lagovirus europaeus*

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Autophagy and apoptosis are key cellular responses to stress, such as viral infections. Autophagy supports cell survival by degrading damaged cytoplasmic components and eliminating intracellular pathogens. Viral infection and replication impose significant stress, often activating autophagy as an antiviral defense. It also aids in antigen processing and presentation, enhancing adaptive immunity. Apoptosis, a form of programmed cell death, can be triggered by viruses to restrict replication and spread. However, excessive or dysregulated apoptosis contributes to viral disease pathogenesis. Both processes may be simultaneously activated, interacting synergistically or antagonistically to determine cell fate. Current studies emphasize their role in viral infections and the importance of understanding their interplay. Rabbit hemorrhagic disease (RHD), caused by *Lagovirus europaeus*/GI.1 and GI.2, is a severe infection primarily affecting hepatocytes and serves as a model of acute liver failure and viral hemorrhagic fevers. We demonstrated that GI.2-induced RHD activates both autophagy and apoptosis not only in the liver but also in non-target organs. This suggests autophagy may help counteract tissue damage caused by excessive apoptosis. These findings provide novel insight into RHD pathogenesis and the interaction between autophagy and apoptosis during viral infections. They also suggest potential therapeutic targets to reduce multi-organ damage and propose candidate biomarkers for monitoring disease progression.

Oral presentations

O.17.1

Developing modular virus-like particles as vaccine platforms for challenging pathogens

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Virus-like particles (VLPs) are multiprotein structures that resemble *via*ble virus particles in conformation and therefore are potent triggers of immune response in mammals. Being non-infectious and non-replicative, VLPs are attractive candidates for safer vaccines. Modular VLPs are composed of proteins displaying universal attachment moieties allowing for further modification with antigens of interest. Such modular VLPs might be of particular use for creating multivalent vaccines or vaccines against challenging pathogens, when preparations produced by "traditional" approaches were not effective. Moreover, modular VLPs serving as ready-to-use vaccine platforms offer an attractive solution during epidemics/pandemics, since they can be easily and rapidly adapted to newly emerging pathogen stains.

Here we present two types of modular VLPs, where antigens are anchored to the viral scaffolds by two different methods. First VLP originates from a bacteriophage and displays on its surface large trimeric antigens: Receptor Binding Domain from spike protein from SARS-CoV2, attached by Spytag-Spycatcher technology. Second VLP originates from a feline parvovirus, to which linear antigens from Feline Infectious Peritonitis Virus are tethered using click chemistry conjugation. Both approaches resulted in efficient functionalization of the two VLPs, which was confirmed using size-exclusion chromatography, electrophoretic separation, dynamic light scattering and cryo-electron microscopy.

O.17.2

Development of the universal chemically-defined medium for the analysis of „stressed" bacterial EVs in the context of undernutrition

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Undernutrition represents a significant global health challenge, impacting vulnerable groups such as the elderly and chronically ill. Its influence on the gut microbiome is complex, causing quantitative and qualitative shifts that alter inter-bacterial and host interactions. Recent insights suggest that bacteria under nutritional stress produce changed extracellular vesicles (bEVs), which could serve as universal messengers modulating the gut environment.
This project aims to elucidate the role of undernutrition-induced stress on bacterial EV production, focusing on key nutritional deficiencies and excess of bile salts. We are developing a universal chemically-defined medium (CDM) optimized for the reproducible culture of commensal strains. Using CDM we can easily change its composition and analyse bacterial growth and the resulting bEVs without the need of analysing medium-derived EVs. CDM enables systematic comparison of bEVs, including proteomic and transcriptomic profiling to identify functional cargo involved in microbiome-host interactions and to investigate their biophysical properties and cargo.
This research aims to establish a standardized platform for the production and analysis of bEVs, fostering a deeper understanding of their role in gut health. These insights may pave the way for novel biomarkers or therapeutic strategies in undernutrition and related digestive pathologies.

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O.17.3

Optimizing pyocyanin production with nanostimulants

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Bacterial phenazines, including pyocyanin (PYO), are redox-active molecules that can be applied in agriculture, electronics, and medicine. Unfortunately, production efficiency is far from optimal, and additional optimization steps should be taken.
The work aimed to optimize the production of pyocyanin in *P. aeruginosa* ATCC 27853 using nanostimulants.
The design of experiments (DoE) was employed to optimize PYO production using zinc oxide nanoparticles (ZnO NPs) and multi-walled carbon nanotubes (MWCNTs) as stimulants. PYO was produced in KingA liquid medium. Optimization parameters included temperature and the concentration of nanomaterials. The product was extracted using chloroform and hydrochloric acid, and its concentration was quantified spectrophotometrically. Bacterial physiology under optimized conditions was investigated using flow cytometry, confocal microscopy, quantitative PCR (qPCR), and spectrophotometry.
PYO production was maximized by using the optimal concentrations of nanomaterials. These stimulants significantly and precisely altered bacterial physiology, including biofilm formation, cellular stress response, and transmembrane transport.
In conclusion, the use of nanostimulants in bioprocess engineering opens up novel areas of research that can serve as a tool for the intensified production of desired metabolites.

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O.17.4

Clinical picture of selected cytokine secretion in pediatric patients with urosepsis caused by *E. coli*

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Urosepsis is a clinical condition that may develop as a result of an activation cascade that leads, among others, to enhanced synthesis and secretion of cytokines. The presence of bacterial antigens in the urinary tract of a child stimulates a rapid immune response, production of cytokines, nitric oxide and the influx of neutrophils.

The aim of this study was to assess the cytokine profile in patients with urosepsis.

The analyses were performed using blood plasma and urine of young patients who developed urosepsis caused by *E. coli* ESBL (-). Flow cytometric assessment of the levels of selected cytokines were performed.

With the exception of IL-12p70 and IL-2, the analyzed cytokines were present in both plasma and urine of the studied patients. Urinary IL-6, IL-8, IL-1β, and IL-17A concentrations (as opposed to IL-10) were significantly higher than plasma values. Plasma Th1/Th2 index (IFN- γ /IL-4 ratio) was opposite to the value determined in urine.

It seems that anti-inflammatory processes may be triggered with a delay, that may result in the exacerbation of clinical symptoms. Interestingly, the high urine concentration of IL-8, which plays a chemotactic role towards neutrophils and may lead to increased oxidative stress contributing to tissue damage and intensification of inflammation was observed.

Acknowledgements

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Plant Biology

Lectures

L.18.1

How plants sense invaders?

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Plant immune system consists of two tiers that rely on distinct types of receptors. The cell surface located Pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs). Whereas intracellular nucleotide-binding leucine-rich repeat receptors (NLRs) detect pathogen-derived effectors. NLRs either directly bind the effectors or sense modifications they introduce into host targets. The proper function of NLRs requires a chaperone complex consisting of HSP90, SGT1 and RAR1 whose role is to maintain receptors in an inactive but signaling-competent form. Upon activation, NLRs assemble into large protein complexes, called resistosomes. Their formation initiates a multicomponent defense response, that often leads to onset of programmed cell death at pathogen entry sites. This strategy efficiently restricts the infection, but it requires tight spatiotemporal regulation. Our studies allowed us to propose two mechanisms controlling this process. We have shown that nucleocytoplasmic equilibrium of some NLRs, including tobacco N immune receptor is determined by phosphorylation status of SGT1. We have also revealed that NL, a splice isoform of tobacco N immune receptor inhibits formation of N resistosome. Fascinatingly, these early steps of plant immunity are often manipulated by the effectors. HopBF1 effector of bacterium Pseudomonas syringae nicely illustrates this concept. We have shown that HopBF1 is an atypical kinase targeting plant HSP90 and thus interfering with the host defense response.

Oral presentations

O.18.1

The new, comprehensive repository for plant rich-glycine proteins-construction, challenges and perspectives

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Glycine rich proteins (GRPs) with a modular structure belong to potent regulators of plant gene expression. Little is known about the role of GRPs in heat response. At least 50 various *grp* genes differentially expressed under heat and heat recovery were identified in cauliflower (*Brassica oleracea* var. *botrytis*) leaf transcriptome. Due to the record annotation quality, we constructed a repository, which will enable re-analysis of transcriptomic data to retrieve additional stress responsive *grp* genes.

A total of 766 non-redundant GRP records from several model and crop plant species as well as data on *grp* genes and transcripts, GRP properties, classification, sequence motifs, subcellular location and orthologs were deposited. The distribution of MW and pI allowed for the clear clustering of GRPs, especially from classes I, IV and V. Subcellular targeting prediction indicated that numerous GRPs interact with the intracellular membrane system or are secreted. The class IV contains medium-sized acidic or slightly basic proteins, most likely targeted to mitochondria. On the contrary, classes I and V are enriched in ER-targeted small, basic GRPs.

In the future, the repository will be transformed into an *online* database and other features of GRPs will help record annotation. The development of a neural network model based on language models should allow for the independent verification of GRP classification, and GRP subcellular targeting will be predicted with other *in silico* tools.

O.18.2

Exopolysaccharides from *Hymenoscyphus fraxineus* induce cell wall remodeling and activates immune response in ash and Arabidopsis

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Ash dieback, caused by the invasive fungal pathogen *Hymenoscyphus fraxineus*, poses a major threat to ash tree populations in Europe. Understanding how plants respond to fungal infection is critical for uncovering resistance mechanisms. This study investigated the effects of fungal exopolysaccharides (EPS) on cell wall integrity and defense responses in *Fraxinus excelsior* and *A. thaliana*. We found that EPS extracted from *H. fraxineus* contain mixed-linkage glucans and significantly trigger the expression of defense-related genes. However, this induction was markedly reduced in xyloglucan-deficient Arabidopsis mutants, suggesting that xyloglucan, a key structural component of the plant cell wall, plays an important role in mediating immune responses. Cell wall compositional analysis revealed significant alterations in xyloglucan structure and other components, including cellulose and glucose, following EPS treatment. In addition, EPS induced oxidative stress, as evidenced by elevated ROS levels and the subsequent deposition of callose, a β-1,3-glucan, at the cell wall and plasmodesmata. Defense signaling analysis identified the jasmonic acid/ethylene and salicylic acid pathways as key regulators of fungal glycosyl hydrolase gene expression, linking hormone signaling to pathogen interaction and carbohydrate metabolism. Taken together, this study provides valuable insights into plant defense strategies and may inform future approaches for enhancing resistance to fungal pathogens.

O.18.3

The role of the stringent response in the regulation of tomato (*Solanum lycopersicum* L.) plant development and responses to stress

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Plant RelA/SpoT Homologs (RSH) are nuclear-encoded and chloroplast-localized proteins, which can be divided into three groups: RSH1, RSH2/RSH3 and CRSH (Ca^{2+}-dependent RSH). RSH1s are considered as major (p)ppGpp hydrolases; RSH2s and RSH3s (*At*RSH2 and *At*RSH3 show ~80% amino-acid identity [a true *RSH3* was lost in the *Arabidopsis* lineage] and are placed together in a single RSH2/RSH3 group) are considered as major (p)ppGpp synthases; and CRSHs are considered as Ca^{2+}-dependent (p)ppGpp synthases. These proteins are responsible for the metabolism of guanosine tetraphosphate (ppGpp) and pentaphosphate (pppGpp) – nucleotides referred to as (p)ppGpp or alarmones of the stringent response (SR). (p)ppGpp regulate chloroplast transcription, photosynthesis, plant growth and stress responses. However, the physiological significance of (p)ppGpp synthesis remains incompletely understood, especially in plants outside the *Brassicaceae* family. We have characterized the tomato RSH gene family – the species has two genes encoding RSH1 proteins, one RSH2, one RSH3 and one CRSH. Importantly, a true RSH3 protein is encoded in its genome, which makes the plant a good model to understand functional differences between RSH2 and RSH3 proteins. To perform studies on the tomato SR, targeted (using CRISPR/Cas9 technology) *SlRSH2* and *SlRSH3* gene knockouts were generated. Using these mutants, the involvement of the SR in the regulation of tomato plant development and responses to stress has been analyzed.

O.18.4

Inhibition of β-substituted alanine synthase 4;1 from common bean (*Phaseolus vulgaris* L.) by benzoic and salicylic acids

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The nutritionally essential sulfur amino acids, methionine and cysteine, are present at suboptimal levels in legumes. β-Substituted alanine synthase 4;1 (BSAS4;1) is the major isoform of cytosolic cysteine synthase present in developing seeds of common bean (*Phaseolus vulgaris* L.). There is evidence that this enzyme is also involved in the biosynthesis of the non-protein amino acid *S*-methylcysteine, which accumulates in the form of a γ-glutamyl dipeptide. Here, we report the high-resolution structure of recombinant BSAS4;1. Unexpectedly, the crystal structure showed the presence of a molecule of benzoic acid near the active site, which appeared to be co-purified from *E. coli*. Benzoic acid acts as a competitive inhibitor with respect to *O*-acetylserine. Kinetic analysis revealed the presence of multisite inhibition interactions, with possibly four independent binding sites. The apparent K_i value for benzoic acid was equal to 50 μM, and IC50 values for benzoic acid and salicylic acid were equal to 1 mM and 0.7 mM, respectively. Using developing cotyledons grown in vitro, quantification of incorporation of $^{13}C_3$- and ^{15}N-labeled serine into cysteine and downstream metabolites indicated that benzoic acid effectively inhibited cysteine biosynthesis at a concentration of 1.2 mM. The results of experiments tracking the incorporation of ^{13}C-labeled sodium thiomethoxide provided further evidence that BSAS4;1 may be involved in the formation of free *S*-methylcysteine.

Cellular Motility: Mechanisms and Structures

Oral presentations

O.A1.1

How does the reduced availability of an energy source affect skeletal muscles? Zebrafish as a model of human McArdle disease

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Muscle glycogen phosphorylase (PYGM) deficiency in humans results in a metabolic disorder called McArdle disease. It can be caused by mutations in the PYGM gene, resulting in the absence of a functional protein and glycogenolysis disruption. As a result, the energy supply needed for proper muscle function isimpaired. Patients experience symptoms such as muscle damage and pain, leading to an inability to perform physical exertion.
We aim to establish a zebrafish model of McArdle disease to study its metabolic aspects and pathophysiology. We have shown that Pygm levels change during development, which correlates with glycogen levels. Pygm knockdown resulted in the accumulation of glycogen granules and disrupted muscle structure. The pygm-/- zebrafish individuals exhibit both morphological and behavioural alterations that mirror the symptoms observed in human patients. Furthermore, we propose that the role of PYGM should be considered from a broader perspective. PYGM differs from other phosphorylase isoforms in its expression pattern and biochemical properties. It is not only essential for providing energy for muscle contraction, but also plays an important role in other tissues and in various physiological and pathological processes.
In conclusion, the zebrafish pygm-/- line represents a model for studying human metabolic McArdle disease, and serves as a tool for investigating PYGM functions in muscle and other tissues.

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O.A1.2

Myosin VI in *Drosophila* border cell migration

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Collective migration of border cells is a distinctive event occurring during the 14-stage oogenesis in *Drosophila*. At early stage 9, these specialized cells delaminate from the epithelial layer and form a cluster around two non-motile polar cells. During stage 9, the cluster migrates collectively between supporting nurse cells toward the oocyte, reaching it by the end of stage 10 [1]. Over 20 years ago, it was suggested that myosin VI (MVI), a unique molecular motor that moves toward the minus end of actin filaments, is required for border cell migration [2]. Using MVI immunodetection, we confirm the expression of this protein in the border cell cluster, particularly during its delamination and early stages of migration. However, as migration progress MVI signal in the cluster disappears. Furthermore, using confocal and electron microscopy, we provide evidence of efficient border cell migration in females with RNAi-mediated MVI knockdown in border cells and in MVI null zygotic females, demonstrating that MVI is not crucial for *Drosophila* border cell migration.

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O.A1.3

FHOD1 – an actin polymerase – binds to lipid droplets (LDs) and regulates their functioning in human skin melanoma cells

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Skin melanoma is a dangerous malignancy that remains challenging to treat. One of the mechanisms responsible for the acquisition of resistance to treatment is the metabolic reprogramming of cancer cells, which involves, among other things, alterations in lipid droplet (LD) dynamics. LDs serve as a store of lipids that function to support cellular energy demands and survival. There is a growing body of evidence that LDs are essential for the survival of skin melanoma cells.

We discovered that one of the formins - FHOD1, which is an actin polymerase, is targeted to LDs in human skin melanoma cells. Furthermore, by analyzing various truncation versions of FHOD1, we identified the FH3 (DID) domain of FHOD1 as the region responsible for LD targeting. Moreover, we used a High-Content Screening microscope to assess the distribution of LDs in skin melanoma cells expressing various FHOD1 versions. We discovered that some of the analyzed FHOD1 variants act as tethers for LDs. By utilizing siRNA to lower FHOD1 expression, we demonstrated that FHOD1 regulates the number and size of LDs. Finally, we observed that FHOD1 is capable of actin polymerization or F-actin bundling at LD. Additionally, the distribution of LDs in skin melanoma cell lines is dependent on β-actin but not on γ-actin, pointing to the functional diversification of non-muscle actin paralogues.

Our results indicate that FHOD1 regulates the biology of LDs. Thus, we discovered a new function of FHOD1 and the FHOD1's FH3 domain.

O.A1.4

Computational modeling reveals distinct protein stability and interaction defects due to pathogenic DNAI1 variants

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Amino acid substitutions in the WDR domain of DNAI1 constitute a primary cause of primary ciliary dyskinesia (PCD), yet their mechanistic effects remain poorly understood. To address this, we performed an integrated computational analysis of 15 pathogenic DNAI1 variants using molecular dynamics (MD) simulations, MM-GBSA free energy analysis, and FoldX alanine scanning. Our results reveal two distinct pathogenic mechanisms. One class of mutations, exemplified by V335I, targets structurally critical hot spots and leads to severe protein destabilization. In contrast, a second class of mutations, including F556S and A538T, produces proteins predicted to be as stable as, or even more stable than, the wild-type, implicating pathogenicity *via* stable, non-functional conformations that disrupt essential protein-protein interactions required for dynein arm assembly. This framework enables mechanistic classification of pathogenic variants and provides a prioritized list of high-impact candidates (V335I, F556S, C388R) for experimental validation, guiding efforts to distinguish misfolding defects from interaction network disruption.

O.A1.5

Unraveling the biphasic mechanism of electrotaxis

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Electrotaxis, the directed migration of cells in a direct current electric field (dcEF), plays a crucial role in wound healing and development, yet its molecular mechanism remains incompletely understood. We analyzed the dynamics of 3T3 fibroblast electrotaxis, focusing on both early and sustained responses, and observed that 3T3 fibroblasts could change their migration direction within 1–2 minutes of dcEF reversal. Inhibition of Kir potassium channels (notably Kir4.2) suppressed rapid cathodal migration, but this effect was reversible within 1–2 hours, suggesting a biphasic mechanism, with redistribution of membrane receptors sustaining electrotaxis. Using live-cell imaging with fluorescently tagged receptors, we found that, among those tested (EGFR, PDGFRα/β, TGFβR1), only EGFR and weakly PDGFRα redistributed towards the cathode. Although dynamic, EGFR redistribution was delayed relative to the rapid change in migration direction after field reversal. Functional studies confirmed EGFR and ErbB4 as important for electrotaxis, with EGFR acting in a largely ligand-independent manner. Notably, we demonstrated that Kir channels are crucial for the immediate response to dcEF, while EGFR and ErbB4 mediate a slower although still early phase of electrotaxis. However, even when both components were inhibited, electrotaxis was gradually restored over time, highlighting the presence of a more complex, multimodal mechanism underlying long-term directional responses in 3T3 fibroblasts.

O.A1.6

GLR channels are involved in chloroplast movements in *Lemna trisulca*

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GLutamate Receptor-like (GLR) channels are nonselective, ionotropic, ligand-gated receptors that form multimeric transmembrane structures in plant cells. They are homologous to ionotropic Glutamate Receptors (iGluRs) that play a key role in neurotransmission in the central nervous system of vertebrates. GLRs are involved in seed germination, pollen tube growth, reproduction and chemotaxis, stomata regulation, ion transport, long-distance signaling, responses to salt stress, wounding and pathogens.
A double-beam photometer was used to quantitatively assess chloroplast movements in the aquatic angiosperm, *Lemna trisulca*. The method is based on the fact that chloroplast redistribution changes light transmittance through the leaf. We used two classical inhibitors, CNQX and MK-801. In animal cells they block AMPA and NMDA channels respectively. The inhibitory approach was chosen because no mutants of *L. trisulca* are available so far. Chloroplast movement was inhibited by the MK-801, indicating the involvement of NMDA-like channels in the studied process. This inhibition depends on pH and requires alkaline conditions. On the contrary, CNQX did not change parameters of chloroplast movements in both mild alkaline and acidic conditions.
We have shown that GLRs are involved in the regulation of the strong blue light-activated chloroplast response controlled by phototropin 2. We also show that plant GLR channels are pH-dependent, similar to glutamate receptors present in animal cells.

Mitochondria and Membranes: Function and Regulation in Cellular Signalling

Oral presentations

O.A2.1

Pore dewetting as a common mechanism in gating and inhibition of large-conductance calcium-activated potassium (BK_{ca}) channels

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Large-conductance calcium-activated potassium (BK_{Ca}) channels are widely expressed in mammalian tissues and organelles, where they play essential roles in regulating membrane excitability, calcium signaling, and cell survival. Mutations of BK_{Ca} cause severe diseases, highlighting its significance as a therapeutic target. Despite the identification of several BK_{Ca} inhibitors and activators, none have reached clinical application due to issues with toxicity or off-target effects. A detailed understanding of their mechanisms of action is therefore crucial for developing better modulators.

Paxilline (PAX) is a well-known high-affinity pore blocker of BK_{Ca} channels. We identified another, weaker natural inhibitor – dibenzoylmethane (DBM). Electrophysiological experiments, combined with molecular dynamics simulations, support a pore-binding mechanism for DBM. To explore the structural determinants of its activity, we also tested DBM analogs, finding that diphenyl substitution contributes to BK_{Ca} inhibition. The BK_{Ca} channel exhibits some of the highest conductance values among potassium channels. Notably, during gating, the channel pore does not close fully in a physical sense but instead expels water *via* changes in its hydrophobicity – a phenomenon known as hydrophobic gating. Our findings support a model in which both PAX and DBM exploit this intrinsic gating property, inducing a more hydrophobic, dehydrated environment to achieve effective BK_{Ca} channel inhibition.

O.A2.2

BK_{Ca} Channel as a Novel Modulator of DNA Dmage Response in Human Bronchial Epithelial Cells Exposed to Particulate Matter

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Although particulate matter (PM) is a well-recognized genotoxic environmental agent, the molecular mechanisms underlying its harmful health effects remain poorly understood. The respiratory epithelium, as the primary site of PM deposition, acts as a protective barrier and is enriched in potassium channels that are essential for maintaining airway surface liquid homeostasis. In HBE cells, large-conductance calcium-activated potassium (BK_{Ca}) channels – located at the apical plasma membrane and within the inner mitochondrial membrane – play a key role in this regulation. In this study, we investigated the potential involvement of the BK_{Ca} channel in the DNA damage response (DDR) following PM exposure. While DDR pathways have been extensively characterized, the role of ion channels in these processes remains largely unexplored. To address this, we employed BK_{Ca}-depleted HBE cells (HBEΔαBK_{Ca}) as a physiological model. Exposure to standardized PM in HBEΔαBK_{Ca} cells resulted in decreased clonogenic survival, elevated ROS levels, PARP1-dependent apoptosis, cell cycle alterations, and an increase in DNA double-strand breaks compared to wild-type (HBE WT) cells. qPCR analysis revealed upregulation of genes involved in both single-strand break repair and double-strand break repair, suggesting a compensatory activation of DDR pathways. In conclusion, this study provides the first evidence of a critical role for the BK_{Ca} channel in modulating the DNA damage response to particulate matter.

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O.A2.3

Mitochondrial adaptations to hypoxia and reoxygenation in human endothelial cells

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Ischemia-induced hypoxia in the brain and heart remains one of the most prevalent causes of death. Because reperfusion is thought to exacerbate cellular damage initiated by ischemia, we investigated the effects of hypoxic conditions on mitochondria in human endothelial cells, as well as the effects of in vitro reoxygenation following anoxic exposure (A/R).

Mitochondria from hypoxic cells showed reduced membrane potential, decreased respiratory rates, and elevated production of reactive oxygen species, associated with an increased coenzyme Q (CoQ) reduction level. Although A/R further impaired mitochondrial function, the effects were less pronounced in hypoxic mitochondria compared to normoxic controls, indicating their enhanced tolerance to A/R-induced stress. Under hypoxic conditions, we observed a significant decrease in CoQ levels, along with increased assembly of respiratory complexes I, III, and IV into supercomplexes, which likely facilitate CoQ-mediated electron transfer [1]. These results indicate that hypoxia induces mitochondrial adaptations that promote tolerance to oxygen fluctuations.

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O.A2.4

The cytosolic ribosomal protein Rpl40/eL40 supports mitochondrial function

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Accurate stoichiometric production of ribosomal proteins ensures optimal protein synthesis efficiency. *RPL40A* and *RPL40B* are two paralogous genes of the yeast *Saccharomyces cerevisiae*, which encode ribosomal proteins of the large subunit of the cytosolic ribosome that have identical amino acid sequences. Deleting one paralog results in the other paralog being upregulated at the transcript and protein levels. Interestingly, the overexpression of a single paralog in the absence of the other produced distinct yet complementary effects on the mitochondrial proteome. Mitochondrial biogenesis depends on the production of proteins on cytosolic ribosomes, so identifying ribosomal components that modulate mitochondrial protein production could reveal how cells adapt mitochondrial function to meet their needs. Using label-free quantification, we analyzed changes in the proteome of mitochondria from *rpl40a*Δ and *rpl40b*Δ strains compared to the wild type. Deletion of *RPL40A* resulted in the upregulation of proteins involved in cristae formation and respiratory chain complex (RCC) assembly, as well as respiratory chain complex (RCC) subunits. In contrast, proteins involved in lipid metabolism and mitochondrial import machinery were less abundant in the *rpl40b*Δ strain than in the wild-type strain. Our results demonstrate that changes in the composition of the mitochondrial proteome in *RPL40* deletion strains influence their distinct responses to cellular stress.

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O.A2.5

The mitoBK Channel as a Player in the Epithelial Response to Urban Particulate Matter

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The bronchial epithelium forms a barrier between the external environment and the internal milieu and is chronically exposed to pollutants – key contributors to respiratory pathologies.Potassium channels are critical for maintaining lung homeostasis through the regulation of ion transport, mucus secretion, and epithelial integrity. Among these, mitochondrial large-conductance Ca^2-activated potassium (mitoBK Ca) channels play a pivotal role in modulating mitochondrial membrane potential, respiratory chain activity, ROS signaling, matrix volume, and calcium homeostasis. Our previous work identified functional mitoBK Ca channels in human bronchial epithelial (HBE) cells. In the present study, we investigated their role in response to particulate matter (PM) exposure. Using CRISPR/Cas9 gene editing, we generated a 16HBE14o cell line deficient in the KCNMA1 gene (HBE Δα). These cells exhibited impaired mitochondrial respiration, disrupted ATP synthesis *via* oxidative phosphorylation, and altered organization of the electron transport chain. Transcriptomic profiling of wild-type and HBE Δα cells exposed to various concentrations and durations of PM revealed significant BK Ca -dependent alterations in mitochondrial gene expression. PM exposure induced distinct transcriptional responses in each cell line. These findings underscore the essential role of BK Ca channels in sustaining mitochondrial function and epithelial resilience under environmental stress.

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Applied Biotechnology

Oral presentations

O.A3.1

Superparamagnetic Iron Oxide Nanoparticles (SPIONs): A Versatile Platform for Capture and Detection of Biomolecules

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Superparamagnetic iron oxide nanoparticles (SPIONs) serve as a versatile platform for the capture and detection of biomolecules, including proteins, miRNAs, and antibodies. Our most recent work demonstrates the successful integration of SPIONs into biomimetic virus-like particles (VLPs) displaying the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. These engineered magnetic VLPs retain the bioactivity and immunogenic properties of the RBD, allowing for efficient and specific binding to target antibodies. The magnetic core allows for rapid separation from complex biological matrices, streamlining sample processing and enriching target analytes. In proof-of-concept studies, the SPION-RBD VLP system exhibited robust immunodiagnostic performance, efficiently capturing SARS-CoV-2-specific antibodies and supporting their sensitive and specific detection. This innovative approach not only enhances diagnostic workflows but also opens new possibilities for multiplexed biomarker analysis and point-of-care applications, leveraging the combined advantages of magnetic manipulation and VLP-based molecular recognition.

O.A3.2

Patient-derived induced pluripotent stem cells-based model uncovers cardiomyocyte-specific dystrophin Dp427 preservation and Dp116 expression in Duchenne Muscular Dystrophy caused by atypical splicing of DMD gene

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Duchenne muscular dystrophy (DMD) is caused by mutations in the *DMD* gene. We modeled a rare splice-site mutation (c.9975-1G>T in intron 68) using patient-derived hiPSCs and generated a CRISPR/Cas9-corrected isogenic control. Both lines were differentiated into cardiomyocytes (hiPSC-CMs) and skeletal muscle cells (hiPSC-SMs). The mutation led to skipping six nucleotides from exon 69, causing an in-frame deletion of Tyr3325 and Arg3326 in Dp427. Western blotting showed near full-length Dp427 in DMD hiPSC-CMs but strongly reduced levels in hiPSC-SMs. Surprisingly, the Dp116 isoform—previously considered non-cardiac – was detected in hiPSC-CMs for the first time. Despite Dp427 expression, confocal imaging revealed membrane abnormalities in DMD hiPSC-CMs absent in corrected cells, suggesting impaired dystrophin–β-dystroglycan interaction. Functional assays revealed altered β-adrenergic responsiveness: DMD hiPSC-CMs showed increased beating frequency and faster repolarization after isoproterenol stimulation. Our study highlights a rare case of partial dystrophin preservation *via* non-canonical splicing, ectopic Dp116 expression in hiPSC-CMs, and functional impairment despite near-normal Dp427 levels. Such hiPSC-based model enables precise investigation of mutation-specific mechanisms and therapeutic targeting in DMD.

Acknowledgements

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O.A3.3

Targeting the immune response and redox imbalance in endometriosis with resveratrol and its natural analogs

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Endometriosis is a chronic inflammatory disease in which endometrial-like tissue grows outside the uterus. Macrophages associated with endometriosis promote cell proliferation, angiogenesis, and pain generation by releasing pro-inflammatory cytokines. Since crosstalk between macrophages and endometriotic cells sustains disease progression, targeting macrophage plasticity may offer a therapeutic avenue. This study evaluated the effects of resveratrol and its natural derivatives on immune dysfunction and oxidative stress in endometriosis. An inflamed co-culture of 12Z endometriotic cells and M1 macrophages differentiated from THP-1 monocytes was used. Inflammatory and oxidative markers, cytokine secretion, and ROS levels were analyzed by qPCR, ELISA, Cytometric Bead Array, and multiplexed flow cytometry with bioimaging. Stilbenes-treated macrophages exhibited reduced expression of key proinflammatory cytokines and mediators, as well as a lowered cellular oxidative status, depending on the specific resveratrol derivative used. Notably, pterostilbene, the most effective compound in regulating ROS balance, decreased the percentage of ROS-positive cells from 96% to 48%, while also increasing the expression of antioxidant enzymes such as SOD and GPX. These findings suggest that resveratrol and its analogs may modulate immune response and redox imbalance in endometriosis, supporting alternative therapeutic and dietary interventions in endometriosis-related immunopathology.

O.A3.4

A microfluidic model of the blood–brain barrier for electroporation-mediated permeability modulation

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One of the major challenges in developing therapies for neurodegenerative diseases is overcoming the blood–brain barrier (BBB), a highly selective interface that separates the circulatory system from the central nervous system. Potential answer to this challenge may be brought by electroporation, i.e. precise application of electric pulses. In this study, a 3D microfluidic BBB-on-a-chip model was designed and tested to evaluate the impact of electroporation on BBB permeability. The microsystem consists of three parallel channels (neuronal, vascular, and medium) that are not physically separated, and is equipped with a pair of electrodes designed by us. Microglial cells and astrocytes are embedded in hydrogel in the central channel, while brain microvascular endothelial cells and pericytes are seeded in the vascular channel. During optimization of model, barrier integrity was assessed using FITC-labeled dextrans (4 and 70 kDa), and cell *via*bility was evaluated using calcein-AM and propidium iodide staining. Electroporation was performed with various pulse parameters to modulate BBB permeability to choose conditions when propidium iodide uptake is increased without compromising cell *via*bility. Microscopy revealed the formation of a BBB-like structure; however, further validation is required to confirm its functional integrity.

Acknowledgements
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O.A3.5

Make Your Data Speak: Simplified Approaches to High-Dimensional Flow Cytometry Analysis

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Analyzing multiparametric high-dimensional flow cytometry datasets typically demands specialized computational expertise and relies heavily on hyperparameters specific to each algorithm. The absence of trained bioinformaticians in the cytometry field impedes the comprehensive analyses and reproducibility of results. To address these limitations, we conducted a comparative assessment of available data analysis methods aiming to bridge the computational gap and accelerate biological discoveries. Recent efforts have focused on developing automated analysis methods. By guiding researchers in selecting suitable data analysis methods, we aim to enhance their confidence in reporting experimental results and promote broder adoption of these technologies in biology and medicine. We compared available automated methods (CRUSTY, CytoBatchNorm, CYANUS and MARMOT) with code-based approaches (CATALYST, PICAFlow and Spectre) using in-house generated datasets from human and murine immune cells. Our study examines recent methodologies not only in terms of performance but also features such as user-friendly interfaces, intuitive workflows, and comprehensive documentation that contribute to the accessibility of these advanced analytical tools. We aim to provide guidelines and explanations for non-bioinformatician researchers to comprehend the necessity of each analysis step and confidently execute transparent and reproducible unsupervised analyses.

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O.A3.6

Exploiting the properties of human early β-cells to boost generation of insulin producing cells *in vitro*

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The endocrine β-cells are the only cells capable of producing insulin, a hormone essential for glucose metabolism. Loss or dysfunction of β-cells leads to diabetes. Current therapies rely on insulin delivery or glucose-lowering drugs with lifelong monitoring. Thus, significant efforts aim to generate a robust β-cell source for replacement therapy. Data from animal models and early human trials suggest that transplantation of β-cells derived from human pluripotent stem cells (SC-β-cells) offers a promising physiological treatment. Despite recent progress, stem cells cannot yet be reliably coaxed into functional β-cells in sufficient purity, number, and costs. We identified SPOCK2, an extracellular matrix (ECM) protein, as an inhibitor of immature β-cell proliferation. SPOCK2 KO SC-β-cells showed increased INS, NKX6-1, and PCSK1 expression, indicating enhanced maturation. Notably, SPOCK2 deficiency improved glucose-stimulated insulin secretion (GSIS). This effect was linked to increased MMP2 activity, which activated the β-integrin–FAK–c-JUN pathway. Recombinant MMP2 treatment promoted short- and long-term SC-β-cell expansion and boosted GSIS both in vitro and in vivo, matching the performance of human islets. Our findings reveal a critical ECM-mediated mechanism controlling SC-β-cell proliferation and function and offering a novel strategy to improve β-cell production for diabetes therapy.

Oral presentations

O.A4.1

Flow Cytometry – Beyond the Limits of Imagination

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The rapid development of new intelligent analytical methods and techniques has revolutionized the medical and life sciences. In this area, flow cytometry continues to hold a prominent place. Despite the emergence of new ways to study cell biology, cytometry has not yet reached the peak of its capabilities. New intelligent technologies used in the construction of the devices themselves not only significantly increase their research potential, but also enable the incorporation of cytometric techniques into advanced research and analytical systems supported by artificial intelligence, robotics, and highly developed automation. Flow cytometry is an essential element complementing the holistic approach to studying living organisms in multiomics analyses, combined with genomics, proteomics, metabolomics, immunomics, or bioinformatics. Currently, it forms the basis of biomedical research in diagnosing various diseases and the research and development of treatment. However, it is also used in ecosystem and microbiological research, the food industry, and agriculture. The utilitarian nature of flow cytometry is also demonstrated by the fact that it has recently been planned to exceed the second cosmic velocity and send a properly constructed device on a cosmic mission to the Moon and Mars.

O.A4.2

Identification and profiling of planarian multinucleated cells using imaging flow cytometry

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Cellular polyploidy plays a key role in various physiological and pathological processes, including development and regeneration. Freshwater planarians, such as *Schmidtea mediterranea*, are well-established models in regeneration research due to their ability to reconstruct entire organisms from small body fragments – a capacity driven by a population of pluripotent adult stem cells, called neoblasts. While neoblasts have been extensively studied, the presence and potential role of polyploidy in planarian regeneration remain largely unexplored.

We employed imaging flow cytometry and fluorescence-activated cell sorting to identify and characterize a stable population of multinucleated cells in *S. mediterranea* under both regenerating and non-regenerating conditions. Analysis of nuclearity, as well as cell and nuclear size, indicates that these cells may arise through processes such as cell fusion and/or endomitosis. Moreover, gene expression profiling suggests that these multinucleated cells are likely undifferentiated.

Together, our findings provide a foundation for future investigations into the biological significance of multinucleated cells in planarian regeneration, and contribute to a broader understanding of regenerative processes across phyla.

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O.A4.3

Before you proceed with cultivation step: flow cytometry in microbiology uncovered

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Flow cytometry is a high-throughput, quantitative single-cell analysis technique that is increasingly used in microbiological research. As a culture-independent method, it enables the detection of *via*ble but non-culturable (VBNC) cells - microorganisms that remain metabolically active despite being unable to grow on standard media (undetectable using culture – based techniques). This allows for a more accurate assessment of the structural and functional complexity within microbial populations.
The use of specific fluorescent dyes and reagents makes it possible to measure a range of cellular parameters, including membrane integrity, membrane potential, enzymatic activity, and nucleic acid content. Proper sample preparation - such as filtration, sedimentation, centrifugation, and enzymatic disaggregation - is essential for effective isolation of microbial cells from various environmental and industrial matrices.
Instrument configuration is a critical component of flow cytometric analysis. Proper threshold settings, detector adjustments, and strategies to distinguish cells from background noise are necessary to ensure measurement reliability. Flow cytometry is widely applicable in environmental, industrial, and clinical microbiology, and also enables sorting of the defined microbial subpopulations based on the measured parameters, offering powerful opportunities for functional and diagnostic studies.

O.A4.4

Extracellular Vesicle Analysis by Flow Cytometry: Challenges and Opportunities

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Extracellular vesicles (EVs) are heterogeneous, membrane-bound particles released by most cell types, found in various biofluids. They play crucial roles in physiological and pathological processes, carrying diverse cargo. Flow cytometry is widely used for EV analysis, offering multiparametric, single-particle characterization. However, EVs' small size presents significant technical challenges, demanding robust experimental design and essential controls.
A PubMed-based literature review was performed to assess original research articles from 2024–2025 using the terms "Extracellular Vesicle" AND "Flow Cytometry", excluding reviews.
Among 274 articles, 23% mentioned flow cytometry without direct EV analysis; 14% used bead-based methods. Only 22% adhered to MIFlowCyt-EV reporting guidelines. Crucially, essential controls (e.g., detergent lysis, aggregate checks) were reported in just 26% of studies. Furthermore, 54% lacked proper sizing calibration, often misinterpreting bead size, and only 28.6% reported dilution controls.
Despite standardization efforts (ISAC, ISEV), considerable variability persists in instrument setup, controls, and data validation. These findings highlight a critical need for greater awareness, improved training, and consistent protocols to enhance data quality and reproducibility in EV flow cytometry. Future work should prioritize harmonized reporting, automated calibration, and strict adherence to guidelines to advance EV research and its clinical relevance.

O.A4.5

Where Science Sharpens Its Edge: Core Labs as Strategic Enablers

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In an era of rapidly evolving research technologies and the emergence of new scientific niches, core facilities are becoming increasingly essential to modern science. Researchers employing advanced techniques such as imaging (including fluorescence and electron microscopy), proteomics, genomics, mass spectrometry, or flow cytometry are progressively turning to specialized scientists and technicians working within centralized laboratories for expert support. These facilities provide not only cutting-edge instrumentation but also the know-how to optimize its use. Beyond the obvious benefits – such as centralized expertise, fast service response, and reduced instrument downtime through preventive maintenance – core labs also offer economic advantages. When properly scaled and managed, they can deliver significantly lower per-sample costs compared to individual research groups operating independent equipment. While countries such as Germany, the UK, France, the US, or the Czech Republic have long recognized the strategic role of core labs, in Poland and several neighboring countries the concept remains underdeveloped or poorly implemented. Yet, a mature core facility, driven by a skilled team, can not only accelerate research but push the boundaries of science – often by repurposing instruments in innovative, off-label ways beyond their intended design.

Posters

P.1

Mapping N-DRC Composition and Function in *Tetrahymena*: How Individual Subunits Orchestrate Axonemal Integrity and Ciliary Motility

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Cilia are intricate evolutionary conserved microtubule-based organelles projecting from many eukaryotic cells, categorized by function and structure into motile and non-motile types. The axoneme, the core structural scaffold of cilia, includes constituents such as the nexin-dynein regulatory complex, N-DRC, which collaborates with other components to regulate ciliary motility. Defects or mutations in ciliary proteins are associated with numerous human disorders, collectively known as ciliopathies.
Here, we unravel the composition of the N-DRC complex and define the roles of its subunits in regulating ciliary beating in Tetrahymena. Using DRC-KO/CoDel Tetrahymena cells, we demonstrate how loss of individual DRC subunits impairs cell motility and ciliary beating. Moreover, we show that core subunits of the complex are essential for maintaining its structural integrity, where loss of any of them causes severe structural damage to the entire complex and consequently impairs motility.
The protein-protein interaction studies between various DRC subunits validated their proposed arrangement, which had previously been suggested by integrated modeling and cryo-EM analyses. Moreover, the comparative mass spectrometry analyses revealed that levels of some uncharacterized proteins also significantly decreased in mutant cells, and their proximity to the N-DRC complex was confirmed through BioID assays, identifying them as putative N-DRC components or interactors to its adjacent complexes.

P.2

MLK4 contributes to oncogenesis and cross-talk with the tumour microenvironment in breast cancer

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Recent research shows that tumors comprise cancer cells alongside diverse immune, stromal, and extracellular matrix components, collectively known as the tumor microenvironment. While this environment clearly influences cancer cell malignancy, the mechanisms behind these interactions remain unclear.
Our group demonstrated that Mixed-Lineage Kinase 4 (MLK4) contributes to malignant progression and therapy resistance in different tumors. We showed that MLK4 is frequently upregulated in breast cancer and that high expression of MLK4 promotes migration and invasion of breast cancer cells. Our work also revealed the MLK4-regulated cytokine secretion pattern during chemotherapy. However, the role of MLK4 in regulating the tumor microenvironment has never been investigated. Here, we show that MLK4 activates the phenotypic changes of breast cancer cells induced by macrophages, the major components of the tumor microenvironment. We used co-cultures of breast cancer cells and macrophages to show that MLK4-mediated cross-talk between these cell types promotes cancer growth and invasion. We also investigated the MLK4-dependent mechanisms of interaction between breast cancer cells and macrophages by identifying paracrine signaling factors. In summary, our research demonstrated how MLK4 contributes to oncogenesis and cross-talk with the tumor microenvironment and provided a rationale for the therapeutic targeting of MLK4 in breast cancer.

P.3

The endonuclease activity of MCPIP1 influences renal cancer progression through *CTNNB1* regulation and post-transcriptional modification of β-catenin

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Renal cell carcinoma is one of the most common urological neoplasms in the world, with ccRCC being the most common subtype, accounting for approximately 75% of RCC cases. To overcome these challenges, acquiring a comprehensive understanding of the underlying biology of ccRCC is crucial. Among the endonucleases, which have a suppressive effect in breast cancer and renal cell carcinoma, is the MCPIP1. Our recent study revealed that MCPIP1 may act as a tumor suppressor in ccRCC that prevents EMT by stabilizing Wnt inhibitors and decreasing the levels of active β-catenin.

In this study we present that the endonuclease activity of MCPIP1 might mediate tumor progression by controlling the transcriptional and post-transcriptional modification of β-catenin. The level of inactive, degradation-promoted, phosphorylated β-catenin (Ser45) decreases dramatically in advanced stages of ccRCC compared to early clinical stages. However, the level of active β-catenin increases significantly with the progression of the disease. Microarray analysis indicated that the level of genes binding to the *CTNNB1* gene promoter encoding β-catenin may affect the progression of ccRCC. Furthermore, the results confirm the involvement of MCPIP1 endonuclease activity in the regulation of the level of these transcripts.

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P.4

Insights into the Non-Antioxidant Functions of the NRF2/KEAP1 Axis

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NRF2 is a stress-responsive, cytoprotective transcription factor that is negatively regulated by KEAP1. Both its expression and activity decline with age. Our findings demonstrate that NRF2 plays a vital and multifaceted role in endothelial cell (EC) biology. NRF2 regulates angiogenesis by sequestering KEAP1, thereby stabilizing podosomes—an unexpected effect independent of the NRF2 transcriptional activity. Silencing NRF2 in endothelial cells triggers premature senescence, characterized by robust protein S-nitrosation (SNO) in the absence of oxidative damage. Mechanistically, we identified KEAP1 to interact with GAPDH and NOS to form a functional SNO-generating complex. This leads to S-nitrosation of NOX4, which inhibits oxidative damage and prevents EC death. In vivo, genetic ablation of NRF2 promotes the development of abdominal aortic aneurysms (AAA). However, this effect is reversed by endothelial-specific deletion of miR-34a. Loss of miR-34a enhances angiotensin II–induced, MTA2-dependent EC proliferation, potentially accounting for the protection against AAA observed in miR-34aΔEC mice despite their endothelial dysfunction. Together, these results highlight the essential role of the NRF2/KEAP1 axis in vascular biology and reveal non-canonical functions beyond NRF2 mediated classical gene transactivation and KEAP1-dependent NRF2 repression.

P.5

Metformin preventive action in autoimmune myocarditis: Single-cell transcriptomic profiling of PBMC in EAM mouse model.

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Myocarditis is a common condition which leads to severe complications – cardiomyopathy, nonischemic heart failure, and death. Among others, autoimmune overdrive is one of the causes. Metformin, exhibits anti-inflammatory and immunomodulatory properties, which makes it a promising candidate as a therapeutic factor in autoimmune myocarditis (AM). Since the definitive AM diagnosis requires cardiac MRI and endomyocardial biopsy, there is a need for less invasive, more cost-effective diagnostic and monitoring methods.
The aim was to assess metformin's cardioprotective actions in AM and identify prognostic biomarkers for non-invasive monitoring. The BALB/c mice were immunized to induce experimental AM (EAM) and received preventive metformin treatment from day 0 post-immunization. Single-cell RNA sequencing was performed with 10x Genomics technology.
Sequencing of 180 074 PBMC cells from timepoints: day 0, day 14 and day 21 revealed 15 PBMC clusters: 3 subsets of T cells: CD4+ and CD8+, monocyte, megakaryocytes, 2 subsets of B cell, NK cells, neutrophil/granulocytes, mast cell/basophil subsets, plasmacytoid dendritic cells, erythroid progenitors, plasma cells and hematopoietic progenitors. Differentially transcribed genes were pinpointed for each experimental condition. Candidate biomarkers were identified for further studies evaluating their usefulness for fast and non-invasive monitoring of treatment progression.

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P.6

Targeting EF-P lysinylation by crystallographic fragment screening.

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Multidrug resistance (MDR) in Gram-negative bacteria, including pathogens such as *Acinetobacter*, *Salmonella*, and *Shigella*, poses a serious global health threat. The translation elongation factor EF-P plays a critical role in resolving ribosomal stalling at polyproline motifs, which are common in resistance-related proteins like efflux pumps. EF-P requires a specific post-translational modification for activation—typically, β-lysylation of a conserved lysine residue, catalysed by the ATP-dependent ligase EpmA. Deletion of either the *efp* gene or its modifying enzyme has been shown to compromise *Salmonella enterica*'s ability to withstand environmental stresses, including exposure to antibiotics. Targeting this modification could reduce bacterial fitness and enhance antibiotic effectiveness. However, no inhibitors of EpmA are currently available.
This project applies crystallographic fragment screening to discover low-molecular-weight compounds that bind to critical sites on EpmA, laying the ground for structure-guided drug development. Recent advances in high-throughput X-ray crystallography and computational tools like PanDDA now enable efficient screening of large fragment libraries. Previous studies on related human enzymes have shown high hit rates, supporting the approach. By mapping druggable sites on EpmA and screening homologous enzymes, this research aims to advance understanding of EF-P regulation and bacterial resistance. Ultimately, identifying EpmA inhibitors may lead to novel antimicrobial strategies against MDR pathogens.

P.7

Mapping the Uncharted: Expanding the lncRNA vertebrate catalogs through capture long-read RNA sequencing

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Accurate gene annotation is crucial for linking genome sequences to biological function. While the GENCODE consortium has provided high-quality human and mouse genome annotations for over two decades, long non-coding RNAs (lncRNAs) remain significantly under-annotated and fragmented across various catalogs, limiting their utility in functional genomics. To overcome this gap, GENCODE has undertaken its most comprehensive lncRNA annotation effort to date. Using full-length, targeted long-read sequencing from matched embryonic and adult tissues in both species, combined with manual curation, this initiative identifies orthologous lncRNAs and greatly expands the reference catalog. The project reveals 17,931 novel human genes (140,268 transcripts) and 22,784 novel mouse genes (136,169 transcripts), marking a two- to six-fold increase in transcript annotations – the largest since the human genome was first sequenced. These lncRNAs show evolutionary conservation, defined promoter regions, and enrich the interpretation of millions of previously unmapped genomic signals such as transcription start sites, chromatin marks, and transcription factor binding sites. Notably, this effort also triples the number of human disease-associated lncRNAs with identifiable mouse orthologs, enhancing cross-species functional studies. The expanded GENCODE annotations represent a significant advance in genome interpretation, offering a vital resource for understanding lncRNA function, conservation, and their roles in health and disease.

P.8

The role of the potassium and chloride transport in the development of inflammation induced by particulate matter

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Particulate matter (PM) is an increasing health threat, known to induce inflammation through its immunomodulatory effects on bronchial epithelial cells, notably by stimulating cytokine release such as TNF-α and IL-6. To investigate the role of ion channels in this response, three bronchial epithelial cell lines were used: wild-type (HBE WT), a line lacking the α-subunit of the BKCa channel (HBE $\Delta\alpha BK_{Ca}$), and CFTR-deficient cells (CFBE). Cells were exposed to various PM concentrations and analyzed for ROS production, IL-6 and TNF-α secretion, mitochondrial respiration (OCR), intracellular Ca^2 levels, and TEER. PM exposure markedly increased ROS and proinflammatory cytokine release, especially in HBE $\Delta\alpha BK_{Ca}$ and CFBE cells. TNF-α induced the strongest IL-6 response in these modified lines, indicating a key role for ion channels in inflammation. Mitochondrial dysfunction was observed as reduced respiratory capacity in both BK_{Ca} and CFBE lines. PM also elevated intracellular calcium and decreased TEER, with CFBE cells showing the highest barrier disruption. These findings suggest that impaired potassium and chloride transport increases epithelial vulnerability to PM-induced damage, including oxidative stress, inflammation, mitochondrial impairment, and barrier dysfunction. Modulation of BK_{Ca} channels and targeting inflammatory pathways may offer new therapeutic approaches for protecting airway health from environmental pollutants.

P.9

Spatial Organization of cAMP Signaling at the Nuclear Envelope Regulates Neuronal Structural Plasticity in Retinal Ganglion Cells

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Compartmentalization of cAMP/PKA signaling is crucial for neuronal survival and regenerative responses. We identify soluble adenylyl cyclase (sAC/AC10) as a key source of perinuclear cAMP in retinal neurons, where it binds scaffold protein mAKAPα. Co-immunoprecipitation confirmed a specific interaction between sAC and mAKAPα in the retina and neurons. Using FRET-based PKA biosensors targeted to the nuclear envelope (AKAR4-nesprin), we demonstrated that perinuclear PKA activity is significantly higher than cytosolic activity upon forskolin stimulation. Knockdown of sAC (shRNA-Adcy10) reduced PKA signaling in both compartments and impaired neurite outgrowth under basal and depolarizing conditions, highlighting the essential role of sAC in activity-dependent structural plasticity. Furthermore, disruption of AC10 anchoring to mAKAPα using a AC-binding domain (AC-BD) peptide impaired depolarization-induced axon elongation, despite preserved sAC expression. This confirms that the spatial organization of cAMP signaling – and specifically the perinuclear localization of sAC *via* mAKAPα – is critical for effective neuronal responses to external stimuli. This highlights the importance of spatially restricted cAMP signaling *via* mAKAPα-bound sAC in neuronal responses. Targeting this microdomain may enhance regeneration after optic nerve or CNS ischemic injury.

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P.10

Physicochemical Characterization and Biochemical Safety Assessment of Commercial Intravenous Fat Emulsion Enriched with Two Natural Compounds

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Intravenous fat emulsions (IVFE) used in parenteral nutrition (PN) provide essential fatty acids and energy but their phytosterol-rich composition has been linked to intestinal failure-associated liver disease (IFALD). Bioactive compounds like fisetin (FIS) and curcumin (CUR), which modulate oxidative stress (*via* Nrf2 pathway) and inflammation (*via* NF-κB inhibition), may provide hepatoprotection when added to IVFE.

This study aimed to characterize the physicochemical properties and assess the biochemical safety of IVFE (Lipidem) enriched with FIS and CUR at three concentrations: c_1 = 0.01 mg/mL, c_2 = 0.1 mg/mL, c_3 = 1 mg/mL. Physicochemical analyses included mean droplet diameter (MDD), percentage of fat residing in globules >5 μm (PFAT5), zeta potential, osmolality, pH, and injectability. Moreover, hemolytic activity and cytotoxicity on human hepatocyte-derived THLE-2 cells (MTT assay) were evaluated for biochemical safety.

Formulations at c_1 and c_2 complied with US Pharmacopeia standards, maintained stable physicochemical parameters suitable for intravenous use, and showed negligible hemolysis (<3 %). In MTT assay, c_1 and c_2 were non-toxic, while c_3 caused a marked *via*bility decrease, likely linked to mitochondrial dysfunction and oxidative imbalance.

These findings indicate that IVFE with low-dose FIS and CUR is biochemically safe and offers properties relevant to IFALD, supporting further study of their effects on hepatic metabolism and redox balance in the context of PN.

P.11

Novel container for secured transport and storage increase the quality of isolated of human total RNA samples

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It is known that the appropriate storage of RNA samples is as critical as their isolation and purification. Many factors, such as temperatures above 4°C, oxygen access, and the presence of active nucleases triggering RNA degradation, influence results of RNA analyses. We present the new type of container for the secured transport of biological material and describe its role in extending RNA sample quality. The novelty of this tool is its unique construction making it both secondary and external packaging and highly resistant to mechanical factors.The aim of this study was to evaluate the usefulness of the container for storing samples for quantitative RNA analyses. The changes in the expression of selected human leucocyte housekeeping genes (ACTB, GAPDH, and Rack1) using RT-qPCR and RT-dPCR were analysed. RT-dPCR analysis evidenced that the container retains a higher count of analyzed gene copies per μL of samples during 5 h of incubation time. The container ensures a low maintenance temperature for several hours, making it useful for sustaining the conditions for transporting biological samples. It helps to retain the quality of total RNA over time so that RNA samples can be used to accurately analyze biological material that has been transported for several hours.

Acknowledgements
Technical construction of the container is subject of the utility model pending application in the Patent Office of the Republic of Poland (no. W.132412).

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P.12

Dissecting the role of ETV4 and ETV5 in human pluripotent stem cell state transitions

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Pluripotent stem cells (PSCs) exist along a spectrum from naïve to primed states, each characterized by distinct molecular and functional properties. While ETV4 and ETV5, members of the PEA3 subfamily of ETS transcription factors, are established regulators of pluripotency in murine systems, their roles in human PSCs remain unclear. In this study, we generated ETV4 and ETV5 knockout (KO) human PSC lines using CRISPR/Cas9 and cultured them under chemically defined conditions supporting naïve and primed states. Single-cell RNA sequencing was performed across primed, resetting, and naïve stages. ETV5-KO cells exhibited enhanced resetting efficiency, with a higher proportion of cells in naïve-like clusters and elevated SUSD2 expression. ETV4-KO cells showed lower efficiency of resetting but retained early naïve markers such as PRDM14. Differential expression analysis revealed disruptions in genes involved in neurodevelopment, cell adhesion, and signal transduction. Motif enrichment analysis showed a gain of chromatin regulation-related motifs in both KO lines and a loss of SMAD3/4 binding motifs, suggesting altered TGF-β signaling. These changes may converge on key pathways such as LIF/STAT3 and FGF/MAPK, with differential upregulation of TBX3, necessary for the primed-to-naïve transition. Overall, our findings reveal novel and distinct roles for ETV4 and ETV5 in regulating the transition between pluripotency states in human PSCs.

P.13

Suspects – small RNAs – are they responsible for cold stress response in soybean?

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Soybean, an annual crop belonging to the Fabaceae family is rich in fats, protein, and vitamins. Serves a major value for animal feed and also constitutes an important part of the human diet. Originating from subtropical and tropical regions, soybean is sensitive to cold, though some variants are more tolerant. However, exposure to cold in crucial development phases can lead to reduced yield and seed quality. Small RNAs are a diverse group of molecules with various functions, among which miRNAs are best known for their regulatory roles. An emerging class of small regulatory RNAs are tRNA-derived fragments (tDRs), 14–50 nucleotides long, produced by cleavage of precursor tRNAs. Our study aims to identify and characterize small RNAs, as cold stress-responsive factors. Two varieties of soybean were chosen: Erica – cold-sensitive, and Augusta, cold-resistant. Both of them were exposed to short-term and prolonged stress at different developmental stages. Samples from roots, cotyledons, and first trifoliate leaves were collected. Differential expression of small RNAs was assessed using high-throughput sequencing. A total of 24 small RNA libraries were prepared: 2 biological replicates, 2 treatments (stress, control) and 3 tissues (roots, cotyledons, trifoliates). The resulting data were analyzed to identify differentially expressed transcripts and small RNAs in response to stress.

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P.14

Deficiency of *Blmh* and Homocysteine Metabolism causes ER Stress, UPR Activation, and Apoptosis in Mouse Neuroblastoma N2a-APPSwe Cell Model of Alzheimer's Disease

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Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by endoplasmic reticulum (ER) stress, unfolded protein response (UPR) and apoptosis. Hyperhomocysteinemia (HHcy) is a condition of elevated plasma total homocysteine (tHcy). HHcy and related metabolites such as Hcy thiolactone (HTL), *N*-homocysteinylated (*N*-Hcy)-protein and bleomycin hydrolase (BLMH), an enzyme that detoxifies HTL are associated with AD. However, it remains unknown how *Blmh* deficiency, Hcy and its metabolites promote ER stress, UPR and apoptosis in AD development.
Aim: To study the effect of *Blmh* gene silencing and Hcy, HTL and *N*-Hcy-protein treatment on ER stress, UPR and apoptosis gene expression.
Method: N2a-APPswe cells expressing human APP transgene were treated with Hcy, HTL, or *N*-Hcy-protein for 24 h in methionine-free media. For gene silencing, *Blmh* siRNAs were transfected using Lipofectamine RNAi Max in Opti-MEM for 48 h. ER stress, UPR, and apoptosis markers were analyzed by Western blot and RT-qPCR.
Results: The *Blmh* silencing or treatment with Hcy, HTL, or *N*-Hcy-protein in N2a-APPswe cells increased GRP78, ATF3, CHOP, BAX, and Caspase-3 levels, while reducing the anti-apoptotic protein BCL-2. mRNA changes mirrored protein expression patterns.
Conclusion: The *Blmh* deficiency and Hcy, HTL and *N*-Hcy-protein may promote AD development by inducing ER stress, UPR and apoptosis.

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P.15

Targeting viral RNA G-quadruplexes by antisense oligonucleotides – a promising antiviral strategy?

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Influenza A virus (IAV), a major RNA virus threat, possesses highly conserved secondary structures within its genome that are crucial for the replication cycle. Among these, G-quadruplexes (G4s) – non-canonical arrangements in guanine-rich regions – seem to be important regulators of the viral life cycle. This makes them attractive targets for novel therapeutics, and we hypothesize that disrupting these IAV G4 motifs is a promising antiviral strategy.
Our previous paper describes the identification and biophysical confirmation of G4 structures within the influenza A/California/04/2009 (H1N1) viral RNA (vRNA). Based on these data, we designed and synthesized a corresponding series of antisense oligonucleotides (ASOs) targeting three G4 motifs. This ASO library was synthesized using solid-phase phosphoramidite chemistry. The unmodified ASOs and ASOs with locked nucleic acid (LNA) and 2'-O-methylated modifications were prepared to improve their stability and binding affinity.
Subsequently, we examined the conformational changes of G4s upon ASO binding. To this end, the native polyacrylamide gel electrophoresis (PAGE) experiments were conducted. They revealed that the designed ASOs successfully form complexes with the target G4s. Additionally, we are currently optimizing the fluorescence-based approaches to gain deeper insight into ASO-induced stabilization/destabilization. The data obtained will guide the selection of the most promising ASOs for further biological evaluation of their antiviral activity.

P.16

Targeted siRNA-NPs to reverse fibrosis in ischemic cardiomyopathies

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Cardiovasculardiseases, particularly ischemic cardiomyopathies, remain the leading cause of morbidity and mortality worldwide. A major challenge in ischemic heart disease is progressive myocardial fibrosis and scar expansion, which ultimately leads to impaired cardiac function and increased risk of death. Despite the clinical relevance of this process, no effective anti-fibrotic therapies are currently available. This project aims to address this therapeutic gap by developing a nano-based delivery system using siRNA-loaded alginate sulfate nanoparticles (siRNAAlgSNPs). These nanoparticles are designed to specifically target activated cardiacfibroblasts and reverse their profibrotic phenotype following myocardial infarction. The proposed platform represents a novel strategy for molecular modulation of cardiac fibrosis and holds strong potential for clinical translation, supported by GMP-compatible production workflows.

P.17

Salicornia europaea as a microbiological biotechnological toolbox for pathogen suppression and saline soil restoration

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Salicornia europaea, a halophyte adapted to saline environments, offers a rich source of microbiological tools with biotechnological potential. Within the Sali-Food and SaltyBEATS projects, interdisciplinary studies investigated its endophytic and rhizosphere microbiomes, emphasizing antimicrobial activity, plant growth promotion, and the restoration of salt-degraded soils.
Selected microbial strains produced bioactive microbial volatile organic compounds (mVOCs) that inhibited key pathogens such as Escherichia coli, Listeria monocytogenes, and Salmonella enterica, supporting their potential as natural biopesticides and bioinoculants in agriculture and food systems. Metagenomic data from five European countries confirmed selective colonization of *S. europaea* by halotolerant endophytes with pathogen-suppressing and stress-alle*via*ting capabilities.
As part of the SaltyBEATS initiative, saline ecosystem microbiomes are further studied for their role in ecological restoration. Soil and rhizosphere profiling underscore the contribution of halophytes and their associated microbes to biodiversity enhancement and soil functionality recovery. These microbial communities, functioning as biological "engineers", provide tools for sustainable agriculture and environmental resilience.

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P.18

Characterization of MMEJ-dependent deletions in cancer genomes.

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Microhomology-Mediated End-Joining (MMEJ) is a DNA repair mechanism based that mediates double-strand break repair. Due to its reliance on end resection and microhomology alignment, MMEJ can result in complex rearrangements, insertions, deletions, and multinucleotide variants (MNVs). Understanding this process is particularly important in homologous recombination-deficient (HRD) cancers, where overreliance on MMEJ contributes to genomic instability.
This study aimed to characterize MMEJ activity in HRD tumors and evaluate its role as an alternative DNA repair mechanism. Although our analysis encompassed multiple tumor types, ovarian cancer – owing to its frequent HRD – served as a primary model for analyses. We developed an algorithm to classify indels potentially arising through MMEJ. Whole-genome(WGS) and whole-exome sequencing(WES) data were analyzed using multiple variant callers to assess dependence on data type and preprocessing. We observed variation in MMEJ-mediated deletion frequency across cancer types, with germ cell, pancreatic, and lymphoid tumors showing highest rates in WES, and germ cell and adrenal neuroendocrine tumors in WGS. Our analysis revealed a link between elevated MMEJ activity and RB1 loss. Survival analysis showed that a higher number of MMEJ-dependent deletions correlated with better prognosis.
Our results highlight MMEJ-dependent indels as potential biomarkers of PARP sensitivity and suggest broader relevance of MMEJ in cancer biology and treatment.

P.19

Transcriptome comparison of differentiating nasal and bronchial airway epithelium from male and female donors

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The human airway epithelium (hAE) covers the respiratory tract and consists of several cell types: basal (BCs), secretory (SCs), goblet (GCs), and multiciliated cells (MCCs). BCs are precursors for all other AE cell types and are crucial for development and regeneration of AE. MCCs and GCs (secreting mucus) enable mucociliary clearance, protecting airways from pathogens. Defects of this mechanism occur in diseases like cystic fibrosis (CF) and primary ciliary dyskinesia (PCD). Understanding AE cell differentiation is key to studying genetic airway diseases. Direct analysis of donor-derived hAE cells is prone to errors, so Air-Liquid Interface (ALI) cultures of primary nasal (hNEC) or bronchial (hBEC) epithelial cells are used for mucociliary epithelium differentiation. While hNECs and hBECs are similar morphologically and functionally, transcriptome differences have been observed, including in disease contexts like CF and COPD. Differences in gene expression between sexes and differentiation stages have also been reported. Our RNAseq-based study compares hNECs and hBECs from males and females at three time points of ALI culture (ALI_0, ALI_6, and ALI_12), aiming to evaluate their interchangeable use in AE research and diagnostics. While the overall differentiation process exhibit a high degree of similarity across all donors and samples, notable differences in gene expression are observed across culture-time, while those related to tissue-, and sex are much less pronounced.

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P.20

Towards drought-resilient soybeans: Insights from small RNA profiling

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Drought is one of the most critical abiotic stresses threatening global crop production. Soybean (*Glycine max*) yields can be reduced by over 50% under dry conditions. This makes drought a major climatic risk that demands effective mitigation strategies to ensure a stable global soybean supply. As climate change increases the frequency and severity of drought events, soybean cultivation is increasingly at risk.
This project investigates the role of small RNAs in regulating gene expression under drought stress. Small RNAs are key post-transcriptional regulators that modulate gene expression by guiding mRNA degradation or inhibiting translation, playing essential roles in plant stress responses and developmental processes. We selected two soybean cultivars with contrasting drought tolerance: Acardia (tolerant, high-yielding, adapted to Northern and Eastern Europe) and Maja (sensitive, early-maturing, requiring adequate moisture). Plants were grown under controlled conditions and subjected to moderate and severe drought stress at the V3 developmental stage for five days. Leaf and root tissues were harvested for RNA extraction, followed by the preparation and sequencing of small RNA and mRNA libraries. The resulting data were analyzed to identify differentially expressed transcripts and small RNAs in response to drought stress.

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P.21

Impact of bacterial exopolymers on microalgae cultivation

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As primary producers, unicellular algae play a significant role in maintaining the ecological balance of an ecosystem. The interactions between microalgae and bacteria influence the ecological functionality and microbial diversity of microbial consortia. Microalgae–bacteria interactions increase the production of extracellular polymeric substances (EPS). According to the literature, EPS are crucial in microalgal–bacterial consortia, particularly in processes such as the biosorption and biodegradation of environmental pollutants. EPS are complex biopolymers that are primarily composed of polysaccharides, proteins, nucleic acids and lipids. Research suggests that the specific interactions between bacterial and microalgal cells may depend on the composition of extracellular polymeric substances. The presence of bacterial EPS can influence algal growth, metabolic activity and tolerance to environmental stressors. This study assessed the growth parameters, photosynthetic activity, and changes in photosynthetic pigment content and metabolite accumulation of unicellular green algae in the presence of bacterial exopolymers.

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P.22

Application of *in silico* methods to assess HVEM protein druggability

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The BTLA/HVEM complex acts as an immune checkpoint, which under physiological conditions is responsible for regulating the immune system. Overexpression of immune checkpoint proteins in cancer cells is one of the mechanisms by which they evade immune surveillance. It has been demonstrated that blocking immune checkpoints is the most promising approach to enhancing the immune response in the tumor microenvironment. Thus, the BTLA/HVEM complex is an attractive target for drug design in the context of immunotherapy. Although monoclonal antibodies and protein-based peptides targeting BTLA and HVEM, respectively, have been reported, there are no known small-molecule inhibitors targeting these proteins to date.

To provide deeper insights and identify structural features responsible for the "undruggable" status of the BTLA/HVEM system, *ab initio* molecular dynamics (MD) simulations were conducted, followed by extensive molecular docking studies. Based on the results obtained, the seven most promising compounds were selected and forwarded for experimental evaluation. Microscale thermophoresis and ELISA methods were applied, and the data obtained showed that none of the tested compounds blocked the formation of the BTLA/HVEM complex. In conclusion, the utilization of *in silico* methods enabled the exploration of a broad chemical space, combined with experimental evaluation, provided crucial insights that convincingly uphold the "undruggable" status of the HVEM protein.

P.23

General characteristics and potential involvement of FUS in the processing of SNORA77 into its short derivatives: sdRNAs 1_707-5p and 1_707-3p

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In recent years, small nucleolar RNAs (snoRNAs) have been shown to contribute to cancer development, acting as both oncogenes and tumor suppressors. They can also be processed into shorter, stable fragments called sno-derived RNAs (sdRNAs), which are increasingly recognized as potential biomarkers and therapeutic targets. However, the significance of sdRNA expression in glioblastoma (GBM) - one of the most aggressive brain tumors - remains unknown. Some miRNA precursors overlap with snoRNA loci, and sdRNAs share a similar length with miRNAs, suggesting partially convergent biogenesis pathways, but the actual mechanisms behind snoRNA processing remain unclear. In this study, we analyzed the expression of sdRNAs and their parental snoRNAs in GBM patient tissues, selecting sdRNAs 1_707-5p and 1_707-3p (derived from SNORA77) for further analysis. Both sdRNAs showed predominantly nuclear and chromatin-associated localization, unlike canonical miRNAs. Based on this, we applied a non-canonical approach focused on RNA-binding proteins rather than Dicer-dependent processing. Given prior findings that FUS knockout affects snoRNA levels, we hypothesized that FUS may be involved in sdRNA biogenesis. Indeed, we demonstrated that FUS binds SNORA77 and may facilitate processing of sdRNA 1_707-5p. Under hypoxic conditions – commonly present in the GBM microenvironment - we observed decreased sdRNA and FUS levels, along with SNORA77 upregulation, suggesting that sdRNA processing is regulated by both protein interactions and stress conditions.

P.24

Effect of *Conidiobolus coronatus* infection and fungal β-carboline metabolites on Interleukin 2 levels in *Galleria mellonella* hemocytes

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Insect models share key features with mammals, including signaling pathways, metabolism, structural components, and innate immunity. As such, they are increasingly used to study human diseases and assess drug toxicity in a cost-effective and time-efficient manner. However, immunological parallels between vertebrates and invertebrates remain incompletely understood. In mammals, interleukin-2 (IL-2) is a critical regulator of immune responses, promoting T cell proliferation and maintaining immune homeostasis. Although cytokines are evolutionarily conserved, little is known about their analogs in insects. *Conidiobolus coronatus*, a mammalian pathogen, shows strong entomopathogenicity against *Galleria mellonella*. Its metabolites, harman and norharman, influence insect hemocytes—cells central to insect immunity. This study assessed how *C. coronatus* infection and β-carboline alkaloid exposure affect IL-2 levels in *G. mellonella*. Larvae were exposed to fungal spores or treated with harman/norharman (750–1250 ppm) topically or *via* diet. IL-2 levels were measured by ELISA, fluorescence microscopy, and flow cytometry. Fungal infection did not increase IL-2 levels. However, alkaloid treatment significantly elevated IL-2 expression, regardless of administration route. These findings suggest that harman and norharman may activate cytokine-related immune mechanisms in insects. *G. mellonella* thus represents a promising model for studying interleukin-like activity and insect immunity.

P.25

Mitochondrial transport of catalytic RNAs and targeting of the organellar transcriptome in human cells.

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Mutations in the small genome present in mitochondria often result in severe pathologies. Different genetic strategies have been explored, aiming to contribute to rescue such mutations. A number of these were based on the capacity of human mitochondria to import RNAs from the cytosol and were designed to repress the replication of the mutated genomes or to provide the organelles with wild-type versions of mutant transcripts. However, the mutant RNAs present in mitochondria turned out to be an obstacle to therapy and little attention has been devoted so far to their elimination. Here, we present the development of a strategy to knockdown mitochondrial RNAs in human cells using the transfer RNA-like structure of the Brome mosaic virus or the Tobacco mosaic virus as a shuttle to drive trans-cleaving ribozymes into the organelles in human cell lines. We obtained a specific knockdown of the targeted mitochondrial ATP6 mRNA, followed by a deep drop in ATP6 protein and a functional impairment of the oxydative phosphorylation chain. Our strategy opens a powerful approach to eliminate mutant organellar transcripts and to analyze the control and communication of the human organellar genetic system.

P.26

Exploring CAG Repeats in Non-Coding RNAs: Characteristics and Potential Impact on Neurodegeneration

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Trinucleotide repeats are present in many expressed sequences, including protein-coding and non-coding RNAs. A specific feature of these tracts is their length polymorphism in the population and the potential to cause diseases when expanded. CAG repeats have been associated with brain function, and mutations of these sequences in specific genes are responsible for neurodegenerative disorders. In this study, we focused on RNAs with at least 10 CAG repeats, which are more likely to vary in length in the population and/or fulfil a functional role in the molecule. We identified human RNAs in reference datasets: 131 protein-coding transcripts, 28 long non-coding RNAs (lncRNAs), and 102 circular RNAs (circRNAs). These mRNA, lncRNA, and circRNA sequences originated from 34, 10, and 20 genomic loci, respectively.

Most CAG-containing circRNAs were derived from mRNA exonic sequences. For several of them, we verified the back-splicing junction sequence and circRNA expression in human brain and liver tissue. For the selected ATXN7 locus, we identified 9 circRNAs with CAG repeat tract and investigated their potential to be implicated in pathological pathways in spinocerebellar ataxia type 7 (SCA7). Our preliminary results suggest that this circRNAs should be investigated in more detail in the context of disrupted pathways in SCA7.

Acknowledgements

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P.27

miR-181 and miR-26a overexpression impact on the participation of ESCs in mouse skeletal muscle regeneration

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Satellite cells (SCs) play a pivotal role in skeletal muscle regeneration. However, under different pathological conditions , e g. VML or muscular dystrophies, SCs may not be sufficient to support full muscle reconstruction. For this reason, other cells, as well as different factors which could support their regenerative potential or muscle regeneration itself, are tested for potential therapies. Here, we analysed the impact of miR-181 and miR-26a on mouse embryonic stem cells (ESCs) transplanted to injured mouse skeletal muscle. Selected miRNAs support myogenic differentiation by reducing the expression of such factors as SIRT1, which inhibits this process. We studied effects of the transplantation of ESCs transfected either with miR-181 or miR-26a mimics or control ESCs (MOCK) on the structure of regenerating muscles. We also performed ICC staining to determine localisation of injected cells or their derivatives, as well as qPCR to assess changes in the expression of genes regulating muscle regeneration.

P.28

Tracking Autoimmune Myocarditis Progression Through Single-Cell Transcriptomic Profiling of Peripheral Blood Mononuclear Cells

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Autoimmune myocarditis (AM) is a heart disease involving immune dysregulation. Despite progress in understanding its pathogenesis, the complexity of immune infiltrates in cardiac tissue remains unclear. Single-cell RNA sequencing (scRNA-seq) has revolutionized research by enabling detailed analysis of immune cell heterogeneity. The aim of this project was to understand molecular mechanisms underlying AM and identify potential biomarkers in peripheral blood mononuclear cells (PBMCs), which could improve diagnosis and support treatment.
A mouse model of experimental autoimmune myocarditis (EAM) was used – BALB/c mice were immunized with αMyHC peptide and complete Freund's adjuvant. PBMCs were isolated at multiple disease stages: control (day 0), acute (day 10, 14), subacute (day 21), and myopathy phase (day 40). scRNA-seq was performed using 10x Genomics and Illumina technologies.
We identified 14 clusters of immune populations, incl. Naive CD4+ T cells, Monocytes, Naive B cells, CD8+ T cells, Regulatory T cells, Neutrophils, Megakaryocytes, Mastocytosis, Plasmacytoid DC and Memory T and B cells. Notably, monocytes responsible for recruiting immune cells into heart tissue, increased in number during EAM progression, highlighting their role in inflammation and potentially in tissue repair. Therefore, as they could have diagnostic value, careful attention should be given to them.

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P.29

Investigative study of the novel members of the CYP81F enzyme family from Brassicaceae plants

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Glucosinolates are sulfur-containing defense compounds in Brassicales order, classified into aliphatic, aromatic or indole glucosinolates. The core IG biosynthesis leads to indol-3-ylmethyl glucosinolate (I3G), which is modified by four *A. thaliana CYP81F* monooxygenases. CYP81F1, CYP81F2 and CYP81F3 mediates formation of 4OHI3G, while CYP81F4 functions in biosynthesis of 1OHI3G, which are subsequently methoxylated by IG *O*-methyltransferases. Our recent study revealed, species closely related with *A. thaliana*, such as of *Capsella*, *Camelina* and *Neslia* genera, lost *CYP81F2* and *CYP81F4* genes, but gained novel genes *CYP81F5* and *CYP81F6* with unknown functions.

In this study, we performed analysis of available genomic sequences of Brassicaceae species to identify *CYP81F* orthologs. Putative *CYP81F5* orthologs are found in *Thlaspi arvense*, and in two species from Isatideae tribe. Moreover, we found *CYP81F6* orthologs in *Arabis alpina*, *Boechera stricta* and *Malcolmia maritima*.

Additionally, we investigated if and at which positions CYP81F5 and CYP81F6 from *Capsella rubella* hydroxylate I3G in planta. We expressed these enzymes in *cyp81f2/f4 A. thaliana*, deficient in 1MI3G biosynthesis and accumulates strongly reduced amounts of 4OHI3G and 4MI3G in leaves, but hyper-accumulates the CYP81Fs substrate, I3G. Metabolic analysis of generated transgenic lines indicated *Cr*CYP81F5 is able to hydroxylate I3G to produce 4OHI3G. It has been also concluded that *Cr*CYP81F6 is not capable of modifying I3G.

P.30

Age, sex and duration of anhydrobiosis affect ROS levels differentially in released storage cells and intact specimens of *Paramacrobiotus experimentalis*

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Reactive oxygen species (ROS) are regarded to be crucial for mitochondrial involvement in cellular adaptation and stress resistance. ROS can serve as intracellular signals but also lead to oxidative stress when generated excessively. Tardigrade anhydrobiosis is a well-known example of the ability to survive extreme dehydration, which is linked to ROS levels, yet prolonged or repeated anhydrobiosis episodes can exacerbate ROS-mediated damage. It is known that in animals ROS levels are lower in females and increase with age, but the impact of tardigrade age and sex on ROS level changes related to anhydrobiosis remains poorly known. Moreover, it is not known whether the tardigrade "ROS response" is cell autonomous or requires an organismal response. Here, we studied how the duration of anhydrobiosis as well as the animal age and sex affect ROS levels using intact animals (IA) and released storage cells (RSC) of the sexually dimorphic tardigrade *Paramacrobiotus experimentalis*. The ROS levels were influenced differently by animal age, sex, and duration of anhydrobiosis as well as differed between IA and RSC, suggesting important effect of extracellular environment. Furthermore, the ROS level predictive power as survival indicator depended on age. Thus, the "ROS response" can be regarded as an integrative read-out of tardigrade stress dose and life-history state although the differences between intact animals and released storage cells should be considered in relevant research.

P.31

Proteomic predictors of regional lymph node metastasis in rectal cancer

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Regional lymph node metastasis (RLNM) is a key prognostic factor in rectal cancer (RC), affecting both overall and disease-free survival. However, reliable pre-treatment predictors of RLNM are still lacking. This study aimed to identify proteomic signatures of RLNM using a label-free LC-MS/MS approach in tumor and margin tissues collected from 40 RC patients (stage T2–3, M0), classified into RLNM+ (n=20) and RLNM– (n=20) groups. Proteomic analysis was performed using an Orbitrap qExactive mass spectrometer in DDA mode.

More than 2000 proteins were identified, of which 120 showed significantly different abundance between groups. Tissues from RLNM+ patients displayed increased levels of proteins associated with metastasis, including VCAN and matrisome components such as ANXA2, THBS1, and THBS2. Additionally, over 1000 proteins (including VCAN, CUL3, OLFM4, MMP9, PON2, S100A8/9) differentiated tumors and proximal margins depending on RLNM status. These proteins were linked to cancer-related pathways, including ECM-receptor interaction, immune response, leukocyte migration, neutrophil degranulation, and altered energy metabolism.

The study revealed a distinct proteomic profile of primary rectal tumors associated with RLNM, which may serve as a basis for developing predictive tools applicable before treatment.

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P.32

Doxorubicin-induced cytotoxicity and apoptosis in human adipose-derived mesenchymal stem cells

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Adipose-derived stem cells (ADSCs) are mesenchymal, multipotent somatic stem cells. Due to their regenerative potential, ADSCs are considered promising candidates for the reconstruction of tissue. However, the regenerative capacity of these cells may decline, potentially due to the detrimental effects of agents such as doxorubicin. DOX disrupts DNA replication and transcription processes. Although it primarily targets cancer cells, doxorubicin also affects normal, non-cancerous cells, leading to side effects. The present study aimed to evaluate apoptosis and cellular senescence in ADSCs following exposure to doxorubicin.

Initially, the *via*bility of the ADSCs was assessed. Based on these results, three concentrations were selected for subsequent experiments. Apoptosis was evaluated using the RealTime-Glo™ Annexin V Apoptosis Assay. In addition, β-galactosidase staining was performed to assess cellular senescence. Finally, cell cycle distribution was examined by FACS.

The results showed that DOX induced apoptosis after 24 hours of treatment. Furthermore, DOX alters the activity of β-galactosidase in cells. Cell cycle analysis revealed that the drug increased the percentage of cells in the sub-G1 phase and reduced the number of cells in the S phase.

In summary, the results suggest that low concentrations of DOX affect the proliferative activity of ADSCs. This suggests that even low doses of the drug could reduce the regenerative potential of stem cells.

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P.33

Identification of natural protein variants in the patients's saliva with oral cancers

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Naturally occurring variants at the genetic level or at the level of gene products (e.g., proteins) differentiate individuals of the same species. Differences between individuals are most often studied at the genetic level and only to a very limited extent at the level of gene products (e.g., proteins). The study of individual differences is becoming increasingly important as the basis for so-called personalized medicine. Using our database and bioinformatics tools (www.alicedb.ug.edu.pl), we analyzed saliva samples from patients with various types of parotid gland tumors and oral cavity cancer to identify natural variants of protein sequences that could. Mass spectrometry and bioinformatics methods were used to analyze the proteomes and peptidomes of saliva from: 12 individuals from the control group (CG), 12 patients with salivary gland Mixed tumor mixed salivary gland tumors (SGMT); 12 patients with salivary gland Warthin tumor (SGWT) (data from own measurements) and 25 patients with oral squamous cell carcinoma (OSCC) (data from the PRIDE repository PXD020211). In total, approximately 1,000 natural protein variants were identified in all analyzed samples, some of which may have significant diagnostic potential. For example, the natural variant T298A of the Keratin 13 protein was identified in over 65% of samples from the SGMT, SGWT, and OSCC groups and in only one sample from the control group (CG).

P.34

Extracellular vesicles as an alternative platform for CRISPR-Cas system delivering.

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The CRISPR-Cas system is a popular tool for genome editing, characterized by its simplicity of design and flexibility. Despite impressive advances in the development of CRISPR-based tools, their clinical applications are limited, mainly due to inefficient delivery methods. To date, viral vectors remain the most effective method of cell transduction, but they are not suitable for clinical use due to immunogenicity and the risk of insertional mutagenesis. This has led to growing interest in non-viral methods, in particular extracellular vesicles (EVs), which are characterized by low toxicity and the ability to transport nucleic acids and proteins. In our work, we used a passive method of loading EVs with the CRISPR system. For this purpose, HEK293T cells were transfected with a two-color reporter array (pX330x6(x7)/Green for sgRNA targeting Myocd1 and SP-Cas9-VPR-Tomato). EVs were isolated by PEG precipitation and characterized by nanoparticle tracking analysis. We found that EVs produced by transfected HEK293T cells were loaded with Cas9 at both the protein and RNA levels. Furthermore, they contained Myocd1 gene RNA, which was targeted by the Cas9-VPR system to activate its expression. EVs showed no cytotoxicity in cultured human cardiac fibroblasts and mesenchymal stem cells. qPCR revealed significant overexpression of Myocd1 in cells treated with EVs. Thus, EVs loaded with the CRISPR-Cas system represent a promising and non-toxic tool for delivering genes to target cells.

P.35

Circular RNAs as regulators of alternative splicing and potential biomarkers in lung cancer

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Circular RNAs are covalently closed transcripts that regulate gene expression by sequestering microRNAs, scaffolding RNA binding proteins, serving as templates for translation and modulating alternative splicing. Dysregulation of circRNAs contributes to oncogenesis, metastasis and therapy resistance, and lung cancer often shows distinct circRNA signatures with prognostic value. This review summarizes current knowledge on circRNA biogenesis and molecular mechanisms, with emphasis on their impact on splice isoform choice and protein function. We discuss methodological advances for detection and validation, including long read sequencing and functional perturbation assays, and highlight major gaps: limited functional characterization, lack of standardized analysis pipelines and sparse translational studies. Finally we outline the translational potential of circRNAs as biomarkers and therapeutic targets and propose priorities for future research.

P.36

Synergistic insights from microcalorimetric and structural studies of *R. etli* asparaginases

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L-asparaginases catalyze the hydrolysis of asparagine into ammonia and aspartic acid. They are classified into three structural groups and display substrate affinities ranging from the low micromolar to the high millimolar range. In addition to their primary activity, many asparaginases exhibit co-activities toward other substrates. Certain asparaginases are of particular medical and industrial importance. Our research focuses on rhizobial rep-resentatives of the recently described Class 3 asparaginases: the constitutive ReaIV and the inducible ReAV. These enzymes are metalloenzymes with no structural similarity to other known asparaginases. To study them, we combine X-ray crystallography with isothermal titration calorimetry (ITC). This approach enabled us to iden-tify conditions sufficient for obtaining a crystalline complex of ReAV with its substrate, to provide a structural explanation for the unexpected acrylamide reactivity of ReAIV observed by ITC or to clarify how the reaction product, which was found in the structure of ReAV, influence the enzyme kinetics.

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P.37

MicroRNA-126a as a Potential Modulator of Skeletal Muscle Regeneration

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Skeletal muscle regeneration is efficient but can be compromised in conditions such as cancer, AIDS, diabetes, muscular dystrophies, neurodegenerative disorders, aging, or severe injury. Strategies to enhance regeneration are therefore of significant interest. Previous studies by Brzoska, Mierzejewski, and colleagues demonstrated that cytokines such as SDF-1 (stromal-derived factor-1) and microRNAs regulating SDF-1 and NOTCH signaling are critical for stem and progenitor cell migration and myogenic differentiation. Based on these findings, we hypothesized that microRNA-126a overexpression may enhance the regenerative potential of myogenic cells by modulating interactions between the vascular niche and muscle progenitors. In vitro studies were performed using human skeletal muscle (hSKM) myoblasts, terminally differentiated myotubes, and iPSC-derived myoblasts transfected with microRNA-126a. Analyses included histological and cytological assessment, RT-PCR, Luminex-based secretome profiling, and next-generation sequencing (NGS). Comparative morphological evaluation of gastrocnemius muscle sections from wild-type, mdx, and microRNA-126 knockout mice provided preliminary in vivo context. Expression of myogenic regulatory factors (MRFs) and other regeneration-associated genes was examined. Preliminary observations suggest that microRNA-126a may modulate MRF expression and secretory activity, warranting further investigation into its role in skeletal muscle regeneration.

P.38

Site-directed mutagenesis of EcAIII stabilization loop: Kinetic and Structural Insights

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L-asparaginases are enzymes that catalyze the hydrolysis of L-asparagine into L-aspartate and ammonia. L-asparaginases are employed in cancer treatment, especially in managing acute lymphoblastic leukemia (ALL) [1]. Type III L-asparaginases, in contrast to currently used therapeutic enzymes, lack L-glutaminase activity responsible for adverse effects; however, type III L-asparaginase exhibit lower substrate affinity compared to existing biopharmaceuticals. Site-directed mutagenesis allows for the targeted modification of enzyme catalytic activity or stability, leading to improved functional properties [2].The aim of this study was to obtain and characterize EcAIII mutants with modifications in the sodium-binding stabilization loop. The mutations were designed based on a PSSM matrix calculated using sequences of EcAIII orthologs. Proteins were expressed in *E. coli*, purified *via* affinity chromatography, and characterized biochemically (Nessler and GOT/MD methods), biophysically (nanoDSF), and structurally (X-ray crystallography). Two variants showed a significant improvement in L-asparaginase activity. These findings pave the way for the development of safer and more effective biopharmaceuticals based on type III L-asparaginases, offering promising prospects for future research.

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P.39

Structural basis for functional cooperation between Pol γ and RNase H1 proteins in the processing of mitochondrial RNA primers

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Human mitochondrial DNA (mtDNA) is a circular, double-stranded molecule of 16.6 kb whose faithful replication and maintenance are essential for cellular homeostasis. Defects in these processes are associated with various mitochondrial diseases. The strand-displacement model of mtDNA replication proposes that leading-strand synthesis initiates at the heavy-strand origin (OH), and lagging-strand synthesis begins once the replication fork exposes the light-strand origin (OL), about two-thirds around the genome. After replication, RNA primers at the 5' ends of nascent DNA must be removed to prevent accumulation of pathogenic mtDNA species. Our recent studies revealed functional interactions between several mitochondrial enzymes, including RNase H1 and DNA polymerase γ (Pol γ). Under certain conditions, their interaction may support RNA primer processing and facilitate replication. We show that while RNase H1 alone leaves 1–3 ribonucleotides at RNA/DNA junctions, Pol γ stimulates its activity to promote complete RNA removal. Using a gap-filling assay, we defined the substrate length needed for co-occupancy. Complex formation was monitored by biolayer interferometry and isolated by size exclusion chromatography. Although cryo-EM was limited by sample quality, AlphaFold modelling provided insight into ternary complex architecture, supporting a cooperative role for Pol γ and RNase H1 in primer removal during mtDNA replication.

P.40

Chimeragenesis as a new approach in anticancer treatment

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L-asparaginases are enzymes that catalyze hydrolysis of L-asparagine into L-aspartate and ammonia. Since the 1960s, these proteins have been used in the treatment of acute lymphoblastic leukemia (ALL), exploiting the inability of malignant lymphoblasts to synthesize L-asparagine independently. By depleting circulating L-asparagine, L-asparaginases induce apoptosis in leukemic (ALL) cells. However, the currently used type II L-asparaginases derived from E. coli and D. dadantii are associated with significant side effects, largely due to their L-glutaminase co-activity [1]. Recent studies suggest that type III L-asparaginases, despite their lower catalytic efficiency, may serve as promising alternatives in ALL treatment [2]. This study aimed to modify kinetic properties of type III L-asparaginases through chimeragenesis. Six chimeric variants of type III were designed, expressed, and purified using affinity chromatography. These new proteins were characterized for their L-asparaginase and β-aspartyl peptidase activities, thermal stability was also determined. Two chimeras demonstrated improved enzymatic performance and show potential for future therapeutic use.

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P.41

Lipid-Mediated Regulation of Human Mitochondrial Ligase IIIα by Cardiolipin and Pol A

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Mitochondria are vital for metabolic and bioenergetic processes in eukaryotic cells. They contain their unique DNA (mtDNA), which is crucial because damage to it can cause serious diseases. Like in the nucleus, pathways involved in mtDNA maintenance include DNA replication, recombination and repair. The final step in all these processes is the formation of a DNA nick, which is sealed through ligation. Only one DNA ligase, Lig IIIα, is found in human mitochondria; therefore, it is entirely responsible for DNA nick processing.

Recent research indicates that lipids play roles beyond their structural function, also in human mitochondria. The inner mitochondrial membrane mainly contains phosphatidylcholine (PC), phosphatidylethanolamine (PE), and cardiolipin (CL). Cardiolipin is crucial for maintaining mitochondrial shape, stability, and dynamics, regulating key processes such as apoptosis and formation of respiratory supercomplexes[3].

In this study, we demonstrate that human mitochondrial Lig IIIα binds CL and PE but not PC. We demonstrate that CL but not PE binding to Lig IIIα inhibits ligation; however, it is reversible through interaction with Pol A. We attempt to elucidate the roles of Lig IIIα terminal, flexible domains: the BRCT and zinc finger (ZnF) in mediating this interaction. It appears that binding to CL restricts its flexibility, mimicking DNA binding behaviour of Lig IIIα and suggesting a stable, regulatory association.

P.42

Production and purification of recombinant proteins of the FGF4 subfamily

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Protein members of the FGF4 subfamily, namely FGF4, FGF5 and FGF6, belong to the canonical fibroblast growth factors. Here, recombinant proteins FGF4, FGF5 and truncated FGF6 (sFGF6) were produced in bacterial expression system and purified from the soluble fraction using heparin affinity chromatography. In case of FGF4, we observed cleavage, which led to the formation of a shorter version of the protein. For all the proteins obtained, we confirmed their proper folding and determined their thermodynamic parameters and affinity to heparin. By analyzing MAPK activation, we verified that the obtained proteins are biologically active. We then analyzed their stability in conditioned medium. FGF4 and sFGF6 proteins proved to be less susceptible to degradation than FGF5 protein. Analysis of FGFR-dependent signaling pathways revealed that the kinetics of PLCγ1 and FRS2α phosphorylation was slower in response to sFGF6 stimulation compared to FGF4 and FGF5 in U2OS cells stably expressing FGFR1. Next, we assessed the ability of FGF4 subfamily proteins to stimulate cell migration by performing a scratch wound healing assay. All purified proteins were shown to induce migration at the same level and significantly accelerate wound healing compared to untreated cells. In summary, we overexpressed, purified, and characterized recombinant proteins belonging to the FGF4 subfamily.

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Bioinspired *in vitro* mineralization models in tissue regeneration

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Biomineralization is a biologically controlled process regulated by extracellular matrix components such as proteins, proteoglycans, and glycosaminoglycans (GAGs). Bone and dentin consist mainly of collagen fibrils and hydroxyapatite crystals, while otoliths and otoconia are composed of calcium carbonate and organic matrix. Crystal properties and polymorph selection are directed by macromolecules. We used bioinspired in vitro models based on ultrathin dental and otolith matrix sections to study individual molecular contributions in a native-like context. To assess GAG function in mineralization, we enzymatically removed GAGs from demineralized sections of mouse dentin, cementum, and ligament. Proteomic analysis confirmed minimal impact on protein content. TEM analysis showed that GAG removal reduced mineralization more than protein digestion, suggesting a key role for GAGs in promoting mineralization. A similar model will be applied to study otolith/otoconia development, with clinical relevance for age-related balance disorders such as BPPV and antibiotic-induced vestibular damage. These models may inform new strategies for tissue regeneration and vestibular dysfunction treatment.

P.44

WNT/β-catenin signaling plays an important role in the survival and migration of triple-negative human breast cancer cells

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Breast cancer is the leading cause of cancer death in women and the most frequently diagnosed malignant tumor. In the present study, the role of the canonical WNT/β-catenin signaling in the regulation of cell proliferation and mitochondrial activity was investigated in a breast cancer model. The alterations in cell *via*bility, migration, pro-apoptotic properties, cell cycle progression, and mitochondrial activity were analyzed in the MDA-MB-231 cells after treatment with LiCl and XAV939. MTT assay results demonstrated a dose-dependent cytotoxic effect of LiCl, reaching an IC_{50} at 10 mM, while XAV939 did not significantly reduce cell *via*bility at concentrations up to 40 μM. The cell cycle analysis demonstrated accumulation of cells in the S phase following LiCl treatment, whereas XAV939 induced G1 phase arrest. LiCl promoted cell migration, while XAV939 suppressed it. Flow cytometry analysis revealed an increase of the early apoptotic cells after treatment with XAV939, while LiCl increased the *via*ble cells percent. Moreover, marked changes in the mitochondrial potential and mitochondrial mass were detected in the treated cancer cells. The presented results demonstrate the significant role of Wnt/β-catenin signaling in the regulation of breast carcinoma cell proliferation and confirm its potential as a promising target for anticancer therapy.

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P.45

Exploring the relationship between translation and mRNA structure

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Despite years of research, the relationship between translation and RNA structure remains a topic of debate. It is still unclear whether RNA structure directly affects translation efficiency or whether ribosomal activity shapes RNA structure in cells. Addressing this question requires a comprehensive, transcriptome-wide analysis in a well-characterized organism.
We applied the selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) method to provide a global secondary structure map of the yeast transcriptome. We investigated mRNA structure features under native and stress conditions, impact of RNA structure on translation efficiency and ribosome remodeling properties. Coupling chemical probing with next-generation sequencing and advanced bioinformatics tools enables examining hundreds of heterogeneous RNAs across various conditions.
Our data revealed that mRNA coding sequences (CDSs) tend to be less structured in the native in vivo state than ex vivo, suggesting destabilizing influence of cellular processes. Polysome profiling showed impaired translation under glucose starvation and vanillin treatment. Interestingly, we did not observe CDS structure stabilization in the presence under both stress conditions. We also found a characteristic pattern of SHAPE reactivity in the codon START region that suggests local mRNA structure relaxation facilitating AUG recognition by the ribosome.

P.46

The Impact of Dihydrotestosterone on LEAP2 Expression in the Female Hepatocyte Cell Line Hepa 1-6

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Polycystic Ovary Syndrome (PCOS) is an endocrine disorder affecting about 10% of women globally. It is characterized by presence of ovarian cysts that contribute to absent or irregular ovulation. Clinical signs include hyperandrogenism and obesity. In this study, we examined expression and function of a peptide, Liver-Expressed Antimicrobial Peptide 2 (LEAP2), which has been implicated in regulation of food intake.
Recent evidence suggests that circulating LEAP2 levels are decreased in individuals with PCOS, indicating an association between LEAP2 deficiency and PCOS. Therefore, we aimed to investigate impact of hyperandrogenic conditions, induced by dihydrotestosterone (DHT), on an in vitro model of female hepatic function. Our goal was to enhance understanding of LEAP2's role and assess its potential as a biomarker for PCOS.
Murine hepatocyte Hepa 1-6 cells were treated with varying concentrations of DHT. Following treatment, gene and protein expression were analyzed using qPCR, Western blotting and ELISA. To evaluate effects of DHT and LEAP2 on hepatic steatosis, cells were cultured with fatty acids in presence of DHT alone or in combination with LEAP2.
Our findings revealed that DHT upregulates LEAP2 gene expression, which is accompanied by decrease in intracellular LEAP2 protein and concurrent increase in LEAP2 secretion into culture medium. Moreover, while DHT treatment promoted lipid accumulation in Hepa 1-6 cells, co-treatment with LEAP2 attenuated this steatotic effect.

P.47

Does expression of large-conductance calcium-regulated potassium channel prevent senescence?

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Activation of mitochondrial potassium (mitoK) channels has been described in numerous studies as exerting cytoprotective effects during ischemia-reperfusion injury. Although mitoK channel activators are employed in the treatment of various cardiovascular diseases, their cytoprotective efficacy in aged animals remains controversial. In previous study we showed that the expression of large-conductance calcium-activated potassium (BK_{Ca}) channel protein and channel activity is significantly decreased in senescent vascular smooth muscle cells (VSMCs, Głuchowska et al. 2023). Herein, we report a 8-fold increase in β-galactosidase activity (aoβGal) in U87-MG cells lacking functional BK_{Ca} channels (U87-MGΔ). Moreover we compare aoβGal in VSMCs, U87-MG and U87-MGΔ cells after senescence-inducing treatment.
Both senescent phenotype and loss of the BK channel results in a differential expression of mRNA of encoding the proteins involved in the regulation of mitoROS levels, apoptosis and mitochondrial transport. Understanding the impact of BKCa channel loss on ROS production and detoxification can possibly provide valuable insights for the treatment of cardiovascular and age-related diseases.

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P.48

Metal ions and small heat shock proteins – interaction models, studies, and possibilities

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Small heat shock proteins (sHSP) are synthesized in cells upon an increase in temperature, but also contribute to cell protection in various ways. For example, crystallins, which belong to the family of small HSPs, are present in the eye lens, and the polymer structures they form are responsible for the transparency of the lens and its protection at high temperatures. Since the interaction of crystallin with metal ions such as zinc or copper could be crucial for the maintenance of these properties, it is worthwhile to determine the binding sites of metal ions and the stability of such complexes. Since these proteins polymerize easily, a simpler way to study them is to examine their peptide fragments and their analogs.
HSPB1, a sHSP with a conserved alpha-crystallin domain, presents some similarities to a fragment of an unstructured region to not only crystallins, but also ferritin, the iron storage protein. The peptide fragments of HSPB1 and their Ala-analogs were studied in terms of thermodynamic stability, with the use of various methods of analytical chemistry to determine the most important amino acid residues, which may be involved in metal ions binding. It was shown that various approaches may result in different results, but altogether, the full characteristics of the metal ion interaction with specific peptide fragments of HSPB1 were achieved.

P.49

Split intein-assisted fluorescent tagging system for protein engineering and expression analysis

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Protein engineering enables precise modifications of proteins *via* site directed mutagenesis, random mutagenesis, domain swapping, loop insertion, or fusion design to improve enzyme activity. However, mutations in coding sequences may affect both function and expression levels. Therefore, kinetic characterization—rather than crude extract activity alone—is essential in enzyme optimization workflows, especially in high-throughput screening (HTS).

Although some enzymes possess intrinsic chromophores or prosthetic groups enabling direct quantification, most require purification prior to kinetic analysis. Conventional techniques such as ELISA, western blotting, or mass spectrometry, while precise, are not easily scalable for HTS applications. To address this limitation, various fluorescence-based strategies have been developed, including fusion to full-length fluorescent proteins, fragment complementation systems (e.g., GFP1–10/GFP11), or co-expression using polycistronic vectors.

This study presents a dual-function tagging system combining a self-associating split intein (NpuN/NpuC) with a split fluorescent protein (ffGFP1–10/ffGFP11). The design enables autocatalytic removal of the tag and simultaneous quantification of the protein of interest based on fluorescence. It is compatible with solid-phase purification and HTS platforms.

This strategy offers an efficient solution for protein expression monitoring and rapid selection of functional enzyme variants without the need for full protein purification.

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Split Intein Based Tag For Quick Protein Purification and Tag Removal

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Recombinant protein production and purification are key processes in modern biotechnology. Various expression systems, including advanced cell-free platforms, have been developed to meet the demand for high-quality proteins. A critical step in protein of interest (POI) characterization is purification, often facilitated by affinity tags. However, for therapeutic proteins or sensitive research contexts, complete tag removal is required- typically achieved by specific protease recognition sites for targeted cleavage and release of the native POI or its near-native form. Intein-based tags offer a powerful alternative. Inteins can undergo self-catalyzed cleavage under defined conditions, allowing controlled release of the POI without using exogenous enzymes. A key advantage of this system is tunable splicing, enabling precise control of cleavage timing and specificity. Recently, the Npu intein has been engineered to enable even greater functionality. Splitting the intein into mutual high-affinity N- and C-terminal fragments enabled a novel purification strategy. In this approach, the N-terminal intein fragment is immobilized on a solid support, while the POI is fused to the C-terminal fragment. Upon binding, the complex remains stable until cleavage conditions are applied, releasing the POI with minimal host protein contamination. This split intein-based strategy is rapid, cost-effective, and highly specific, reducing steps and streamlining purification in research and industry.

P.51

Diet-induced hyperhomocysteinemia alters ovarian gene expression and reproductive function in mice

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Elevated homocysteine (hyperhomocysteinemia, HHcy), is linked to hormonal imbalance and reproductive dysfunction. To study diet-induced HHcy, female C57BL/6J mice received methionine (Met 1%) or homocysteine (Hcy 0.1%) in drinking water for 6–8 weeks. HHcy was confirmed by HPLC. Hcy mice gained more weight, and both diets increased ovarian weight, with a higher ovarian-to-body weight ratio in Hcy mice.

Ovarian single-cell transcriptomics revealed upregulated *Cyp17a1* in theca cells, suggesting increased androgen synthesis, while key genes involved in ovulation (*Adamts1*), steroid metabolism (*Akr1c18*), and signaling (*Cyyr1*) were downregulated. Granulosa cells showed diet-specific changes: *Ccn5* decreased in Hcy, *Mgp* and *Agt* in Met, indicating altered follicular microenvironments. Both diets decreased circulating progesterone and estradiol levels, reflecting transcriptomic evidence of impaired steroidogenesis.

Estrous cyclicity was disrupted, with significantly prolonged length and extended fertile phases in both HHcy groups vs. controls. Y-maze tests showed increased working memory errors in Hcy mice, while open field behavior was unchanged.

In summary, diet-induced HHcy impairs ovarian steroidogenesis, folliculogenesis, and luteal function, potentially reducing fertility. These findings highlight the critical role of one-carbon metabolism in reproduction and warrant further in-depth investigation of its influence during the peri-conception period.

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P.52

Liposomal Cannabidiol and Celecoxib Modulate Wnt/β-Catenin and NF-κB Pathways in U-87 MG Astrocytoma Cells

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Astrocytoma remains one of the most aggressive and therapeutically resistant brain malignancies. The Wnt/β-catenin pathway drives tumor progression by enhancing proliferation, invasion, and resistance to apoptosis, while NF-κB signaling promotes survival and inflammation-related growth.

Given the need for innovative therapeutic strategies, we investigated the effects of liposomal formulations of cannabidiol (CBD), celecoxib (CELE), and their combination (CBD+CELE) on the Wnt/β-catenin and NF-κB pathways in astrocytoma U-87 MG cells. Encapsulation of these compounds in liposomes can enhance cellular uptake and bioavailability, providing a novel platform for targeted delivery.

CBD downregulated gene expression of *CTNNB1*, *CCND1*, *BIRC5*, *c-MYC*, and *NF-κBp65/p50*, while upregulating *AXIN2*. Similar effects were observed for CELE. The CBD+CELE combination induced a more pronounced decrease in nuclear β-catenin levels and significantly inhibited nuclear NF-κBp65/p50 levels.

Liposomal delivery improved efficacy, and CBD+CELE exhibited synergistic inhibition of both Wnt/β-catenin and NF-κB pathways, indicating potential therapeutic benefit. These findings suggest that liposomal CBD, particularly in combination with CELE, effectively disrupts oncogenic signaling in GBM and may represent a promising strategy for astrocytoma therapy.

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P.53

Interactions of the Starmaker protein with glycosaminoglycans and their synergistic role in biomineralization

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The Starmaker protein (Stm) plays a key role in the biomineralization of otoliths in fish, where it may interact with other components of the organic matrix, including glycosaminoglycans (GAGs). The effects of such interactions on this crucial biological process remain largely unknown. Therefore, this study aimed to investigate the potential synergistic role of Stm and three selected GAGs – hyaluronic acid, chondroitin sulfate, and heparin – in calcium carbonate crystallization. Recombinant Stm was produced and purified, and its interactions with GAGs were analyzed through *in vitro* biomineralization assays. Scanning electron microscopy analysis revealed that the presence of hyaluronic acid in combination with Stm significantly altered the morphology of calcium carbonate crystals, leading to increased density and size, with individual crystals appearing to merge into larger, more organized structures. This morphology pattern was not observed in samples containing Stm or hyaluronic acid alone, suggesting that these components play a synergistic role in crystal formation. Our findings contribute to a better understanding of otolith biomineralization mechanisms and may, in the future, support the development of methods for regenerating human otoconia or engineering novel biomaterials. Our next goal is to explore additional protein–GAG interactions that may influence inner ear biomineral formation.

P.54

Spectral flow cytometry of extracellular vesicles enables tissue-specific biomarker discovery

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Extracellular vesicles (EVs) are critical mediators of intercellular communication and minimally invasive biomarkers for disease diagnosis and monitoring. However, their nanoscale size and molecular heterogeneity present challenges for measure and high-throughput analysis. We applied spectral flow cytometry and nanoparticle tracking analysis (NTA) to characterize EVs of different origines such as cancer cell lines, serum from healthy donors and NSCL cancer patients and EVs from atherosclerotic plaques. EVs were isolated using ultracentrifugation or size-exclusion chromatography and immunophenotyped using fluorescence-conjugated antibodies against canonical EV and tissue-specific markers. Cytometry was performed based on the ISEV and ISAC/MIFlowCyt-EV guidelines and recommendations to perform high standard reproducible experiments. Expression of Tetraspanin markers: CD9, CD81, CD63 by flow cytometry showed different staining profiles dependently on EVs origin. For immunophenotyping two strategies have been tested: direct staining and immune phenotyping based on MACSPlex exosome beads-based array. We will present the whole pipeline of standardization, proper instrument setup, necessary controls, as well as sample preparation from different tissues and final experiment design. The tissue and disease-specific EVs landscapes will be presented. We will also discuss the limitations of spectral flow cytometry to identify EVs specific signatures.

P.55

FIS1, *OPA1*, and *SIRT3* – genes involved in mitochondrial dynamics in non-small cell lung cancer

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Recent research on lung cancers has focused on mitochondrial dysregulation related to increased fusion and impaired fission, resulting in mitochondrial network disruption. Sirtuin 3 is an mtDNA repair protein that counteracts oxidative stress, which can affect mitochondrial dynamics. Our study aimed to evaluate the expression of mitochondrial dynamic regulators – for division *FIS1* and fusion *OPA1*, and the DNA-repairing *SIRT3* - in non-small cell lung cancer (NSCLC) patients and controls.

The study group comprised 57 LC patients and 28 controls. *SIRT3, FIS1, OPA1* relative expression were evaluated in peripheral blood lymphocytes, and SIRT3 immunoexpression in serum.

SIRT3 and *OPA1* expression were significantly higher in controls than in the NSCLC group ($p=0.012$; $p=0.003$). SIRT3 was downregulated in women ($p=0.004$). SIRT3 immunoexpression varied with cancer progression—higher in patients with node involvement (N1–N3) vs. N0 ($p=0.03$). Correlation analysis in controls revealed that *SIRT3* expression was positively correlated with *FIS1* and *OPA1*, age, and smoking. In NSCLC, *SIRT3* correlated with *FIS1* expression, and its serum level with BMI.

Smoking impacts mitochondrial dynamics: *FIS1* expression in LC increased with the number of pack-years smoked. A positive correlation between BMI and SIRT3 immunoexpression in LC patients indicates that higher SIRT3 serum levels may be a positive prognostic marker in cancer survival. More studies are needed to confirm its prognostic value.

P.56

Pol γB interacts with Pol β in human mitochondria under non-oxidative conditions and regulates its dRP lyase activity to facilitate short-patch DNA repair

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Earlier studies showed that DNA polymerase β (Pol β) localizes to mitochondria in certain cell types and participates in base excision repair (BER). Here, we detected Pol β in mitochondria of human lung epithelial cells (BEAS-2B) and found that it specifically binds Pol γB, the accessory subunit of DNA polymerase γ, but not the catalytic subunit Pol γA. Biophysical assays revealed that the N-terminal lyase domain of Pol β mediates this interaction. Functionally, Pol γB stabilizes the Pol β-dRP Schiff base intermediate, slowing but not preventing β-elimination. Notably, Pol γB does not affect 1-nt gap filling by Pol β but reduces its strand displacement activity. Under oxidative stress, the Pol β-Pol γB interaction is abolished in cells, despite both proteins remaining in mitochondrial fractions. We propose that under physiological conditions, Pol γB transiently stabilizes Pol β at lesion sites, thereby promoting short-patch BER, whereas during substantial oxidative stress, Pol β is susceptible to forming DNA-protein crosslinks (DPCs) with oxidized abasic sites, compromising its function. These Pol β-DPCs are resolved by the mitochondrial 5'-exo/endonuclease EXOG *in vitro*. Collectively, our findings provide the first evidence that Pol γB modulates enzymatic activity of Pol β, thereby regulating mitochondrial DNA repair.

P.57

Neuroprotective and antiapoptotic activities of *Salvia aethiopis* extract from cultivated plants in rats with scopolamine-induced model of dementia

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Salvia aethiopis is a relatively understudied species from the Lamiaceae family, known for its cognitive-enhancing (nootropic) properties. Previous *in vitro* studies show *reported nootropic and anticancer potential. Its effects on neuroprotection, however, remain underexplored.*This study aimed to explore the neuroprotective and antiapoptotic effects of S. *aethiopis* extract in a rat model of scopolamine (Sco)-induced dementia. Male Wistar rats (200–250 g) were divided into four groups: Control (0.9% NaCl, i.p./p.o.), Sco (2 mg/kg, i.p.), S. aethiopis (100 mg/kg, p.o.), and Sco + *S. aethiopis*. Scopolamine was administered intraperitoneally for 11 days. The extract (from aerial in vitro-grown parts) was given orally for 21 days, including a 10-day pretreatment. We assessed memory-related proteins: pCREB and BDNF in the cortex and hippocampus. Bcl-2 (antiapoptotic) and BAX (proapoptotic) expression was evaluated in the cortex. Sco decreased BDNF, pCREB, and Bcl-2 levels and increased BAX. *Treatment with S. aethiopis* reversed these effects. In healthy rats, it elevated pCREB and Bcl-2 and reduced BAX levels. In conclusion, *S. aethiopis* extract exhibited neuroprotective and antiapoptotic properties in this model.

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Unicellular organisms in discoveries of cilia tip composition and function

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Motile cilia, organelles believed to be present in the Last Common Eukaryotic Ancestor, are assembled by organisms from nearly all modern lineages except for some fungi and the majority of seed plants. During the eukaryotic evolution, cilia underwent functional specialization that resulted in the formation of, besides typical motile cilia, other cilia types: motile nodal cilia and immotile sensory cilia of different types. However, the structure and function of cilia are retained in various, even very distant eukaryotic lineages. The main structural components of all types of cilia are microtubules accompanied by numerous multiprotein complexes. Their differential distribution leads to the formation of specialized ciliary zones, such as a transition zone at the cilium base, a middle segment, containing, motor or sensory complexes, and a distal segment called a ciliary tip.

While the components of the transition zone and middle segment were broadly studied, much less is known about the protein composition of the ciliary tip and functions of its components. The ultrastructural analyses using electron microscopy revealed significant differences in the architecture of the distal tip in sensory and motile cilia. On the other hand, there is some similarity in the composition of ciliary tip in motile cilia assembled by various, evolutionarily distant organisms. Interestingly, recent data indicate that ciliary tip proteins identified in unicellular model organisms, are located in motile cilia in vertebrates and, in some cases seem to be common for all types of cilia.

P.59

Structural insights into human L-strand mitochondrial DNA replication

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Mitochondrial DNA (mtDNA) replication is essential for cellular energy production and requires synthesis of both the heavy (H) and light (L) DNA strands. The two strands use distinct mechanisms: H-strand replication depends on helicase activity, while L-strand replication occurs on pre-unwound single-stranded DNA, making the interaction between DNA polymerase gamma (Pol γ) and mitochondrial single-stranded DNA-binding protein (mtSSB) critical. Earlier biochemical studies showed that mtSSB can boost Pol γ activity by stimulating primer extension and increasing replication rate, but also revealed that mtSSB bound to DNA can impede Pol γ movement, stalling replication. These contrasting effects suggest the Pol γ–mtSSB interaction is highly dynamic and influenced by the structural conformation of the replication complex.
In this study, we investigate the molecular and structural basis of Pol γ–mtSSB interactions. Using biochemical assays, we identify the specific DNA-binding site of mtSSB necessary for stable Pol γ–mtSSB complex formation under conditions that mimic L-strand replication. Furthermore, through cryogenic electron microscopy (cryo-EM), we aim to resolve the structure of the complete Pol γ–mtSSB–DNA complex to elucidate the molecular mechanism of L-strand replication and its implications for replication fork orientation.

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Effect of hypoxia and autophagy inhibition on chemoresistance and proliferation in senescent colon cancer cell model

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Autophagy and therapy-induced senescence (TIS) are known chemoresistance mechanisms, with TIS believed to induce the phenotype also present in cancer stem cells. Varied culture conditions: normoxia (21% O_2) and hypoxia (1% O_2) were used to determine if combined autophagy inhibition and hypoxia has a senolytic effect or hinders the ability of senescent cells to proliferate. The main experiments were performed on colon cancer HCT116 and SW480 cell lines treated with irinotecan (IRINO) to induce senescence, and with hydroxychloroquine (HCQ) as well as bafilomycin A1 (BAFA1) to inhibit autophagy. IRINO was capable of inducing senescence, based on increased fraction of polyploid and G2/M phase-arrested cells as well as SA-β-gal positive cells, coinciding with a decreased expression of stem cell markers (e.g. CD133) upon senescence. qPCR experiments in HCT116 cell line depict the changes in the level of expression of proliferation and metabolism-related genes in hypoxia and upon treatment with HCQ as well as BAFA1. BAFA1 provided a cytotoxic effect and delayed proliferation in senescent HCT116/SW480 cell lines. Moreover, hypoxia may impair entering senescence and maintain epithelial-to-mesenchymal transition. As such, both regulation of autophagy and hypoxia display an effect in senescent and stem-like phenotype regulation, indicating the need to consider the role of newly introduced parameters in cancer treatment.

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Phytochemical Analysis and In Vitro Anthelmintic Activity of *Thymus capitatus* Essential Oil Against *Haemonchus contortus*

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Sheep haemonchosis is a disease that causes serious losses in livestock production, particularly with the increase of cases of anthelmintic resistance around the world. This justifies the urgent need of alternative solutions. The aim of this study was to determine the chemical profile, in vitro anthelmintic properties of *Thymus capitatus* essential oil. To evaluate the, in vitro, anthelmintic activity of the *T. capitatus* EO on *Haemonchus contortus*, two tests were used: egg hatch assay (EHA) and adult worm motility (AWM) assay. Chromatographic characterization of T. cap itatus composition using gas chromatography coupled to mass spectrometry (GC-MS) demonstrated the presence of carvacrol (81.16%), as the major constituents. The IC 50 values obtained was 1.9 mg/mL in the EHT. In the AWM assay; T. capitatus essential oil achieved 70.8% inhibition at 1 mg/mL after 8 h incubation. The results of present study, demonstrate that *T. capitatus* EO possess a significant anthelmintic properties. Furthermore, it could be an alternative source of anthelmintic agents against gastrointestinal infections caused by H. contortus in livestock.

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Halo-Tag as a tool for determining the subcellular localisation and activity of the ROMK2 channel

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ROMK2 is a channel responsible for potassium transport within the kidney. Previous experiments conducted in our laboratory have shown that ROMK2 could potentially form complexes with the lipid kinases AGK and DGKε, present in mitochondria and endoplasmic reticulum, respectively (Krajewska *et al.*, 2024). However, before these interactions can be studied in depth, identifying the subcellular distribution and activity of ROMK2 is crucial.

To address this issue, we decided to use the Halo-tagged variant of ROMK2. HaloTag, a genetically modified hydrolase, covalently binds with synthetic linkers containing a chloroalkane group. Such linkers can be functionalised with fluorescent dyes, allowing further study of the activity and localisation of the ROMK2-HaloTag fusion protein (Los *et al.*, 2008).

In this work, we quantified the colocalisation of the ROMK2-HaloTag fusion protein with markers of the plasma membrane and endoplasmic reticulum in transiently transfected HEK293 cells. To this end, we used confocal microscopy and labelled live cells with TMR Direct (a fluorescent ligand bound to HaloTag) and CellMask Green (plasma membrane stain). Similarly, the colocalisation of ROMK2-Halo labelled with TMR Direct and an ER marker was carried out. In addition to localisation, HaloTag can be used to measure ROMK2 channel activity with the use of the Halo-Tag reactive, thallium-specific fluorescent dye Thallos HTL (Ion Biosciences). The feasibility of such measurements will be discussed.

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Bioprinting with Meniscus dECM, Collagen-Based Nutraceuticals, and Single-Cell Transcriptomics: New Perspectives for Biomaterials and Regenerative Medicine

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Collagen-based biomaterials are increasingly relevant in regenerative medicine and nutraceuticals. This study integrates three approaches: (1) characterization of marine spongin collagen, (2) development of bioinks from decellularized porcine meniscus extracellular matrix (dECM), and (3) creation of a single-cell transcriptome atlas of the meniscus. Proteomics, NMR, and Raman confirmed spongin's similarity to mammalian collagen, identifying types I and III, with HPLC-MS revealing unique halogenated di- and tri-tyrosine crosslinks. We also developed a scalable protocol for dECM-based bioinks using homogenization, hydrolysis, supercritical CO_2 extraction, and lyophilization. Despite DNA levels exceeding standard thresholds, the bioink showed excellent biocompatibility, challenging current decellularization benchmarks. Our single-cell transcriptome atlas revealed four main cell types – chondrocytes, endothelial, smooth muscle, and immune cells – with five chondrocyte subclusters. Red-zone chondrocytes display mesenchymal-like regenerative properties, while white-zone chondrocytes focus on cartilage matrix maintenance. The strong cellular similarity between porcine and human menisci validates the pig as a translational model for orthopaedic applications. This integrative approach advances collagen biomaterial applications in tissue engineering and meniscal repair.

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Exploring miR-146a function in Melanoma Cells

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MicroRNAs (miRNAs) are small noncoding RNAs that post-transcriptionally inhibit gene expression. MiRNAs may participate in both tumorigenesis and tumor suppression, and they also modulate the radiosensitivity of cancer cells. MiRNA-146a is an inflammation-associated miRNA that can influence oxidative stress responses, potentially affecting the outcome of radiotherapy in cancer cells. The aim of this study was to examine the function of miR-146a in Me45 melanoma cells. Small RNA-seq revealed that miR-146a was the highest expressed miRNA in Me45 cells. We inhibited miR-146a using lentiviral vectors that also encoded GFP, enabling us to track the transduced cells using flow cytometry in a GFP competition assay. When miR-146a was silenced, the number of GFP-positive Me45 cells decreased compared to co-cultured wild-type cells, suggesting that miR-146a has pro-survival role in Me45 cells. This effect was not present when Me45 cells were transduced with a negative control. Then, Me45 cells were exposed to 4 Gy of IR, resulting in cell cycle arrest at the G2/M phase and induction of apoptosis. By small RNA-seq, we identified several miRNAs altered after IR in Me45 cells, but we did not observe changes in miR-146a levels. In conclusion, miR-146a silencing reduced cell survival of Me45 melanoma cells. Ongoing experiments are focused on the involvement of miR-146a in radiosensitivity of melanoma cells.

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P.65

Hold on tight: N-terminal region enables low-temperature activity of phage-derived Efa DNA polymerase

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During viral infection cycle, its genome, consisting of DNA or RNA, must be replicated prior to assembly and release of progeny virions. While phages – viruses that infect bacteria – can rely on host replication machinery, many encode their own replication proteins, including DNA polymerases. Presumably, they are tailored to phage's specific demands, such as unusual nucleotides, challenging genome topology or fast life cycle.

The recently characterized *Enterococcus faecalis* phage vB_EfaS-271, isolated from urban sewage, encodes a B-family DNA polymerase called Efa. Our studies show that it exhibits typical 5'-3' polymerase and 3'-5' exonuclease activities and adopts canonical right-hand architecture, comprising palm, fingers, thumb, and exonuclease domains. Additionally, it contains an N-terminal region, structurally and functionally similar to a single-stranded DNA-binding (SSB) monomer.

We demonstrate that Efa remains catalytically active at temperatures as low as -10°C. Removal of the N-terminal region results in a pronounced loss of activity with decreasing temperature. Truncated variant of Efa shows significantly reduced DNA binding with rapid substrate dissociation. These results suggest that cold adaptation of Efa polymerase depends on the N-terminal region, which maintains tight contact with DNA even at low temperatures, enabling completion of synthesis under extreme conditions.

P.66

The nuclease activity of human EXOG is regulated through its interaction with cardiolipin containing membranes

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Mitochondria are essential organelles for cellular energy production. They house a small genome (mtDNA) anchored to the inner mitochondrial membrane (IMM). The proximity of mtDNA to the electron transport chain makes it susceptible to damage by reactive oxygen species, generated as by-products of oxidative phosphorylation. To counteract this, mitochondria are equipped with a specialized set of enzymes, known as the 'repairosome', capable of robust DNA repair. Human EXOG, a mitochondria exclusive nuclease, is an essential component of mitochondrial base excision repair (mtBER) due to its precise 5'-3' exonuclease activity and broad substrate specificity. In cells, EXOG is located along the cristae indicating an association with the IMM. However, the exact nature of this association remains unclear. Here, we unveil a novel interaction between the truncated, enzymatically active EXOG and lipid membranes. Using *in cellulo*, *in vitro* and *in silico* approaches, we reveal an interaction between EXOG and Cardiolipin (CL) that relies primarily on membrane contact and is influenced by local charge. This interaction impedes EXOG-DNA binding, consequently inhibiting the nuclease activity of the enzyme. Moreover, we find that the membrane bound EXOG promotes the recruitment of other members of the mtBER. We propose that, under physiological conditions, EXOG is partially inert on the membrane surface, bound to CL, while also serving as a platform for mitochondrial repairosome assembly.

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Integrative multi-omics analysis reveals molecular mechanisms of cognitive deficits in a mouse model of dietary-induced hyperhomocysteinemia

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Hyperhomocysteinemia (HHcy) is a risk factor for neurodegenerative and psychiatric disorders, but its molecular effects in the brain remain unclear. Female C57BL/6J mice were fed control, methionine (Met, 1%), or homocysteine (Hcy, 0.1%) diets for 16 wks, with HHcy confirmed by urinary tHcy (HPLC). Region-specific gene and protein expression in cortex and hippocampus were investigated using spatial transcriptomics (10xGenomics) and label-free proteomics (LC-MS/MS), alongside assessment of functional outcomes through behavioral tests (NOR, Y-maze, motor).

Both diets induced mitochondrial dysfunction, shown by downregulation of OXPHOS components and remodeling of cytoskeletal and synaptic proteins, mainly in the cortex. The Met diet upregulated immediate-early genes, stress-related lncRNAs, and oxidative defense proteins, while reducing synaptic plasticity markers. The Hcy diet triggered activation of DNA damage response, pro-inflammatory, and stress pathways, alongside suppression of mitochondrial and antioxidant proteins. Proteomic data also revealed impaired autophagy in both brain regions. These molecular alterations likely contribute to deficits in long-term recognition memory, spatial working memory, and motor coordination seen in HHcy mice in this study.

Our integrative multi-omics and behavioral analyses uncover mechanisms by which HHcy disrupts synaptic and metabolic balance, driving cognitive and motor deficits, and offer new insights into its role in neurodegeneration.

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P.68

Is there a connection between retrogenes, retrotranspozons and spermatogenesis?

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Pyocyanin production by *P. aeruginosa* strains isolated from the "Dziewoklicz" municipal bathing site in Szczecin

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Pyocyanin (PYO) is a bacterial phenazine produced by *Pseudomonas aeruginosa* bacteria. This natural and biodegradable compound can be utilized in agriculture as a biopesticide or as an electron transfer enhancer in microbial fuel cells.
The work aimed to check and compare the production potential of pyocyanin dye by environmental strains *P. aeruginosa* isolated from the „Dziewoklicz" bathing area in Szczecin with the reference strain *P. aeruginosa* ATCC 27853.
Water samples were taken from the "Dziewoklicz" bathing area in Szczecin. Isolation was carried out by membrane filtration according to PN-EN ISO 16266:2009. Bacteria were characterized using the API 20NE test. Biofilm formation capabilities and antibiotic resistance were also assessed. Pyocyanin production was conducted in KingA liquid medium. The product was extracted with chloroform and hydrochloric acid. The concentration of PYO was measured spectrophotometrically.
The study has shown that collected strains could be assigned to four distinct groups based on their physiological features. Production capabilities varied, although one of the strains exceeded the production of the reference strain. In conclusion, municipal bathing areas can be a source of *P. aeruginosa* strains that can be potent pyocyanin producers. Under optimized conditions, environmental isolates can become major producers of pyocyanin for use in agriculture or environmental protection.

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P.70

Molecular mechanisms in NBIA: insights into pathogenesis and disease diversity

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Neurodegeneration with Brain Iron Accumulation (NBIA) is a heterogeneous group of rare, progressive neurodegenerative disorders. The most common NBIA subtypes include MPAN (Mitochondrial membrane Protein-Associated Neurodegeneration), PKAN (Pantothenate Kinase-Associated Neurodegeneration), BPAN (Beta-Propeller Protein-Associated Neurodegeneration), and PLAN (PLA2G6-Associated Neurodegeneration). Although the causative genes for each subtype have been identified, most are not directly involved in iron metabolism. Moreover, the significance of iron accumulation itself as the primary pathogenic mechanism has been increasingly questioned. The presented results are part of a larger research project aimed at elucidating the molecular mechanisms underlying NBIA, identifying key cellular dysfunctions, and selecting potential therapeutic targets. Our findings to date are consistent with the hypothesis emphasizing the pivotal role of lipid metabolism disturbances in NBIA pathogenesis and suggest that the individual disorders comprising this group should not necessarily be approached uniformly in terms of research and therapeutic strategies.

P.71

Role of MOTHER OF FT AND TFL1 in Seed Development and Germination in *Medicago truncatula*

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Seeds are highly specialized structures that enable survival of plant progeny in variable climates. The conditions present during their development on the mother plant determine a number of factors, e.g. depth of dormancy. Deep dormancy prevents germination even if all the proper external conditions are met and it can be lost over time (*afterripening* of seeds). The transition between dormancy and gemination of mature seeds is regulated by the interplay of abscisic acid (ABA) and gibberellin (GA) pathways. One of the components of the ABA and GA signaling pathways recruited to control seed germination is the MOTHER OF FT AND TFL1 (MFT) protein.

We have shown that *MFT* from *Medicago truncatula* is highly expressed in developing seeds and in the embryonic root of mature imbibed seeds. The expression of *MtMFT* in imbibed seeds is related to the ABA pathway and dependent on the current level of dormancy. Furthermore, freshly harvested *mtmft* seeds exhibited significantly higher germination rates compared to WT seeds. However, the germination rates did not differ between after-ripened seeds, suggesting MFT's role in maintenance of dormancy rather than the process of germination itself. Our results also suggest MtMFT's role in so called "thermoinhibition", another key mechanism that contributes to the evolutionary success of seeds. We have also determined the subcellular localization and the crystal structure of MtMFT.

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Ultrasensitive profiling of the *TSC1* mutation landscape in the TSC skin tumors

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Aim: Tuberous sclerosis complex (TSC) is due to loss-of-function mutations in *TSC1* or *TSC2*. In TSC, tumors occur in various tissues, including facial angiofibroma (FAF) in skin. Here, an ultrasensitive profiling of *TSC1* mutations in TSC skin tumors was performed.

Methods: A Multiplex High-sensitivity PCR Assay (MHPA) was developed, enabling *TSC1* mutation detection at extremely low frequency (<0.1%). *TSC1*-MHPA was applied to 43 samples, including 37 skin biopsies; the results were compared with prior *TSC2*-MHPA skin analysis results (Klonowska, JCI 2022).

Results: *TSC1*-MHPA of 7 FAFs from *TSC1* patients showed that UV-induced mutations are much less common in *TSC1* than in *TSC2* (average 1.0 vs. 7.4 muts per 2mm biopsy, p=0.0003). *TSC1*-MHPA of 8 non-TSC normal skin samples (high UV exposure) also showed that UV mutations are less common in *TSC1* than in *TSC2* (average 3.0 vs. 13.4 muts, p=0.0004). No UV CC>TT mutations were seen in 10 foreskin newborns' samples (never UV exposed). CC/GG content in *TSC1* vs. *TSC2* sequences suggests a higher UV mutation propensity in *TSC2*.

Conclusions: UV mutations are less frequent in *TSC1* than in *TSC2*, consistent with lower severity of FAF involvement in TSC patients with *TSC1* vs. *TSC2* mutations. The difference in the sequence composition of *TSC1* and *TSC2* contributes to a higher propensity for UV mutations in *TSC2*.

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Studying *ELAC2* knockdown effects in regenerating *Schmidtea mediterranea via* single-cell transcriptomics

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The endonuclease ELAC2 is essential for tRNA 3'-end processing and maturation of mitochondrial transcripts in metazoans. We recently discovered that silencing of the *ELAC2* ortholog in *Schmidtea mediterranea* (*Smed-ELAC2*) leads to delayed regeneration, including impaired nervous system development. To investigate the molecular basis of this phenotype, we studied transcriptomic changes induced by *Smed-ELAC2* silencing at the single-cell level. Preliminary analysis uncovered stage-specific shifts in lineage dynamics. Graph-based trajectory inference and RNA velocity analysis revealed altered lineage topology and flow patterns, particularly within neuronal, muscle, epidermal, phagocyte, and parenchymal trajectories. Velocity fields indicated stalled or rerouted progenitor differentiation toward these lineages, accompanied by shifts in the balance of proliferative versus differentiated states. These changes suggest that *Smed-ELAC2* knockdown disrupts the coordinated progression of regeneration, leading to delays in tissue patterning and structural integration. Beyond defining ELAC2-dependent effects, these data also contribute to a broader single-cell-level understanding of regeneration in *S. mediterranea*.

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Dual-Functional Silk Spheres for Targeted Delivery of Oligotherapeutics to VEGFR-Positive Cells in the Tumor Microenvironment

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siRNA enables efficient silencing of target gene expression, but its delivery to the cells is limited and requires a delivery system. Targeting VEGF receptors, overexpressed in both cancer and endothelial cells of the tumor microenvironment, to deliver siRNA is a promising strategy for cancer treatment. We designed bioengineered silk proteins VE1MS1 and VE2bMS1 to target VEGFR1 and VEGFR2, respectively, and blended them with MS2KN silk, designed to bind nucleic acids. Agarose gel electrophoresis confirmed the siRNA binding affinity to the silk blends. Then, silk nanospheres were formulated, and their physicochemical properties, cytotoxicity, and siRNA loading to the spheres were established. Flow cytometry revealed that VEGFR-targeting spheres loaded with siRNA efficiently bound to VEGFR-overexpressing H1975 and HCC4006 non-small-cell lung cancer, and HUVEC endothelial cells. Cellular internalization of VE1MS1 and VE2bMS1 spheres and intracellular delivery of siRNA were confirmed by confocal microscopy. qRT-PCR analysis showed that HIF-1α-siRNA delivered *via* silk nanospheres lowered target gene expression in H1975 and HUVEC cells. *HIF-1a* silencing modulated the expression of factors related to tumor progression and angiogenesis at the mRNA level. Our results show that VEGFR-targeting silk spheres are a promising tool for delivering siRNA to VEGFR-overexpressing cells and inducing silencing effects.

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Towards all-hPSC multilineage pancreatic organoids for disease modelling and developmental studies

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Diabetes mellitus results from dysfunction or loss of pancreatic β-cells and affects over 820 million individuals globally, causing more than 2 million deaths annually. Human pluripotent stem cells (hPSCs) offer promising potential for β-cell replacement therapy, with hPSC-derived β-like cells demonstrating the ability to mitigate hyperglycemia in murine models. Yet, challenges persist in reliably deriving functionally mature β-cells with sufficient yield and clinical feasibility.

In vivo pancreatic islet development occurs through precise interactions within the cellular niche, including mesenchymal, endothelial, and neuronal cells. We and others have shown that these niche cells accelerate functional maturation and increase the survivability of β-cells. However, feasible *in vitro* models that faithfully recapitulate the physiological human pancreatic niche are lacking.

Here, we created a molecular roadmap of early human pancreatic mesenchyme development. For this, we compared publicly available single-cell RNA sequencing data from early human embryos (PCW3-6) against our scRNA-seq data from an existing splanchnic mesoderm derivatives differentiation protocol. We performed trajectory analysis to identify and validate novel signaling pathways, transcription factors, and their regulons driving pancreatic mesenchyme specification. We then used this knowledge to derive pancreatic mesenchymal progenitors *in vitro* and co-cultured them with pancreatic progenitor spheroids.

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Gene Expression Changes in Huntington's Disease and the Therapeutic Effects of Genistein

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Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an excessive number of CAG trinucleotide repeats in the HTT gene, leading to the production of mutated huntingtin protein (mHTT), which forms poorly soluble aggregates. These mHTT aggregates accumulate primarily in neuronal cells, resulting in a gradual loss of motor, psychological, and cognitive abilities.

In our study, we utilized HEK 293T cell lines with 24, 41, 53, and 84 CAG repeats (HEK, HEK41, HEK53, HEK84), as well as the R6/1 mouse model of HD (116 CAG repeats). Additionally, both the cellular and animal models were treated with genistein- a flavonoid capable of inducing autophagy and reducing mHTT levels. Genistein can cross the blood-brain barrier, making it a potential therapeutic agent for neurodegenerative diseases, including HD. We performed transcriptomic analysis, confirmed the expression levels of selected genes using RT-qPCR, and examined the levels of specific proteins *via* Western Blot.

Our findings indicate that the number of altered expressed genes increases with the number of CAG repeats in the HTT gene. In both the cellular and animal models, genistein modulated the expression of numerous genes-normalizing, upregulating, or downregulating their levels, including those related to autophagy. These studies provide insights into the pathogenic mechanisms of HD and the effects of genistein, highlighting the role of autophagy in mHTT degradation and the potential therapeutic efficacy of this compound in future HD treatments.

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Tropomyosin-dependent modulation of fascin interactions with actin filaments

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Changes in the expression of actin regulatory proteins are key factors in the migration and metastasis of cancer cells. In metastatic osteosarcoma cell lines, expression of tropomyosin isoforms encoded by TPM2 is downregulated, while fascin-1 is upregulated. In vitro biochemical analyses showed that recombinant acetylated Tpm2 isoforms differ in their actin affinity. While the actin affinity (Kapp) of Tpm2.1 and Tpm2.3 was similar, Tpm2.4 showed the highest Kapp. The presence of fascin-1 on filaments did not affect the affinity of Tpm2 isoforms. Fascin-1 bound to unregulated actin with a high Kapp, which was decreased by Tpm2 isoforms by 1.5- to 4-fold.At low fascin:actin molar ratios, all Tpm2 isoforms strongly inhibited fascin-1's actin bundling activity, but this inhibition was alle*via*ted at higher fascin concentrations. The resulting actin bundles contained both Tpm2 and fascin-1; however, in the presence of all Tpm2 isoforms, the number of filaments per bundle was reduced. Increasing actin occupancy with Tpm2 partially displaced fascin-1 from the filaments, with the extent of displacement proportional to Tpm2's actin affinity. Pull-down assays revealed that Tpm2 isoforms can directly interact with fascin-1, which may contribute to the mechanism of Tpm-dependent regulation of fascin-1.In conclusion, cytoplasmic Tpm2 isoforms regulate fascin-1's actin bundling activity, a mechanism that likely contributes to the suppression of metastatic phenotypes in osteosarcoma.

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Translation in ALS: The Role of FUS in rRNA Processing and translation efficiency

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder affecting motor neurons. One aggressive familial form is caused by the P525L mutation in the RNA-binding protein FUS, which mislocalizes to the cytoplasm, forms toxic aggregates, and disrupts RNA metabolism. FUS also regulates small nucleolar RNAs (snoRNAs) that guide site-specific rRNA modifications, essential for rRNA processing and ribosome biogenesis. Disruption of these processes may alter ribosome composition and function, contributing to translation defects in ALS. In this study, we investigated translational disturbances and rRNA processing defects in a human cellular model of ALS-FUS. Using induced pluripotent stem cells (iPSCs) derived from ALS patient fibroblasts carrying the P525L mutation and their isogenic wild-type controls, we generated neuronal progenitor cells (NPCs) and compared translational activity and rRNA maturation. Translational efficiency was assessed with the SUnSET assay, revealing changes in global translation in mutant cells. RT-qPCR targeted precursor (45S, 47S) and mature (5.8S, 18S, 28S) rRNAs to detect imbalances in rRNA forms, indicating disrupted ribosome biogenesis. Our findings will support the hypothesis that FUS mutations impair snoRNA-guided rRNA maturation, , which in turn may affect ribosome integrity and protein synthesis in neuronal cells. This may provide further insight into the molecular mechanisms linking RNA metabolism and neurodegeneration.

P.79

Biochemical Responses of Okra to Liquina: A Novel Liquid Nano-Fertilizer

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The development of nanofertilizers offers a promising approach to enhance nutrient delivery efficiency and plant metabolic performance under sustainable agricultural practices. The present study evaluated the biochemical responses of *Abelmoschus esculentus* (okra) plants to Liquina, a novel liquid nano-fertilizer. The foliar spray of Liquina was performed on okra plants at pre- flowering stage under controlled greenhouse conditions and the antioxidant enzyme activity was measured in leaf tissues post treatment. The foliar spray of Liquina enhanced total chlorophyll content (1.12 folds), maintained the same level of catalase activity as compared to control and elevated ROS content (1.2 – 1.6 folds) suggesting stimulation of redox metabolism in okra. Notably, total MDA content decreased to 0.73-fold than control, indicating improved membrane stability and reduced oxidative damage. These findings demonstrate that Liquina modulates redox homeostasis while protecting cellular integrity, highlighting its potential as a sustainable nanofertilizer to improve plant performance under greenhouse conditions.

P.80

TRPV1 and Beyond: The Contribution of TRP Channels to T Lymphocyte Activation and Immune Modulation

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TRP channels (Transient Receptor Potential) form a large and diverse family of cation channels involved in sensing physical and chemical stimuli such as temperature, osmolarity, pH, and oxidative stress. While they are widely studied in neurons and epithelial cells, growing evidence suggests they also play regulatory roles in the immune system. TRPV1, best known as the receptor for capsaicin and noxious heat, has emerged as a candidate modulator of immune cell behavior, especially under stress-related conditions such as inflammation, elevated temperature, and acidosis.

In this study, we investigated TRPV1 expression and function in peripheral blood mononuclear cells (PBMCs), with a particular focus on T lymphocytes. We confirmed TRPV1 presence at both mRNA and protein levels in resting cells and demonstrated functional activity *via* capsaicin-induced calcium influx. Interestingly, TRPV1 expression and responsiveness decreased significantly upon T cell activation. This suggests that TRPV1 is dynamically regulated depending on the immune context.

Overall, our findings support the idea that TRP channels, particularly TRPV1, act as sensors that help immune cells adapt to environmental cues, highlighting their potential as novel targets in immunomodulatory therapies.

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P.81

Key importance of intragenic non-coding sequences in gene expression regulation process in Eukaryotes

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In a typical eukaryotic mRNA molecule, the coding sequence is surrounded by so called untranslated regions at the 5' and 3' ends, called 5'UTR and 3'UTR, respectively. Contrary to the once prevailing opinion, these non-coding regions are very important in the process of proper expression regulation. They are characterised by a complex structure, well adapted to their functions. The key role of the 5'UTR is to enable ribosome binding to the mRNA molecule and initiation of translation. The function of the 3'UTR is to regulate translation termination and post-transcriptional modifications, in particular polyadenylation. It is in this part of the transcript that interactions with microRNAs very often occur. Furthermore, contrary to the common name, ORFs can be found in both of the aforementioned regions, and their importance is increasingly being recognised. Equally important in the control of gene expression are intron sequences that enable alternative splicing and exon shuffling, as well as initiating and enhancing expression through so-called intron-mediated amplification. In summary, it can be concluded that non-translated elements are an equal component of protein-coding genes, and their importance is no less than that of the coding sequences that undergo translation.

P.82

Dynamic transcriptional remodeling of cardiac fibroblast subsets in Experimental Autoimmune Myocarditis unveiled by single-nuclei RNA sequencing

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Dilated cardiomyopathy (DCM) arises due to myocardial inflammation. The experimental autoimmune myocarditis (EAM) model serves as a CD4+ T cell-mediated animal model of acute myocarditis that subsequently progresses to post-inflammatory DCM. The aim of the study was to investigate the transcriptional remodelling of cardiac fibroblasts in a mouse model of EAM using single-nuclei RNA sequencing.
In the analysis we have included 62034 nuclei and identified 12 distinct cell populations. Differential expression analysis revealed that cardiac fibroblasts during the acute myocarditis phase showed increased expression of proinflammatory genes, TNF-alpha and interferon-related pathways. Detailed subset analysis of cardiac fibroblasts revealed a temporally dynamic emergence of transcriptionally distinct subpopulations during disease progression, characterised by divergent gene expression signatures regulating extracellular matrix organisation, inflammatory signalling, and myofibroblast differentiation. These subsets exhibited stage-specific functional specialisation. We have noticed that early-activated fibroblasts showed pro-fibrotic phenotypes *via* the expression of TGF-beta/Smad3 pathways, while late-stage subsets showed increased ECM-modifying enzyme expression, driving pathological remodelling. This study may unlock novel therapeutic approaches for inflammatory heart disease.

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P.83

Restoration of Bicarbonate Transport Underlies Clinical Benefits of Elexacaftor/Tezacaftor/Ivacaftor in F508del Cystic Fibrosis

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ETI therapy has shown clinical benefits in people with CF (pwCF) carrying F508del mutation, primarily attributed to restored Cl^- transport. The effect of CFTR rescue on HCO_3^- secretion remains unknown, limiting our understanding of the full therapeutic potential of CFTR modulators.

We aimed to define CFTR-dependent HCO_3^- transport following CFTR correction under basal and inflammatory conditions. We examined how the CFTR functional rescue relates to clinical outcomes in pwCF.

HNECs from pwCF carrying at least one F508del-CFTR allele were treated with ETI, alone or combined with TNF-α and IL-17. CFTR-mediated HCO_3^- and Cl^- transport was evaluated using short-circuit current (Isc) measurements.

ETI treatment enhanced CFTR-mediated HCO_3^- and Cl^- Isc to a similar degree. Stimulation with TNF-α and IL-17 further amplified HCO_3^- and Cl^- transport. On a per-patient basis, the HCO_3^- transport rescue correlated with improvements in FEV_1, whereas Cl^- rescue correlated with sweat chloride concentration.

ETI restores Cl^- and HCO_3^- transport at similar rates. Both CFTR-dependent HCO_3^- and Cl^- transport independently and additively influence pulmonary disease severity in CF. Incorporating HCO_3^- transport assays into clinical trials may enhance the evaluation of modulator efficacy and aid in optimizing personalized treatment strategies for CF.

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P.84

Exploring the role of transposable elements in the epigenetic regulation of nodulation in *Medicago truncatula*

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Symbiotic nitrogen fixation in legumes occurs in root nodules, which develop after mutual recognition between the host plant and colonizing symbiotic rhizobia. The nodulation process is genetically programmed and involves significant shifts in gene expression, particularly the activation of gene clusters within the so-called "symbiotic islands". Recent studies have demonstrated that in Medicago truncatula, transcriptome reorganization associated with nodule development is epigenetically regulated. DNA demethylation within symbiotic islands occurs during nodule development, whereas these same regions remain highly methylated and transcriptionally silenced in roots. Additionally, numerous transposable elements (TEs) adjacent to the activated genes undergo transient activation at the early stages of nodule development, but become hypermethylated and silenced in mature nodules. These observations led us to explore whether natural variation in TE copy number and genomic positioning could influence the epigenetic landscape and expression of symbiosis-related genes. However, due to limitations in the resolution of short-read sequencing data generated within the HapMap project, insights into structural variation across M. truncatula accessions have remained limited. Moreover, high-quality, chromosome-scale genome assemblies are currently available for only three accessions. To overcome these limitations, we generated chromosome-level assemblies for three additional geographically distinct M. truncatula accessions using a combination of PacBio and Oxford Nanopore long-read technologies. We subsequently performed de novo annotation of both genes and TEs in these new genomes. Our current focus is on characterizing the diversity of transposable elements across accessions-both genome-wide and specifically within symbiotic islands. The findings from this comparative analysis will be presented and discussed.

P.85

Assessment of Neurotoxicity and Oxidative stress in the NSC-34 Cell Line Induced by selected Glycoalkaloids from the Solanaceae Family

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One of the key systems for proper human body function is the nervous system. Disturbances in neuronal activity lead to various diseases or disorders, such as abnormal myelination or impaired interneuronal communication. For metabolically active cells like neurons, the redox status is a crucial functional parameter. Therefore, new bioactive compounds are being actively sought for treating nervous system diseases. Glycoalkaloids (GA) are plant secondary metabolites with strong biological activity. They exhibit analgesic, anti-inflammatory, and antioxidant effects, making them promising pharmacological candidates. Known GA-producing plants include potato (S. tuberosum L.), tomato (S. lycopersicum L.), and sweet pepper (C. annuum L.). GA influence various systems in animal organisms, including the nervous system. However, their cytotoxicity—linked to disruption of cell membranes—can affect both healthy and cancerous cells. The exact mechanism of GA action remains unclear. Studies using the NSC-34 cell line—a hybrid of mouse neuroblastoma and motor neurons—will allow assessment of GA neurotoxicity. Parameters such as cell morphology, cytotoxicity, and oxidative stress will be evaluated. The results may offer insight into GA mechanisms and their potential application in treating neurological disorders.

P.86

Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes and Cardiac Progenitor Cell-Based Therapy in Murine Model of Myocardial Infarction

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Cardiovascular diseases remain the leading cause of death worldwide, with ischemic heart disease frequently resulting in myocardial infarction and, ultimately, heart failure. In severe cases, heart transplantation remains the only effective option. Cell-based therapies aim to restore heart function post-infarction, but poor survival of transplanted cells limits their success. This study investigates the effect of transplantation of hiPSC-derived cardiomyocytes (hiPSCs-CM) and cardiac progenitor cells (hiPSCs-CPC) .

hiPSCs expressing luciferase (Luc) were differentiated to hiPSC-CP (10 days) and hiPSC-CMs (21-25 days) using small molecules modulating the WNT pathway. Myocardial infarction was induced by LAD ligation in NOD/SCID immunodeficient mice. Immediately after the insult $5x10^5$ hiPSC-CMs or CPCs were injected into the hearts at the border of infarct area. Heart function was monitored with VEVO ultrasound, while cell engraftment by IVIS. Animals were observed for three months.

Our findings confirm successful engraftment of hiPSC-derived cardiomyocytes (hiPSC-CMs) into cardiac tissue following myocardial infarction (MI) in a murine model. Injection of hiPSC-CPC was less efficient. Ongoing studies will evaluate improvements in heart function and the integration of transplanted cells with host myocardium.

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P.87

Ocena neurotoksyczności i stresu oksydacyjnego w linii komórkowej NSC-34 wywołanego przez wybrane glikoalkaloidy z rodziny psiankowatych

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Zastosowanie ze skutkiem dla istnienia organizmu ludzkiego jest układem chorobowym. Zaburzenia aktywności neuronalnej prowadzą do różnych chorób lub takich jak nieprawidłowa mielinizacja lub upośledzona komunikacja międzyneuronalna. W przypadku komórek metabolicznych aktywnych, takich jak neurony, również stan redoks jest parametrem funkcjonalnym. Albo też aktywnie zaawansowanych bioaktywnych w celu leczenia chorób systemowych.
Glikoalkaloidy (GA) – wybrane biologicznie wybrane metabolity roślinne – są dobrymi kandydatami. Wykazują działanie przeciwbólowe, przeciwzapalne i antyoksydacyjne, co czyni je kandydatami farmakologicznymi. Znane są podane GA na ziemniak (Solanum tuberosum L.), pomidor (S. lycopersicum L.) i paprykę słodka (Capsicum annuum L.). GA spotykane na różnych układach organizmów zwierzęcych, w tym na organizmach zwierzęcych. Ich znana cytotoksyczność, dostarczana z sieci bezprzewodowej, może być izolowana na zdrowym, jak i niszczycielskim gniazdku. Dokładny mechanizm działania GA pozostaje niejasny. W tych badaniach wykorzystania linii komórkowej NSC-34 – hybrydy mysiego nerwiaka niedojrzałego i neuronów ruchowych – po wydaniu wyłącznika o wyłączeniu neurotoksyczności GA (solaniny, chakoniny i tomatyny). Oceniono takie parametry, jak morfologia komórek, cytotoksyczność i stres oksydacyjny. Wyniki mogą dać przegląd GA i ocenę ich zastosowania w chorobach neurologicznych.

P.88

Assessment of Neurotoxicity and Oxidative stress in the NSC-34 Cell Line Induced by selected Glycoalkaloids from the Solanaceae Family

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One of the key systems for proper human body functioning is the nervous system. Disturbances in neuronal activity lead to various diseases or disorders, such as abnormal myelination or impaired interneuronal communication. For metabolically active cells like neurons, also the redox status is crucial functional parameter. Therefore, new bioactive compounds are actively sought to treat nervous system diseases.
Glycoalkaloids (GA) – highly biologically active secondary plant metabolites – are good candidates. They exhibit analgesic, anti-inflammatory, and antioxidant effects, making them promising pharmacological candidates. Known GA-producing plants include potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.), and sweet pepper (*Capsicum annuum* L.). GA influence various systems in animal organisms, including the nervous system. However, their known cytotoxicity, linked to disruption of cell membranes, can affect both healthy and cancerous cells. The exact mechanism of GA action remains unclear. In this studies usage of the NSC-34 cell line – a hybrid of mouse neuroblastoma and motor neurons – allowed assessment of chosen GA (solanine, chaconine and tomatine) neurotoxicity. Parameters such as cell morphology, cytotoxicity, and oxidative stress were evaluated. The results may offer insight into GA mechanisms and assessment of their potential application in treating neurological disorders.

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Ionizing Radiation and miRNA Biogenesis in Lymphoma: Building a Model Around miR-155

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MicroRNAs (miRNAs) are non-coding RNAs that by inhibiting gene expression may modulate the radiosensitivity of cancer cells. miRNAs are transcribed as primary miRNAs (pri-miRNAs), processed to precursor miRNAs (pre-miRNAs), transported to the cytoplasm and further processing to mature miRNAs. A variable miRNA/pri-miRNA ratio may indicate regulated miRNA processing. In this project, we aim to determine how ionizing radiation (IR) influences miRNA biogenesis in Burkitt lymphoma (BL) cells. Using RNA-seq and small RNA-seq, validated by qRT-PCR at multiple time points, we identified IR-responsive miRNAs in BL that included miR-146a, miR-449a, and miR-155. Pri-miR-155 and pri-miR-146a increased within 1–4 hours post-IR, preceding mature miRNA accumulation. We also identified pri-miRNAs not processed to mature miRNAs in any of the 3 BL cell lines and miRNAs with altered miRNA/pri-miRNA ratios upon IR. Next, we focused on miR-155, an oncomiR in lymphoma transcribed from the MIR155HG gene that also produces lncRNA-155, and the micropeptide miPEP155. To study their IR-induced regulation, we developed a mathematical model of biogenesis from MIR155HG, incorporating levels of unspliced and spliced transcripts (lncRNA-155), miR-155, and miPEP155. In conclusion, IR affected the biogenesis miRNAs in BL cells, including miR-155. We made a preliminary model of biogenesis from MIR155HG upon IR.

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P.90

Chromosomics, genomics and epigenomics of male infertility

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Infertility a□ ects approximately 15% of couples of reproductive age who are unable to conceive within 1 year of regular unprotected sexual intercourse (WHO, 2020). About 7% of males and 12% of females are a□ ected, while the male factor is responsible for 20-30% of all infertility cases worldwide. There are multiple reasons for male infertility, including: genetic factors, chromosomal aberrations, epigenetic mutations, hormonal abnormalities, infections, or reproductive tract abnormalities.
Genetic factors determine ~10-15% of revealed infertility cases, and include a network of ~2000 genes (with approx. 950 causative variants in 250 genes already documented). Chromosomal aberrations frequency in infertile males reaches values several times higher when compared to the whole population (mean: 3.5%, up to 20% in azoospermia). However, still approximately 25% of infertile males reveal unexplained background of the disease. In this group the more and more data are releasing last years and are focusing on the epigenomic changes in spermatogenesis and human sperm cells (DNA, post-translational modifications of histones, non-coding RNAs), followed by disturbances in parental imprinting, as the direct reasons of observed lack of progeny or reproductive failures.
The compilation of main data concerning those three genomic branches will be presented in the context of male infertility.

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P.91

Evaluation of three mitochondrial DNA markers for species identification, genetic diversity assessment, and phylogenetic positioning of five *Hyalomma* tick species from Tunisia

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Hyalomma ticks are key vectors of pathogens impacting human and animal health. This study evaluated three molecular markers—16S, 12S rRNA, and COI—for their efficiency in species identification, genetic diversity assessment, and phylogenetic analysis of *Hyalomma* ticks in Tunisia. Twenty specimens were collected from cattle, camels, and turtles across nine governorates. Morphological identification confirmed five species: *H. scupense*, *H. marginatum*, *H. excavatum*, *H. aegyptium*, and *H. dromedarii*. Molecular analyses supported morphological identification and revealed variable levels of intraspecific diversity. *H. scupense* and *H. aegyptium* showed low diversity, with no variable sites in 16S and 12S rRNA, and only one in *H. aegyptium*'s COI. *H. marginatum* exhibited moderate diversity with four COI variable sites. Higher diversity was detected in *H. excavatum* and *H. dromedarii* across all three markers. Phylogenetic analyses showed that COI offered the highest discriminatory power – except for *H. scupense*—and clearly resolved genetic clusters. These results highlight the value of multi-marker approaches for tick species confirmation and genetic diversity studies. COI, in particular, is a reliable tool for understanding the evolutionary and epidemiological roles of *Hyalomma* ticks, supporting better strategies for tick-borne disease control.

P.92

From genes to personality: variants of serotonin transporter gene in women with alcohol use disorder

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The genetic basis associated with the predisposition to excessive alcohol consumption has been comprehensively discussed in the literature for many years, especially with context of dysregulation of the serotonin system. Recent studies have suggested the impact of genotype differentiation of serotonin gene transporter polymorphism (5-HTTLPR) in the mechanisms of alcohol addiction. We aimed to estimate the influence of 5-HTTLPR on the occurrence of AUD in a group of women and the interaction analysis of different personality traits, 5-HTTLPR and alcohol dependence. In this study, 213 female volunteers (including ones with AUD=101) were analysed using psychometrics tests, while 5-HTTLPR was genotyped by PCR. Based on the performed analyses, we observed significant differences in the distribution of genotypes ($p=0.0230$) and alleles ($p=0.0046$) between the group of addicted and healthy women. We identified significant differences in the prevalence of personality traits between AUD or lack of it including the STAI-T ($p=0.0002$), STAI-S ($p<0.0001$), Neuroticism ($p<0.0001$) and Extraversion ($p<0.0001$). We found significant interaction of 5-HTTLPR and personal traits on the Neuroticism scale in relation to the occurrence of AUD ($p<0.0001$). Further investigation is needed to elucidate the nature of this interaction. The obtained results highlight the role of gene-environment interactions in shaping AUD susceptibility and related predisposition to exhibit traits associated with depressive symptoms such as Neuroticism.

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Structure and dynamics of *S*-adenosyl-L-homocysteine hydrolase from *Pseudomonas aeruginosa*

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Protein dynamics is of key importance to protein function, influencing enzymes' structural integrity and catalytic efficiency. *S*-adenosyl-L-homocysteine hydrolase (SAHase), an essential regulator of cellular methylations, is a striking example of such a phenomenon. The enzyme forms a homotetramer, with each subunit folded into three domains. Substrate- and cofactor-binding domains oscillate during a catalytic cycle between two conformational states: closed (substrate-bound) and open (with a product released). However, the role of regions of the substrate-binding pocket in regulating SAHase dynamics has yet to be fully explained. Moreover, a mode of conformational changes of subunits within the tetramer during the turnover is elusive.

To understand in a more detailed manner the structure and dynamics of SAHases, we performed crystallographic and Cryo-EM Single-Particle Analysis (SPA) of bacterial SAHase. Our results reveal the high complexity of conformational changes of the enzyme during turnover and the role of dynamics in SAHase activity. We demonstrated the importance of amino acid residues in two poles of the substrate-binding pocket, which are unrelated to the catalytic reaction. The flexibility of SAHase allowed us to analyze the enzyme's dynamics using Cryo-EM SPA. The results indicate that within the tetramer, only two subunits can bind a substrate simultaneously.

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P.94

Biopsy proteome-based classification of T cell-mediated kidney allograft rejection

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T cell-mediated rejection (TCMR) remains a challenge in kidney transplantation. Patients are classified as without rejection (NR), with borderline (BR), or acute rejection (AR) corresponding to Banff categories I, III, and IV, respectively. However, classification of borderline cases is uncertain, as some patients may or may not require intervention. Thus, a robust method of TCMR's classification is still needed, which could be achieved *via* mass spectrometry (MS) based analysis of biopsy specimens.

This study aimed to identify proteomic signatures of BR and AR using kidney allograft biopsies and to validate selected biomarkers with immunohistochemistry (IHC).

Of the 2547 proteins, GNB4 and AGXT emerged as significantly differentiating the groups in a quantitative manner, with the highest fold change. Using a binary approach, PDK1 and CD73 delineated the groups best. Based on IHC, only an upregulation of GNB4 in immune cells and PDK1 in macrophages could be observed without changes in the tubular epithelium.

Thus, GNB4 and PDK1 may be of interest for further studies. If these results could be confirmed using a larger cohort of patients, expression analysis of these proteins could be incorporated into histopathological examination in the context of kidney transplant rejection.

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P.95

Biochemical analysis of polysaccharides from calcium carbonate biominerals

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Calcium carbonate is an inorganic salt that serves as a primary structural component of mollusc shells and otoliths in the inner ear of fish. The organic matrix includes glycosaminoglycans (GAGs), although their role in biomineralization remains unclear. The mechanisms of biomineralization are complex, involving tight regulation of calcium carbonate crystal growth and organization. Examples include calcite and aragonite in mollusc shells, nacre, and corals. Understanding the mechanisms underlying calcium carbonate biomineral formation could lead to novel strategies for designing advanced materials and approaches in tissue regeneration. In this study, we investigate the presence of GAGs in common carp otoliths and examine the effect of the organic matrix on calcium carbonate crystal formation. Biomineralization research integrates knowledge from biochemistry, physics, and materials science, offering an interdisciplinary framework for understanding and harnessing nature's strategy for constructing complex mineral structures. A deeper insight into these processes may contribute to the development of methods for regenerating human otoconia. Our next goal is to elucidate how interactions between GAGs and ototoxic drugs influence biomineral degradation.

P.96

The effect of pollution on the electrophysiology of epithelium – insights from Caco-2 cell model

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Nowadays, the topic of environmental pollution and its impact on human health has become critically important. Plastic has been identified as one of the most widespread pollutants, with its presence documented in various ecosystems. The breakdown of plastic litter produces micro- and nanoparticles that are ingested by living organisms and interact with their intestinal barrier. Our knowledge of their effects on the electrophysiology of epithelial tissues remains limited.

The presented research concerns the observed enhanced mucus secretion by Caco-2 cells in response to polystyrene nanoplastic. Ussing chamber studies revealed that the plastic particles alter ion transport across epithelial cell monolayers by decreasing CFTR channel activity, but increasing the activity of CaCC channels, particularly TMEM16A responsible for mucus secretion. The involvement of TMEM16A channel in the observed mechanism was confirmed using its specific agonists and a calcium indicator. Furthermore, this work evaluates the potential cytotoxicity of the nanoplastic as well as its effects on transepithelial electrical resistance.

This research validates that elevated TMEM16A channel activity is responsible for the observed increased mucus secretion, acting as a newly discovered defense strategy of Caco-2 cells against polystyrene nanoplastic.

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P.97

The Role of mitoBK$_{Ca}$ Channels in Epithelial Cell Physiology and Damage.

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Recently, it has been shown that potassium channels in the inner mitochondrial membrane (mitoK) play a role in cytoprotection. Therefore, protecting epithelial cells from damage induced by particulate matter (PM) may be linked to the activation of potassium channels in the mitochondria.
To verify the role of the mitochondrial large-conductance Ca^{2} -regulated potassium (mitoBK$_{Ca}$) channel in cytoprotection in response to stress induced by PM, we conducted a series of experiments using patch-clamp techniques, transepithelial electrical resistance assessments, mitochondrial respiration measurements, fluorescence methods to assess ROS levels and mitochondrial membrane potential, and cell *via*bility assays using trypan blue staining. In the human bronchial epithelial cell damage model (16HBE14o- wt), particulate matter with a diameter of 4 μm (PM4.0) was used.
A better understanding of the relationship between mitochondrial metabolism and cell pathophysiology could aid in the development of effective cytoprotection strategies. By utilizing naturally derived mitoBK$_{Ca}$ channel activators, we may be able to support and enhance these protective mechanisms to counteract the consequences of PM-induced damage.

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P.98

Evolutionary Conservation of Short Linear Motifs in OFD1: Implications for Discovery of Novel OFD1 Interactors and Functions

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OFD1 is a protein involved in many cellular processes, including cilia biogenesis, mitotic spindle assembly, translation, autophagy and the repair of double-strand DNA-breaks. Despite many potential interactors identified in high-throughput studies, only a few have been directly confirmed with their binding sites identified. We performed an *in silico* analysis of the evolutionary conservation of the OFD1 sequence in three clades: 80 Tetrapoda, 144 Vertebrata or 26 Animalia species, and identified 59 short linear protein-binding motifs localized in the OFD1 regions conserved in various clades. Our results indicate that OFD1 contains 14 potential post-translational modification (PTM) sites targeted by at least eight protein kinases, seven motifs bound by proteins recognizing phosphorylated aa residues and a binding site for phosphatase 2A. Moreover, OFD1 harbors both a motif that enables its phosphorylation by mitogen-activated protein kinases (MAPKs) and a specific docking site for these proteins. Generally, our results suggest that OFD1 forms a scaffold for interaction-with many proteins and is tightly regulated by PTMs and ligands. Currently, OFD1 protein partners are identified *via* OFD1 co-immunoprecipitation and mass spectrometry in cell lines, to compare with *in silico* data. Future research on OFD1 should focus on the regulation of OFD1 function and localization.

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P.99

Impact of sperm fractionation on chromosome positioning, chromatin integrity and DNA methylation level in normozoospermic men and reciprocal chromosomal translocations carriers

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Spatial organization of sperm chromosomes plays a key role in the regulation of early embryo development *via* the first contact with ooplasm of the chromosomal regions crucial at the first stages of embryo development.
The aim of this study was to determine whether selection of spermatozoa with good motility and/or morphology, is related to specific positioning of chromosomes, supported by assessment of chromatin quality.
Semen samples from 5 normozoospermic males and 2 reciprocal chromosome translocation carriers were fractionated *via* swim up (motile spermatozoa) and Percoll gradient (90/47%; good motility and morphology). Sperm chromatin integrity was assessed by aniline blue or acridine orange staining or TUNEL assay. FISH was used to analyse the positioning of chromosomes 4/7/8/9/18/X/Y. To check global levels of 5mC/5hmC of sperm DNA, immunofluorescence staining was carried out.
In normozoospermic controls samples, high-quality sperm selection increased chromatin protamination (+24-26%) and 5mC and 5hmC levels (+7-12%), and reduced ssDNA fragmentation (-60-70%). High-quality spermatozoa showed distinct chromosome repositioning, with sex chromosomes shifted to the nuclear periphery, region of the initial interaction with the ooplasm during fertilization. These findings show potential significance for selecting high-quality spermatozoa in assisted reproductive technologies at chromatin and chromosomal level.

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P.100

Effect of Hypoxia on TRPM2 Expression in T Lymphocytes

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The immune system maintains homeostasis, with T lymphocytes orchestrating adaptive responses *via* cytokine release and cytotoxicity. T cell activation is critical for combating infections and tumors. However, in the tumor microenvironment, factors such as hypoxia and oxidative stress impair T cell function. TRPM2 (Transient Receptor Potential Melastatin 2) is a redox-sensitive, non-selective cation channel activated by ADP-ribose, ROS, TNF-α, and Concanavalin A, and is involved in T cell activation, proliferation, and calcium signaling. Its role under hypoxic conditiones remains unclear. This study assessed TRPM2 gene expression in peripheral blood lymphocytes (PBLs) cultured under hypoxia (1% O_2) and chemically induced hypoxia ($CoCl_2$), following CD3/CD28 stimulation. TRPM2, CD25, and CD69 expression was analyzed by qRT-PCR. Results showed increased TRPM2 expression in activated PBLs under both hypoxic conditions, suggesting its involvement in adaptation to low oxygen. CD25 and CD69 confirmed T cell stimulation but were downregulated under hypoxia, particularly chemical hypoxia, indicating impaired activation. TRPM2 may contribute to T cell adaptation in hypoxic stress and represents a potential target in modulating immune responses in hostile environments.

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P.101

Exploring the mysteries surrounding the formation of RCNMV virus-like particles: structural remodelling for delivery applications

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Understanding the rules governing the self-assembly of virus-like particles (VLPs) is critical for unlocking new applications in biotechnology and medicine. In our research, we employ recombinant VLPs derived from the red clover necrotic mosaic virus (RCNMV), which serve as a novel model system for studying spherical VLP formation and analyzing the interactions between capsid proteins and various types of cargo. Like the well-characterized bromoviral and phage models, RCNMV has an icosahedral T=3 symmetry. However, RCNMV exhibits distinct features, including slightly larger dimensions, a relatively short N-terminus involved in RNA genome interactions, and unique genomic RNA structure.

Using techniques such as dynamic and static light scattering, mass photometry, and cryo-electron microscopy, we investigate the properties of these assembled VLPs. Specifically, we examine how factors such as ion presence, protein concentration, buffer composition, and different types of cargo influence the VLP assembly process, including the formation of intermediates and overall assembly efficiency. Furthermore, we evaluate the potential of RCNMV VLPs for RNA delivery to HEK293 cells, assessing their applicability in therapeutic contexts.

Our findings provide valuable insights into the mechanisms of virion self-assembly and supramolecular structure formation, highlighting that RCNMV-derived VLPs assemble through distinct intermediate forms. These results pave the way for further exploration of VLPs in various biotechnological and medical applications.

P.102

miR-181 impact at transcriptome and proteome of differentiating mouse ESCs

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MicroRNAs (miRNAs, miRs) are short, non□ coding RNA molecules involved in the post-transcriptional regulation of gene expression. They play a key role in a variety of physiological processes, such as directing cell fate, regulating proliferation, differentiation, and apoptosis. We use transient overexpression of miR181 as a tool for targeted differentiation of mouse embryonic stem cells (ESCs). Mouse ESCs were transfected with miR181 mimics and then induced to differentiate. The effect of miR181 overexpression at gene and protein levels in differentiating ESCs was analyzed using qPCR, microarray, and proteome techniques. As a result we show that among the processes significantly changed as a result of miR181 overexpression were those connected to neuro- and myogenesis.

P.103

The transmembrane domain of DGKε as a regulator of its activity

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Diacylglycerol kinase-ε (DGKε) is a unique member of the DGK family that specifically phosphorylates a particular species of DAG, namely SAG (18:0/20:4 DAG), to phosphatidic acid (PA) as a part of the phosphatidylinositol (PI) cycle. DGKε is also involved in general lipid metabolism and its activity is linked with Huntington's disease, seizure susceptibility, obesity, and some cancers. Moreover, mutations in the *DGKE* gene lead to a rare hereditary kidney disease called atypical hemolytic uremic syndrome (aHUS). Recently, our group showed that DGKε is a key factor determining the sensitivity of macrophages to bacterial lipopolysaccharide (LPS).
Despite its physiological importance, the regulation of DGKε activity remains poorly understood. DGKε is the only DGK isoform that lacks known regulatory domains and contains a conserved transmembrane α-helix.
We found that m/hDGKε is *S*-palmitoylated at Cys38/40 located at the cytoplasmic end of its N-terminal transmembrane fragment and that Cys38Ala substitution increased mDGKε activity, suggesting an inhibitory effect of *S*-palmitoylation in wild type DGKε. In contrast to Cys38, the mutation of neighboring Pro31 reduced the activity of mDGKε due to hyperpalmitoylation but also enhanced DGKε degradation, indicating that Pro31Ala substitution has a global effect on DGKε folding and stability.
Taken together, our data support the hypothesis that the transmembrane domain of DGKε plays a regulatory role in controlling its enzymatic activity.

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Microbiological evaluation of bacterial strains isolated from selected municipal bathing facilities in Szczecin

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Open bathing areas are exposed to opportunistic pathogenic bacteria such as *P. aeruginosa*, posing a threat to human and animal health. However, their pigment production and enzymatic activity also offer biotechnological potential. Detecting them in public swimming facilities presents both health risks and innovation opportunities.
The study aimed to isolate and analyze bacterial strains from the "Arkonka" and "Dziewoklicz" municipal bathing areas in Szczecin.
A total of 623 samples (water, soil, and swabs) were collected. Bacteria with characteristics of *Pseudomonas spp.* were selected using solid media and membrane filtration. Their morphology, pigment production, biochemical traits, and antibiotic resistance were analyzed.
Bacteria were found in significant numbers in the artificial bathing area, including the pools, faucets, sewage grates, and the walls of the expansion tanks. In the natural bathing area, most bacteria were found in samples from around the beach and recreational areas. The presence of *P. aeruginosa* was confirmed in 19 samples.
The analysis showed that natural bathing areas more often accumulate opportunistic pathogens, however, bacteria such as *Aeromonas*, *Burkholderia*, and other *Pseudomonas* species are commonly found in artificial swimming pools and their surroundings. Safety measures should include water testing and mandatory disinfection of technical systems (tanks, drainage, pumps).

P.105

Amino acid substitutions as the molecular basis for primary ciliary dyskinesia (PCD)

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Primary ciliary dyskinesia (PCD) is a genetically heterogeneous ciliopathy caused by hereditary dysfunction of motile cilia. PCD is caused by defects in proteins essential for cilia functioning or cilia assembly. The majority of deleterious variants identified in PCD patients truncate the proteins, while amino acid (aa) substitutions are less frequent, and their effects on cilia proteins are not always clear.
Dynein axonemal intermediate chain 1 (*DNAI1*) is one of the eight most commonly involved genes in Polish PCD patients. It encodes an important component of the outer dynein arms (ODAs), large multiprotein complexes that drive cilia movement. DNAI1 is one of two very first proteins that enter ODA pre-assembly complex in the cytoplasm. Unlike in other PCD genes, in *DNAI1*, PCD-causing aa substitutions prevail over protein-truncating mutations; interestingly, they accumulate in a WD-40 region (WDR) of the protein.
Here, we aim to analyze the impact of pathogenic aa substitutions on DNAI1 protein structure, stability and interactions. This study uses a combination of in silico predictions, as well as physical and biological methods. The knowledge gained during the project will help to better understand the impact of aa substitutions on the WDR stability, protein-protein interactions, as well as the process of ciliary dynein arms assembly. It will also be the proof-of-concept of an exemplary pipeline aiming to confirm pathogenicity of aa substitutions in other PCD-causing genes.

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P.106

Impact of CacyBP/SIP knockout on diverse cellular processes

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CacyBP/SIP is a protein involved in various cellular functions. In this study, CacyBP/SIP-knockout (KO) HEK293 cells were generated using the CRISPR/Cas9 system. Three clones (5, 9, and 44) with no detectable CacyBP/SIP expression were selected and then used to investigate the influence of CacyBP/SIP on certain cellular processes. At first, the cell proliferation rate was assessed by performing a population doubling assay. The results of this assay showed a slower proliferation rate for CacyBP/SIP-KO cells than for control ones. Then, cell cycle in CacyBP/SIP-KO and control cells, using FACS, was analyzed. An altered distributions of the G1 and S phases for CacyBP/SIP-KO cells were found, which pointed to a potential role of CacyBP/SIP in regulation of cell cycle progression. Following this, MTT assay for CacyBP/SIP-KO and control cells after exposure to H_2O_2 was performed. The results of this assay showed an increased sensitivity to oxidative stress of CacyBP/SIP-KO cells. By applying another method, the wound healing assay, it was found that CacyBP/SIP-KO cells showed impaired migration. Moreover, immunofluorescence staining intensity of vimentin, a marker of cell migration, was weaker and showed a less organized filamentous network in CacyBP/SIP-KO cells than in control ones. In conclusion, our findings indicate a potential role of CacyBP/SIP in certain cellular processes such as cell proliferation, cell cycle, response to stress or cell migration.

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Peripheral Biomarkers as Predictors of Alzheimer's Disease: The Liver-Brain Axis

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Alzheimer's disease (AD) is an aging-related, irreversible neurodegenerative disorder, occurring as a rare familial form (FAD) linked to gene mutations and a more common sporadic form (SAD), mainly influenced by environmental factors. This study investigates the liver-brain axis in amyloid-driven AD development by examining how the Western diet (WD), rich in sugars, saturated fats, and processed food, affects peripheral metabolism and brain amyloid-beta (Aβ) accumulation. Tg2576 (FAD model) and C57BL/6 (SAD model) mice were fed WD or standard diet. WD induced metabolic syndrome, elevated liver enzymes, and non-alcoholic fatty liver disease (NAFLD/NASH). Hepatic fat accumulation, immune infiltration, and liver cell damage were observed. Liver dysfunction impaired Aβ clearance, leading to its accumulation in liver and brain. WD accelerated brain Aβ buildup in Tg2576 and triggered age-related Aβ deposition in C57BL/6. These findings show that peripheral metabolic dysfunction, especially liver injury, may initiate and exacerbate AD pathology. Peripheral blood biomarkers may serve as early, non-invasive indicators of AD risk and progression.

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P.108

CD14 endocytosis and release are differently regulated by POVPC and LPS in mouse macrophages

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CD14, a co-receptor of Toll-like receptor 4 (TLR4), is one of the key proteins involved in the pro-inflammatory response of immune cells to bacterial lipopolysaccharide (LPS) and oxidized phosphatidylcholine species, e.g., POVPC. Recent studies indicated that both POVPC and LPS reduced the CD14 level at the plasma membrane of macrophages promoting its endocytosis. Using a biotin-based assay, we observed that LPS strongly enhanced TLR4 endocytosis but not that of CD14, while POVPC surprisingly reduced CD14 internalization. This finding prompted us to investigate alternative routes of CD14 removal from the plasma membrane. Analysis of an extracellular vesicles (EVs) fraction in supernatants from POVPC- and LPS-stimulated cells indicated that both facilitated the release of CD14 in EVs. In addition, a significant amount of CD14 was found in EVs-depleted supernatants in LPS-stimulated samples, suggesting that LPS also induces shedding of CD14 from the plasma membrane. Indeed, using a biotin-based assay, we proved that LPS induced CD14 shedding and that this process was TLR4-dependent. In contrast, POVPC triggered only a slight increase in CD14 shedding, independently of TLR4. Collectively, our results demonstrate that CD14 surface level is regulated not only by endocytosis, but also by EVs release and shedding, with these routes being differentially modulated by LPS and POVPC. These findings provide new insight into the complexity of CD14 trafficking during macrophage activation.

P.109

New Insights into V-ATPase Dysfunction: Characterization of a Novel Mutation in Patient Human Fibroblasts

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The V-ATPase, also known as H□-ATPase, is a multi-subunit enzyme with proton pump activity, responsible for maintaining cellular pH homeostasis and providing an acidic environment within organelles such as lysosomes. Proper pH regulation is crucial for metabolic reactions and cellular processes, including autophagy and protein trafficking. Mutations in the *ATP6V0A1* gene, which encodes a V-ATPase subunit enriched in the brain, are rare and linked to neurological dysfunctions. The prior described mutation (Arg740Gln) disrupts lysosomal acidification, leading to autophagic impairment. This study reports a previously undisclosed mutation in the *ATP6V0A1* gene identified in a new patient cohort. The affected individuals exhibit neurodegenerative phenotypes, including epilepsy, early-onset dementia, speech delay, and muscle symptoms. We observed reduced lysosomal and mitochondrial function in patient-derived fibroblasts. Furthermore, we optimized a protocol for directly converting fibroblasts to neurons to better characterize the neuronal dysfunction caused by the mutation in the *ATP6V0A1* gene. Our findings expand the knowledge about the spectrum of V-ATPase-related disorders and highlight the critical importance of lysosomal acidification in neurodegeneration, suggesting potential targets for future treatments in these conditions.

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Modulation of Autophagy and EMT by Fisetin and Chloroquine in Pancreatic Cancer Cells

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Pancreatic ductal adenocarcinoma (PDAC) remains among the deadliest malignancies, necessitating exploration of combined therapeutic strategies. This study examines the impact of fisetin, a dietary flavonoid with pro-autophagic and anti-proliferative properties, and chloroquine, a known autophagy inhibitor, on the HS766T pancreatic cancer cell line.

Neither fisetin nor chloroquine exhibited cytotoxicity when administered individually across a concentration range, as confirmed by LDH assays. However, combined treatment at the highest tested concentrations produced a statistically significant reduction in cell proliferation, as determined by MTS assay ($p < 0.05$), suggesting a synergistic inhibitory effect on cellular growth.

Western blot analyses revealed altered expression levels of key autophagy-related proteins (e.g., LC3B-II, p62) and epithelial-mesenchymal transition (EMT) markers (e.g., E-cadherin, N-cadherin, vimentin), indicating that the combination treatment modulates both autophagic flux and epithelial-mesenchymal transition pathways in HS766T cells.

These findings support the hypothesis that dual modulation of autophagy and EMT – *via* fisetin-induced autophagic signalling and chloroquine-mediated blockade – may offer a promising avenue for impairing pancreatic cancer progression. The HS766T cell response highlights the importance of cellular context in autophagy-targeted therapeutic strategies.

P.111

Seasonal (spring vs autumn) analysis of the fine roots metabolome of adult *Pinus sylvestris* trees from a common garden experiment in search of differences caused by their origin

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Changing climate boundaries may exceed the adaptive capacity of plant species because climate change is occurring too rapidly. Understanding the adaptive potential of native long-lived species, particularly their roots – which is still not well understood – is crucial since these species influence the functioning of entire ecosystems. As climate change intensifies, it could lead to the collapse of tree species. This study presents a GC MSMS analysis of fine roots from two mature *Pinus sylvestris* variants – one from Kórnik (KO) and the other from Pieninski Park Narodowy (PPN). These variants were chosen from a large common garden experiment established in 1967, featuring provenances from different climate conditions.

Root metabolomes showed more variation between provenances in spring but became more similar in autumn. Despite the trees' advanced age, PPN from a cooler, wetter location exhibited some changes in root metabolomes, which likely indicate climate-related differences in how reserves are stored and remobilized, even though molecular data point to successful adaptation to the local climate. The observed shifts in fine root metabolomes, particularly in lipids and C- and N-compound levels in the common garden experiment, probably reflect differences in the timing of metabolic activity driven by the adaptive effects of climate variables associated with PPN seed origin.

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Exploring *Streptomyces* – a focus on bioactive compounds to combat drug-resistant pathogens

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Antibiotic resistance is one of the greatest threats to public health. A rapid growth of the number of microorganisms that, despite the administration of drugs, still develop in patients causing life-threatening illnesses, has been observed. This global problem is triggered mostly by the decline in the number of new antibiotics, their inappropriate and excessive use, and the spread of resistance mechanisms. Accordingly, there is still a high demand for compounds with antimicrobial activity.

The study aimed at finding new bioactive substances produced by selected *Streptomyces sp*. Although these bacteria are well-known antibiotic producers, estimates indicate that only up to 3% of these compounds have been identified. Therefore, we checked the activity of substances secreted by *Streptomyces*. Inhibition of the growth of selected pathogens was determined due to antagonism tests. Then, substances produced by selected *Streptomyces sp.* were extracted, and pathogens' sensitivity to these substances was checked using a disc-diffusion method.

All analysed *Streptomyces* secreted antimicrobial compounds. The strains were categorized into five groups due to the spectrum of activity of substances produced. Presented findings suggest that analysed *Streptomyces* secrete different compounds and the examination of extracts confirmed the presence of active substances. Further research on these compounds might contribute to the development of new drugs or other antimicrobial formulations.

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Cytotoxic analysis of cytokinin riboside derivatives on cancer cell lines

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Numerous furanyl- or thienyl-substituted nucleobases, nucleosides, and their analogues have been shown to exhibit promising or at least interesting biological properties. On the other hand, naturally occurring N^6-modified adenosine derivatives (cytokinin ribosides) and their synthesized analogues are an essential class of compounds with a broad spectrum of activities, including antiviral, antiprotozoal, and anticancer. Reports on the cytotoxicity of cytokinin ribosides against various cancer cells have been appearing since the 1960s, continuing throughout the following decades.

In our study, we evaluated the cytotoxicity of cytokinin riboside - N^6-benzyladenosine (BA) and its seven structural analogues, modified by substitution in the phenyl ring and/or the introduction of a nitrogen atom into position 8 of the adenine moiety. BA and its synthesized analogues were examined for their ability to inhibit the growth of a panel of seven cancer (MeWo, MIA PaCa-2, Caco-2, U118MG, HeLaWT, MCF7, and OVCAR-3) and a nontumorigenic cell line (MRC-5). The cytotoxic effect of the studied compounds was assessed after 24, 48, and 72 h of treatment within the concentration range of 0.5–100 μM using the MTT assay. The IC50 values were estimated based on data on cell *via*bility.

The effect of the studied N^6-benzyladenosine analogs depends on the type of chemical modification, concentration, and characteristics of the target cancer cells.

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Biological activity of 8-azakinetin riboside and 8-azaadenosine in cervical cancer and breast cancer cells *in vitro*

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Naturally occurring N^6-modified adenosines (cytokinin ribosides) and their synthesized analogues are a class of compounds endowed with a variety of activities, including antiviral, antiprotozoal, and also anticancer. Reports on the cytotoxicity of cytokinin ribosides, namely N^6-isopentenyladenosine, kinetin riboside, and N^6-benzyladenosine, against various cancer cells, as well as the results of *in vivo* studies, have been appearing since the 1960s throughout the following decades. However, the research into their more cytotoxic and selective analogs is still ongoing.

The cytotoxic effect of the 8-azakinetin riboside (8-azaKR), 8-azaadenosine (8-azaA), and kinetin riboside (KR) was assessed in a panel of seven cancer and nontumorigenic cell lines after 24, 48, and 72 hours of treatment within the concentration range of 0.5–100 μM using the MTT assay. Moreover, cell cycle and apoptosis analyses of the studied compounds, wound healing assays, and immunoidentification analyses of proteins associated with these processes were performed after treating MCF7 and HeLaWT cells with the compounds for 24 or 48 hours.

Our studies revealed the higher effectiveness of 8-azaadenosine on the cell cycle and its influence on the migration process of the MCF7 and HeLaWT cell lines. This demonstrates that the biological activity of N^6-benzyladenosine analogs depends on the type of chemical modification.

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Diacylglycerol kinase-ε affects the LPS-induced proinflammatory responses of macrophages by governing the formation of GPI-anchored CD14

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Diacylglycerol kinase-ε (DGKε) specifically phosphorylates stearic/arachidonic acid-containing diacylglycerol (SAG), generating phosphatidic acid used for the synthesis of phosphatidylinositol (PI). PI and its derivatives regulate numerous cellular processes, including signaling of plasma-membrane receptors. The latter include Toll-like receptor 4 (TLR4) and its co-receptor CD14, which are activated in macrophages by bacterial lipopolysaccharide (LPS). To investigate the role of DGKε in LPS-induced pro-inflammatory responses, DGKε-depleted Raw264 cells were created and subsequently rescued with DGKε-Myc. Depletion of DGKε inhibited LPS-induced signaling, which was restored upon reintroduction of DGKε-Myc. In particular, DGKε depletion abolished the TLR4 signaling pathway that requires CD14-mediated internalization of TLR4 and the engagement of the TRIF adaptor protein in endosomes, as indicated by the absence of IRF3 phosphorylation and downstream production of cytokines. On the other hand, the MyD88-dependent signaling, in which CD14 participation can be dispensable, was only partially impaired. Notably, DGKε-deficient cells lacked mature, GPI-anchored CD14 on the surface, a defect reversed by DGKε reintroduction. Taken together, the data indicate that DGKε controls the generation of the PI pool required for synthesis of the GPI anchor of CD14. Thus, DGKε is a critical determinant of macrophage sensitivity to LPS, acting through regulation of the cell-surface level of CD14.

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BY2 suspension adapted to osmotic stress for over 19 years: a highlight of the molecular mechanisms of adaptation

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Osmotic stress will be most probably a key negative factor influencing functioning of living organisms in incoming decades and plants, which cannot change their habitats will be forced to live in permanently harsh environments. Unfortunately, the molecular adaptive mechanisms which allow plant generations live years in continuously adverse conditions and adapting to them are not fully understood. In this study, suspension cells derived from the roots of *Nicotiana tabacum* (cultivar Bright Yellow-2) were analyzed. Over time, the cells were gradually adapted to increasing salt concentrations and, since 2006, have been maintained in a highly saline environment (190 mM NaCl). The main objective of this work was to investigate the biochemical status of BY-2 suspension cultures in order to identify molecular mechanisms responsible for the adaptation of plant cells to adverse environmental conditions. It was hypothesized that the adaptation process enhances the stability of key metabolic pathways, ultimately leading to the establishment of a new homeostatic state that enables continued cellular function in a changed environment. To test this hypothesis, high-throughput protein and metabolite analyses were conducted, focusing on osmoprotectants, oxidative stress response molecules, intermediate products of major molecular pathways, and proteins involved in protein turnover process.

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Increasing the efficiency of precise editing of the human beta globin gene by CRISPR-Cas9 tethered with ssODN donor template

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The CRISPR/Cas9 system is one of the most applicable genome editing methods, which provides an effective tool for precise editing of desired genes to knock out or repair non-desired mutations. However, the efficiency and specificity of this system still need to be improved. To reach this goal, there are some improvement strategies, including the use of NHEJ inhibitors or the modification of Cas9 enzymes. One of these strategies suggests covalent tethering of single-stranded DNA donor template (ssODN) to Cas9 through the DNA binding domain, which recognises and tethers a specific nucleotide sequence. Adding NLS motives to Cas9 directly translocates the sgRNA/Cas9/ssODN complex into the nucleus. In our work, we used porcine circovirus 2 (PCV) DNA binding domain / Cas9 fusion protein to test three sets of guide/ssODN for human beta globin sequence editing in K562 cells. HDR efficiency for these donor templates was 48.5%, 20% and 14% respectively. The HDR/INDEL ratios were 1.5, 0.5, and 0.3, respectively. Despite not a very significant difference in HDR efficiency between covalently tethered (48.5%) and untethered (44,7%) ssODN, we observed a significant decrease in INDEL with tethered ssODN (32,5%) compared to untethered (40%), which suggests improved editing precision. Therefore, the covalent tethering of single-stranded DNA donor templates to sgRNA/Cas9 RNP complex has the potential to develop more effective strategies for precise genome editing.

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Tissue-specific localization of odorant-binding protein 7 in *Galleria mellonella* larvae following *Pseudomonas entomophila* infection *via* different routes

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Odorant-binding proteins (OBPs), traditionally known for their role in insect olfaction, are increasingly recognized as being involved in processes beyond chemosensation, including immune responses. In this study, we examined the tissue-specific localization and abundance of the OBP7 protein in *Galleria mellonella* (*Gm*) larvae following infection with the entomopathogenic bacterium *Pseudomonas entomophila* (*Pe*), with particular emphasis on how the route of infection influences OBP7 distribution. Larvae were infected either by direct injection into the hemocoel or *via* forced feeding, enabling comparison of systemic versus oral infection routes. Using immunohistochemistry with OBP7-specific antibodies, we observed differential localization of the protein in selected tissues, such as the midgut and fat body, depending on infection status and route. To quantify differences in OBP7 abundance, Western blot analyses were performed on dissected tissues. Additionally, Grocott's methenamine silver staining was employed to visualize bacterial distribution within host tissues, facilitating spatial correlation between pathogen presence and OBP7 localization. These results underscore the multifunctionality of OBPs and highlight the value of *Gm* as a model organism for investigating host-pathogen interactions at the protein level.

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KEAP1-driven Dysfunction of Aging Endothelial Cells

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Cardiovascular diseases (CVDs) are the leading cause of death globally. Endothelial cells (ECs) that line the inner surface of blood and lymph vessels pay critical role in maintaining healthy phenotype of vasculature. Aging dysregulates function of ECs which contributes to a higher risk of CVDs in the elderly. Our data suggest that NRF2 and KEAP1 can be involved in this process.
KEAP1 (Kelch-like ECH-associated protein 1) is a redox-sensitive repressor of NRF2 (*NFE2L2* – nuclear factor (erythroid-derived 2)-like 2) – a transcription factor that mediates the protective response against oxidative stress. But, as we recently proposed, KEAP1 can act independently from NRF2 and along with nitric oxide synthase (NOS) and transnitrosating protein GAPDH be involved in the formation of S-nitrosothiols, leading to changes in protein function. In NRF2-deficient ECs this leads to the accumulation of protein aggregates, loss of function and premature senescence.
Our data show that with age NRF2 level decreases in ECs, which suggests that the same dysfunctional phenotype of ECs can be occurring in physiological aging. Therefore, we study the relation between protein aggregation and cell function in young and aged human-derived primary endothelial cells with the possible involvement of S-nitrosation mediated by KEAP1/NOS/GAPDH SNO complex. Our results indicate that a higher level of protein aggregates appears with age along with the impairment of function, which can be restored by the modulation of KEAP1.

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Different susceptibility of breast cancer cells to C-2028-induced apoptosis in relation to ER, PR and HER2 receptor statuses.

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Breast cancer (BC) classification is based on estrogen (ER), progesterone (PR) and HER2 receptors expression. Due to limited therapies, new treatments are needed. Unsymmetrical bisacridines (UAs), synthesized at Gdańsk University of Technology, are highly cytotoxic and effective against many cancers. This study evaluated the cellular effects of the UAs derivative C-2028 on BC cells with different receptor profiles: BT-474 (ER+, PR+, HER2+), MCF-7 (ER+, PR+, HER2−), MDA-MB-453 (ER−, PR−, HER2+) and MDA-MB-231 (ER−, PR−, HER2−). Cytotoxicity was assessed by MTT, flow cytometry was used to analyze cell cycle progression, membrane integrity, and caspase 3/7 activity after C-2028 treatment and reference compound doxorubicin. Receptors level was examined by Western blot, and nuclear and actin filament changes by microscopy. C-2028 was highly cytotoxic (IC50 0.015–0.1 μM), with lower IC50 in HER2-negative lines. In contrast, the IC50 of doxorubicin was in the range of 0.1-0.3 μM. Apoptosis was induced intensely in MDA-MB-453 and MDA-MB-231, but weakly in BT-474 cells. C-2028 caused death of 50% of MDA-MB-453 cells, compared to 30% by doxorubicin. Confocal microscopy confirmed apoptosis induction by C-2028. Western blot showed receptors' changes after drugs treatment. In conclusion, C-2028 induced apoptosis more effectively than doxorubicin in cells expressing only HER2, suggesting an important role of this receptor in the mechanism of UA action and drug potential in future therapy.

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Structural Basis of Specificity in DNA Recognition by Plant WRKY Transcription Factors

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WRKY proteins are one of the largest families of transcription factors (TFs) found in plants. Biosynthesis of specific WRKYs is induced during certain stages of plant development, as a response to abiotic stress, or upon pathogen infection and have crucial implications for defense responses in plants.
All WRKY TFs posses a unique DNA-binding domain (DBD) with conserved sequence motif, WRKYGQK, responsible for DNA interaction, and stabilizing zinc-binding site. All WRKY TFs recognize the so-called W-box motif [(T)TGAC(C/T)] that usually occurs as tandem repeats. Notably, outside the WRKY-DBD, these proteins are highly diverse due to the presence of additional domains and sequence motifs, e.g. leucine zipper motif. Those regions appear crucial for specific DNA recognition and dictate physiological functions of WRKY proteins.
Despite the great interest, little is known about the 3D-structure of full-length WRKY TFs. Up to date, only structures of DBDs from a few WRKY representatives are available in the Protein Data Bank (PDB).
Here we determined the crystal structures of the At-WRKY18-DBD alone and in the complex with a W-box containing DNA duplex. Our sstructural and DNA binding analyses point out novel aspects of WRKY DNA recognition. In particular, the binding of DNA induces deformation of the B-type double helix, suggesting a requirement for intrinsic DNA flexibility. This may help explain why despite the short W-box consensus, WRKY TFs can precisely control gene expression.

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Detection of OFD1 interactions in HEK293 cells across cell cycle stages

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Cilia are hair-like structures present at the surface of the majority of eukaryotic cells. They are composed of ciliary basal bodies, from which the main part of the cilium grows. Two major existing types of cilia – primary and motile, differ in their function (sensory vs motility) and abundance (single vs multiple per cell).
Mutations in OFD1 (oral-facial-digital syndrome 1) can cause dysfunction of primary and/or motile cilia. OFD1 localized at the ciliary basal body, is essential for cilia biogenesis, cell cycle progression, it also functions in chromatin remodeling and DNA repair. OFD1 localization shifts across cell cycle – from centrosomes and ciliary basal body in G1, through nucleus in S-phase, to ciliary basal body, nucleus and centrosomal satellites in G0. Despite over 300 potential OFD1 interactors identified, few have been experimentally confirmed, and their cell-cycle-specific interactions remain largely unknown.
As part of a broader project dissecting C-terminal-specific OFD1 interactions using CRISPR-Cas9–modified cells with truncating mutations, we employed OFD1 co-immunoprecipitation in human embryonic kidney (HEK293) cells at various cell cycle stages (G0 or G2/M). This approach aims to identify novel or cell cycle phase-specific OFD1 interactors and to better understand their contribution to cilia biogenesis and associated cellular processes.

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Cytotoxic effects of triclosan: the role of membrane structure

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Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol, TCS) is a chemical compound widely used as an antibacterial and antifungal agent in personal care products, cosmetics, and cleaning agents. Despite its popularity, triclosan raises growing concerns due to its effects on human health and the environment. It shows cytotoxic, genotoxic, immunotoxic, and potentially carcinogenic properties, though its molecular mechanisms in human cells remain unclear. The main goal of this study was to investigate how TCS interacts with human cell membranes, as these interactions may be key to understanding its toxicity. The research was conducted using both in vitro human cell models and model lipid membranes. TCS cytotoxicity was assessed (XTT and LDH leakage assays) on HL-60 and SK-N-SH human cell lines. TCS–lipid interactions were analyzed using Langmuir monolayers and single-component membrane models (evaluating collapse pressure, elasticity, and lipid packing density). HL-60 cells showed higher sensitivity, with effects visible at 25 μM. Results from model membranes confirmed observations from live cells. TCS alters membrane lipid properties, especially those of phosphatidylcholine – the main component of native membranes. Its insertion between lipids depends on both the polar head group (phosphocholines most affected, phosphatidylglycerol least) and the lipid tail.

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Gasocrine Hypothesis: A Fundamental Corollary to Cell Theory

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Gases are central to metabolism and are also signaling molecules. Oxygen-sensing gasoreceptors include oxygen-binding hemoproteins with diverse signaling domains (phosphodiesterase, guanylyl/adenylyl cyclase etc.) [1,2]. Some gasoreceptors may act as aquareceptors and the identity of vertebrate and plant oxygen gasoreceptors is still unknown [2,3]. Whether hemoglobin is a (proto)gasoreceptor also warrants a debate and the signaling events mediated by gas-gasoreceptor and gas-riboceptors interactions are proposed as "gasocrine signaling" [2,4]. Based on thought experiments, the following postulates that supplements the cell theory are proposed: 1) All living organisms composed of one or more cells require gasocrine signaling to sense, communicate, survive, and propagate. 2) Gasocrine signaling mediated by gasoreceptor proteins (or perhaps riboceptors), is the most essential cellular and inter-organism signaling. 3) All cells and acellular entities arising from or replicating in pre-existing cells require gasocrine signaling. The implications of the gasocrine hypothesis include, but are not limited to, quorum sensing, embryo development, disease mechanisms, and planetary gas and temperature homeostasis.

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Indole–stilbene hybrids with cytoprotective activity in a model of oxidative stress

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Hybrids are chemical compounds that combine two or more distinct structural components within a single molecule. Indole forms the core structure of many natural compounds, including biologically active alkaloids such as gramine. Stilbenes are natural polyphenols known for their antioxidant properties. We hypothesized that combining alkaloids with polyphenols could lead to hybrids with enhanced antioxidant and cytoprotective activity compared to their individual components.
A series of nine hybrids was synthesized, differing in the length of the alkyl chain and the nature of substituents on the aromatic ring of stilbene. All compounds were characterized using spectroscopic methods (FT-IR, ^{1}H, ^{13}C NMR) and mass spectrometry (EI-MS). *In silico* analyses were performed to evaluate the physicochemical and drug-like properties of the obtained derivatives. Studies using human erythrocytes demonstrated that the hybrids exhibited no hemolytic activity under *in vitro* conditions. Depending on their chemical structure and concentration, the hybrids showed variable cytoprotective activity in an induced oxidative stress model, effectively protecting erythrocyte membrane and hemoglobin from oxidative damage. Cell-free assays revealed that the hybrids exhibited both low radical scavenging activity and low Fe^{2}□ -chelating ability. Based on the results obtained, a probable molecular mechanism responsible for the cytoprotective effects of the hybrids was proposed.

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New strategy for precursor-selective miRNA silencing using short 2'-O-Me/LNA oligomers

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MicroRNAs (miRNAs) with identical or near-identical sequences form families that are often assumed to act redundantly. However, growing evidence suggests that individual family members may exert distinct biological functions, potentially due to differences in their precursor structures. Current knockdown approaches, such as antisense oligonucleotides or miRNA sponges, lack the specificity to discriminate between identical mature miRNAs derived from different precursors, limiting functional dissection of individual family members. Here, we present a novel strategy using short 2'-OMe/LNA-modified oligonucleotides to selectively target specific precursor molecules (pre-miRNAs) and inhibit production of individual miRNAs *in vitro* and *in vivo*. Targeting the apical region of precursor hairpins, we achieve precursor-specific suppression of mature miRNA production. We validate this approach using evolutionarily conserved *Xenopus laevis* and human miRNA families that produce identical mature miRNAs, demonstrating its versatility. This strategy provides a powerful tool to dissect unique biological roles of identical miRNAs derived from different precursors by enabling selective modulation of precursor-specific miRNA expression. Moreover, this approach allows us to explore the role of interplay between a miRNA precursor and interacting factors in shaping miRNA activity.

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Optimization of NPAS4 protein C-terminus expression

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NPAS4 belongs to the family of bHLH-PAS transcription factors. NPAS4is known for its crucial role in maintaining the balance between excitatory and inhibitory synapses in the brain, that is essential for proper neural circuit development and function. The balance disruption has been linked to neurodevelopmental and neuropsychiatric disorders such as autism, schizophrenia, and epilepsy [1,2]. Recently NPAS4 has been proposed as a therapeutic target of many serious diseases including type II diabetes.
This study aimed to screen the most suitable *E. coli* strain and additives for production and purification of NPAS4 C-terminal region. To this end, three *E. coli* strains: ArcticExpress, Rosetta-gami, and LOBSTER, were selected. Following expression, the NPAS4 protein was purified *via* metal affinity chromatography using His-Tag. The most promising fractions were subjected to western blot analysis. This work serves as a foundational step to enable large-scale NPAS4 C-terminus expression and purification for functional and structural studies.

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Lysozyme as a Functional Component of Venetin-1: An Anticancer and Antifungal Nanoparticle from *Dendrobaena veneta*

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Venetin-1 is a biologically active nanoparticle complex with anticancer, antifungal, and immunostimulatory properties, derived from the coelomic fluid of the earthworm *Dendrobaena veneta*. In this study, lysozyme was analyzed as a potential contributor to Venetin-1's bioactivity. The complex was isolated *via* coelomic fluid extraction, thermal treatment, dialysis (6–8 kDa and 12–14 kDa cut-offs), lyophilization, and ultrafiltration. Lysozyme concentration was quantified using ELISA, and its enzymatic activity was measured *via* lysis of *Micrococcus luteus*. The highest activity (1224.62 U/mg) was observed in fractions dialyzed through 6–8 kDa membranes. Immunoblotting with anti-human lysozyme antibodies detected multiple bands (11–132 kDa), suggesting the presence of polymeric forms. FTIR and Raman spectroscopy revealed strong structural similarity to egg white lysozyme, and Cryo-EM analysis provided insight into the nanoparticle's morphology. Zeta potential measurements indicated distinct electrokinetic behaviors of Venetin-1 and lysozyme across pH and electrolyte conditions. These findings confirm the presence and functional contribution of lysozyme in Venetin-1, highlighting its potential as a novel immunomodulatory and therapeutic agent.

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The role of BK channels in regulating cell death and cytokine response of bronchial epithelium exposed to lunar dust simulant.

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Introduction: Large-conductance potassium (BK) channels regulate membrane potential, cell volume, and immune responses. Exposure of bronchial epithelial cells to lunar dust simulant (LD) disrupts epithelial barrier integrity and triggers inflammatory responses. This study investigated BK channel involvement in bronchial epithelial responses to LD at gene, protein, and cell survival levels.
Methods: Wild-type HBE cells (WT) and BK-deficient HBEΔα cells were treated with LD (50–100 μg/ml). RNA-seq analyzed gene expression. Cell *via*bility was assessed using Orangu™ assay and Apoptosis/Necrosis Kit with flow cytometry. Pro-apoptotic proteins and cytokines were measured *via* Western blot and Human Inflammation Antibody Array.
Results: HBEΔα cells showed higher survival after LD exposure compared to WT. Paxilline-mediated BK inhibition also increased survival. RNA-seq revealed lower basal cytokine expression in HBEΔα and a marked upregulation post-LD, confirmed at the protein level. WT showed higher basal IL-1α, IL-6, IL-8, and TIMP-2, which increased after LD. HBEΔα had low basal cytokines but a broad inflammatory and allergic response post-LD. Pro-apoptotic protein expression rose in HBEΔα after LD, but apoptosis rates remained lower than in WT.
Conclusion: BK channels regulate apoptosis and cytokine responses in airway epithelium exposed to lunar dust simulant. Loss of BK prevents apoptosis execution and triggers delayed, dysregulated inflammation.

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Loss of myosin VI in mouse epididymal epithelium manifests as disruption of endocytic pathway

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Myosin VI (M6) is a motor protein essential for maintaining the structural integrity and function of specialized epithelia in mammals. This protein is involved in endocytosis and serves a structural role by anchoring the plasma membrane at the base of microvilli. M6 deficiency in Snell's waltzer mice disrupts the functional organization of specialized epithelia, such as the cochlear sensory epithelium, intestinal microvilli, and renal proximal tubules, leading to deafness, impaired absorption and poor resorption. Furthermore, our recent studies have shown that Snell's waltzer males have reduced fertility by 25%. The epididymal epithelium is another specialized epithelium in which we confirmed the presence of M6. Because the role of M6 is mediated by various adaptor proteins, we demonstrate partner interactions occurring in the epididymis, where Dab2 engages M6 in clathrin-dependent endocytosis and GIPC1 in uncoated vesicle transport. Comparative ultrastructural analysis of control and M6 mutant mice revealed severe defects, leading us to speculate that M6 is essential for maintaining the integrity of endocytic machinery and structural organization of the mouse epididymal epithelium, which support sperm maturation and storage.

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Comparing bacterial and mammalian expression systems to study the mechanism of N4BP1 in the mRNA decapping complex

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Eukaryotic cells regulate gene expression and counteracts foreign RNA through controlled surveillance pathways. A key mechanism is the degradation of uncapped mRNAs in cytoplasmic granules called P-bodies, which house the mRNA decapping machinery. This complex is built around the scaffold protein EDC4, with cofactors like EDC3, DCP1A/B, and the cap-cleaving enzyme DCP2. The ribonuclease N4BP1 was identified as a component of this machinery, but its precise role remains unclear. To investigate N4BP1's function in the decapping complex, we combine bacterial and eukaryotic expression systems. High-throughput cloning in *E. coli* allows rapid screening of multiple constructs to identify those with high expression and solubility. Selected protein fragments are then purified on a larger scale for structural analysis by X-ray crystallography to help elucidate detailed architecture of the decapping components. In parallel, full-length proteins and the entire decapping complex are reconstituted in mammalian non-adherent cell cultures (Expi293 and ExpiCHO). Transfection with Fc-tagged N4BP1 enables purification of intact decapping complex by affinity chromatography. These eukaryotic systems preserve native modifications and interactions, making them suitable for cryo-electron microscopy to visualize near-native assemblies. This integrated strategy will help reveal how N4BP1 associates with EDC4 and contributes to mRNA turnover.

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Neonicotinoids as Obesogens: Uncoupling Neuronal and Metabolic Effects of nAChR Ligands in *Caenorhabditis elegans*

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Amidst a global obesity epidemic, environmental chemicals are increasingly scrutinized as metabolic disruptors. Neonicotinoid pesticides function as potent obesogens, disrupting lipid metabolism even at ultralow, environmentally relevant concentrations. Using *Caenorhabditis elegans*, a model organism with highly conserved metabolic pathways, we quantified lipid stores by measuring the integrated density of Oil Red O staining following exposure to various nicotinic acetylcholine receptor (nAChR) agonists.

Both imidacloprid and thiamethoxam significantly increased fat accumulation. The effect was most pronounced with 0.1 mM thiamethoxam, but remarkably potent activity was also observed at picomolar doses (0.1 pM imidacloprid, 30 pM thiamethoxam), highlighting a non-linear dose-response relationship and the risk of chronic low-dose exposure. This obesogenic profile sharply contrasts with that of the classic agonist nicotine, which exhibited a biphasic effect—increasing lipid levels at 0.01 mM but reducing them at 1 mM.

These findings provide clear evidence that neonicotinoids are powerful metabolic disruptors. The divergent effects of these ligands, initially selected for their distinct neurobehavioral impacts, strongly suggest functional selectivity, whereby different agonists activate distinct downstream pathways despite targeting the same receptor. This challenges current neurotoxicity-based safety paradigms and underscores the need to re-evaluate the metabolic risks posed by these widely used pesticides.

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Combining Cyclophosphamide with Venetin-1: A Proteomic Study of Mouse Spleen Tissue

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hThe coelomic fluid (CF) of *Dendrobaena veneta* is a rich source of biologically active compounds, exhibiting antimicrobial, antifungal, and antitumor properties. However, unprocessed CF is toxic to vertebrate cells, a limitation overcome by heating it to 70 °C. The processed CF, called Venetin-1, shows pro-apoptotic activity against human colon adenocarcinoma and non-small cell lung cancer cells. Proteomic analyses reveal significant changes in the proteome of cancer cell lines and *Candida albicans* following Venetin-1 treatment. While cyclophosphamide (CPA), a conventional chemotherapeutic, is effective, it simultaneously causes severe side effects. This highlights the need for novel therapeutic strategies with fewer adverse effects. Combining CPA with Venetin-1 represents a promising approach. Preliminary studies in mouse lung tissue treated with CPA and Venetin-1 revealed significant changes in the proteome of the tissue, including activation of regeneration and autophagy pathways. Encouraged by these findings, we extended our research to other mouse tissues to further explore the potential of this combination as a support in cancer therapy. Here, we present the results of a proteomic analysis of mouse spleen tissue following administration of CPA and varying doses of Venetin-1.

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Investigating the ectodermal differentiation ability of adipose-derived stem cells

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The regenerative potential of stem cells is being studied extensively in the context of diseases such as neurodegenerative disorders, retinal degeneration, stroke or type 1 diabetes. Tissues of ectodermal origin, such as neural and corneal tissue, remain therapeutically challenging due to limited regeneration and donor shortages. This study investigates the ability of adipose-derived stem cells (ADSCs) to differentiate toward ectodermal lineages.

ADSCs and amniotic epithelial cells (AECs) were used, the latter serving as ectoderm-positive control. Both cell types were isolated from human tissues, identified by flow cytometry and tested functionally. Ectodermal differentiation was induced using a commercial assay and assessed through the detection of specific markers: nestin, OTX2 (immunofluorescence); EN2, SOX1, LNX2, and PAX6 (RT-qPCR); CK14-16 and CK19 (flow cytometry). Additionally, morphology was evaluated using phase-contrast microscopy.

The results showed that, after differentiation, ADSCs appeared to express significantly more nestin and slightly more OTX2 than undifferentiated cells. The expression of *SOX1* and *LNX2* did not change after differentiation; however, we recognized upregulated expression of *EN2* and *PAX6* (statistically irrelevant). CK14-16 and CK19 were not identified in the ADSCs. Morphology analysis revealed clear changes in ADSC shape. In conclusion, adipose tissue is a promising source of cells for ectodermal lineage tissue engineering.

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Extending healthspan in aging nematodes through drug repurposing

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Aging is characterized by a progressive decline in physiological and molecular functions, leading to a reduced quality of life. Healthspan refers to the period of life during which an organism maintains adequate health. This study investigates healthspan modulation in *C. elegans* by repurposing human drugs on aged worms at the post-reproductive phase. The treatment of aged animals takes into account changes in molecular pathways that manifest in the hallmarks of aging. We screened several drugs from different classes, including caloric restriction mimetics, anticonvulsants and painkillers targeting different molecular processes for its ability to improve body movement. Our results show that specific pharmacological interventions improve neuromuscular coordination and mitigate age-related movement losses, suggesting targeted therapeutic strategies for rejuvenation. Investigation of drug-induced molecular changes will provide important guidance for advancing translational approaches to improve quality of life.

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The effect of lead on the membrane protein profile of yellow lupine (*Lupinus luteus*) roots

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Lead represents one of the most serious environmental threats, causing the inhibition of plant growth and development. Cell membranes are the primary target of this metal's toxic action. The aim of this study was to identify and characterize membrane proteins in the roots of lupine exposed to lead nitrate.

Exposure to lead dynamically alters the membrane protein profile in lupine roots. Proteomic analysis revealed changes in the accumulation levels of seven key membrane proteins, including transporters and structural proteins.

Aquaporins PIP2-7 (and PIP1-2) are essential elements of the plant response to lead stress, as their expression levels undergo significant changes. The regulation of water transport through these channels is one of the main targets of lead's action.

The aquaporin response to lead is biphasic and depends on the intensity of the stress. Under moderate stress conditions (150 mgl^{-1} Pb^{2+}), an increase in aquaporin levels or their maintenance at control-like values is observed. This may represent a compensatory mechanism aimed at maintaining cellular water homeostasis and counteracting water deficits caused by root damage. Under severe stress conditions (350 mgl^{-1} Pb^{2+}) and with prolonged exposure, a marked decrease in aquaporin levels occurs. This could result from direct inhibition of their synthesis, accelerated degradation, or may represent a defensive mechanism to limit the uptake of toxic Pb^{2+} ions along with water.

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Radiogenic effects on ion channel function: investigating the role of BK_{Ca} potassium channel in DNA damage response

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Glioblastoma multiforme (GBM) is the most aggressive and common primary brain tumor, classified as WHO grade 4 astrocytoma. Standard treatment involves surgery, chemotherapy with temozolomide, and radiotherapy, but survival rates remain low, emphasizing the need for more effective therapies. GBM shows poor response to chemoradiotherapy, possibly due to enhanced DNA repair mechanisms. Radiotherapy works by inducing DNA double-strand breaks (DSBs), and targeting the DNA damage response (DDR) could enhance tumor radiosensitivity. Ionizing radiation influences ion transport, a key element in DDR, yet most DDR research has focused on nuclear or cytosolic proteins, overlooking membrane-bound ion channels. Recently, potassium channels, especially the BKCa (large-conductance Ca^{2+} - activated K^+) channels, have been identified as "oncochannels" that support tumor progression, therapy resistance, and other malignant features in various cancers, including glioma. These channels regulate potasium levels, cell migration, mitochondrial function, and redox balance. Due to their drug sensitivity, BKCa channels are promising therapeutic targets. Modulating these channels may improve GBM cell radiosensitivity and help overcome resistance to treatment. This study aims to explore the role of BKCa channels in the DDR to develop more effective radiotherapy strategies.

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Loss of MCPIP1 Disrupts Metabolic Gene Expression and Enhances Susceptibility to Liver Cancer

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The incidence of hepatocellular carcinoma (HCC) is rising, with projections exceeding one million cases by 2025. Chronic inflammation leading to fibrosis or cirrhosis is a hallmark of HCC progression. Men are three to five times more likely to develop HCC than women, a disparity that is also observed in mouse models. Monocyte Chemotactic Protein Induced Protein 1 (MCPIP1), encoded by *ZC3H12A*, is a key negative regulator of inflammation, and its absence in hepatocytes promotes fibrosis, immune activation and tumor formation, particularly in female mice. We used hepatocyte-specific *Zc3h12a* knockout (KO) and wild-type (WT) female mice treated with DEN at 2 weeks of age. Animals were sacrificed at 0, 3, 6, and 9 months. RNA-seq analysis revealed substantial transcriptomic differences between KO and WT already at 0 months - prior to DEN treatment. Changes affected metabolic pathways, including bile acid, fatty acid and xenobiotic metabolism. Gene expression changes in time groups were dynamic, with the highest number of differentially expressed genes at 6 months. At 9 months, despite tumor presence in nearly all KO mice and absence in WT, transcriptomic differences were minimal. Altered expression of genes involved in steroid hormone synthesis suggests a potential hormonal contribution to tumor susceptibility in the KO group.

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High-Content Screening for Phenotypic Profiling and Discovery of Pathway Modulators

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High-content screening (HCS) is an advanced imaging-based method used to generate multiparametric phenotypic profiles of cells subjected to chemical or genetic perturbations. Among the commonly applied strategies is the Cell Painting assay, which utilizes six fluorescent dyes to label distinct cellular compartments. This enables the extraction of extensive quantitative features related to morphology, signal intensity, texture, and spatial organization.

By combining multiplexed imaging techniques—including Cell Painting, customized subcellular dyes, and fluorescence in situ hybridization (FISH)—with screening of structurally diverse small-molecule libraries, such as those provided by the EU-OPENSCREEN infrastructure, it becomes possible to investigate chemical space in a biologically relevant context. This facilitates the identification of compounds with distinct phenotypic signatures and supports predictions regarding their mechanisms of action (MOA), cytotoxic potential, and pathway-specific effects.

Case studies demonstrate the utility of high-content phenotypic screening for assessing compound-induced changes in cellular architecture and gene expression. This integrative approach, positioned at the intersection of chemical biology, high-content imaging, and systems-level analysis, contributes to the discovery of novel molecular probes and modulators of cellular pathways.

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Protective Effects of Nobiletin Against IFALD in Human Liver Cells

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Nobiletin, a flavonoid in citrus peels, offers anti-inflammatory, antioxidant, and lipid-regulating benefits. Intestinal Failure-Associated Liver Disease (IFALD) is a severe issue stemming from long-term parenteral nutrition, marked by chronic inflammation, oxidative stress, and disrupted bile acid/lipid metabolism. Soybean oil-based lipid emulsions contribute to IFALD, as does lipopolysaccharide (LPS) from bacterial translocation due to gut nutrient deprivation or catheter-related infections.

Our study aimed to assess nobiletin's hepatoprotective effects in an in vitro IFALD model using THLE-2 human hepatocytes exposed to Intralipid® and LPS. We used non-cytotoxic nobiletin concentrations (10 and 25 μM), confirmed by MTT assay. We evaluated cellular responses *via* MAGPIX multiplex immunoassays for inflammatory signaling, RT-qPCR for gene expression, and Western blotting for protein signaling.

Nobiletin decreased LPS-induced phosphorylation of JNK, ERK, NF-κB, AKT, and P70S6K. It also altered the expression of lipid metabolism genes like AMPKα2, CYP7A1, and ABCA1, and boosted Nrf2 nuclear translocation with increased SOD-1 expression.

These results indicate nobiletin might protect hepatocytes in IFALD-like conditions by modulating inflammatory pathways and improving lipid metabolism.

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Does antioxidant toxicity begin at the cell membrane? – investigating the effects of curcumin and resveratrol on SK-N-SH and HL-60 cells

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Numerous studies on curcumin and resveratrol have demonstrated their antioxidant, anti-inflammatory, anticancer, and neuroprotective properties. However, these compounds exert beneficial effects only at appropriate concentrations. Given that these molecules are hydrophobic, they tend to localize within the lipid of cell membranes. They can alter the physicochemical properties of these membranes, potentially affecting processes such as transport, signal reception, and nerve impulse conduction. The aim of this study was to determine the structural changes in the membranes of SK-N-SH and HL-60 cells induced by different concentrations of curcumin and resveratrol, and to correlate these modifications with the cytotoxicity of the compounds. The study (spectrophotometric method and the Langmuir technique) showed that direct administration of curcumin or resveratrol to cells is toxic and affects the physicochemical parameters of their membranes. The extent of these changes depends on the degree of membrane saturation; the more rigid the membrane, the lower the concentration of the compounds needed to destabilize it. Although the tested polyphenols may exert beneficial health effects under certain conditions, they can also exhibit toxic effects on various cell types. Therefore, establishing safe dosages and exposure times is crucial to fully harness their therapeutic potential.

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Modelling of cellular membranes in studies on the effects of toxic substances present in the environment

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Substances entering cells from the environment initially interact with the membrane, which can directly or indirectly alter their biological effects. It makes no difference whether the organism is animal or plant-based — disturbances in membrane function always carry far-reaching consequences for the overall physiology of the organism.
An effective approach to studying these phenomena involves combining lipid membrane model analysis with examining the action of the same substances on living cells. One example of such a research model is the study of manganese nanoparticles (MnNPs) on wheat and the evaluation of their effects on human cells.
Experiments conducted on wheat have shown that high doses of MnNP can lead to damage to chloroplasts and disruptions in photosynthesis, similar to what is observed in human cell lines, resulting from increased oxidative stress levels.
Membrane models of the examined cells were studied using the Langmuir balance technique and compared to biochemical results from wheat cells of the Alibi and Nimfa varieties, as well as human immune cell lines U-937 and HL-60. The presence of MnNPs was shown to impair the stiffness of phospholipid monolayers, which correlated with, for example, changes in the ratio of chlorophyll a to b in wheat cells and a reduction in *via*bility of the tested immune cells.

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The regulatory role of phosphorylation – Starmaker-like protein in otoliths biomineralization

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Otoliths are calcified structures in the inner ear of fish, composed of calcium carbonate crystals arranged in specific polymorphs. Their proper formation is essential for balance and hearing. A crucial factor influencing otolith biomineralization are proteins, often with inherently disordered structures, such as Starmaker-like (Stm-l) protein.
In our study, we successfully isolated and identified the native Stm-l protein from common carp otoliths. We confirmed that native Stm-l is phosphorylated and possesses a disordered structure. To evaluate the functional significance of phosphorylation, we performed *in vitro* crystallization assays using both phosphorylated and dephosphorylated forms of Stm-l. Importantly, phosphorylated Stm-l promoted the formation of vaterite – a polymorph characteristic of native otoliths, while dephosphorylation altered crystal morphology and favored calcite formation. These results indicate that phosphorylation modulates Stm-l activity, influencing calcium carbonate polymorph selection during otolith development.
Our findings highlight phosphorylation as a molecular switch regulating the function of Stm-l in fish otolith biomineralization, offering new insights into protein-controlled crystallization processes.

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Polystyrene nanoparticles interfere with DNA repair mechanisms in human intestinal Caco-2

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Nanoplastics (NPs) are widespread in terrestrial and aquatic environments, with harmful effects observed in various organisms. Studies on human cells help clarify toxicity mechanisms and assess health risks. NPs may disrupt membranes, generate reactive oxygen species (ROS), and cause DNA damage, including single- and double-strand breaks. Polystyrene (PS) nanoparticles raise concerns due to potential genotoxicity, though mechanisms remain unclear. This study assessed NP effects on human colorectal adenocarcinoma Caco-2 cells- an in vitro model of the intestinal epithelial barrier- exposed to 50–1200 μg/ml. Cytotoxicity was evaluated using the clonogenic assay; genotoxicity *via* alkaline comet assay and flow cytometry (apoptosis, cell cycle). Moderate cytotoxicity was observed, with no significant cell cycle changes or DNA strand breaks, and only a minimal increase in apoptosis. DCFDA staining revealed ROS induction following NP exposure. qPCR analysis showed downregulation of key genes in DNA repair pathways (BER and DSB), indicating impaired repair capacity. The results highlight sublethal but concerning NP effects on intestinal cells and the potential long-term risk of genome instability from chronic exposure.

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Attempt to use a system based on dCas9 and MS2-loop-containing sgRNAs for visualization of the *HTT locus* with CAG repeat expansion

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Expansions of CAG repeats cause several neurodegenerative, hereditary, and currently incurable disorders (polyQ diseases), including Huntington's disease (HD). Double-strand breaks (DSBs) within CAG tracts trigger their instability, leading to both expansions and contractions. The mechanisms behind this instability remain poorly understood. While some DNA repair pathways are implicated, DSB repair within CAG repeats at endogenous loci in human cells has not been sufficiently studied. Understanding these mechanisms is crucial for developing future therapies targeting repeat expansion disorders. In this study, we investigated CAG instability using a CRISPR imaging approach with catalytically inactive Cas9 (dCas9) and MS2-loop-containing sgRNAs to visualize the *HTT locus* in human cells. This system aimed to identify proteins involved in repairing expanded CAG tracts at an endogenous chromosomal site. However, several technical challenges arose. Although multiple nuclear signals were observed, they failed to precisely localize the target locus. Furthermore, a gradual loss of expression of imaging system components was noted over time in culture and after cell banking. These findings highlight the need to optimize dCas9- and sgRNA-MS2-based DNA imaging systems, as the current setup does not yield reliable, specific signals suitable for high-resolution microscopy.

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Effect of intact DNA on SUMOylation of the BRCT domain of PARP1

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Potential of the 5′ terminal domains of RNAs of human laminins and EMCV for therapeutic ribonucleic acid molecules

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Since Covid pandemic the interest in RNA as the therapeutics has grown rapidly. In our work, we focused on the 5' untranslated region of RNA which is responsible for translation initiation and protect the 5' end of RNA from degradation. Both functions are crucial to obtain effective therapeutic molecules. We chose different 5'UTRs as potential elements for artificial RNAs: JK domain of *Encephalomyocarditis virus*, 5'UTR of laminin A2 mRNA, 5'UTR of laminin B1 mRNA and as a reference, we applied the 5'UTR of β-globin mRNA, which drives protein synthesis effectively. To test the efficiency of the chosen 5'UTRs, we produced artificial RNAs *in vitro*. Then, the RNAs were introduced into human cells, HEK293, and comprehensive analysis based on microscopy, spectrophotometry and flow cytometry was performed. It turned out that both 5'UTRs of laminin mRNAs are able to initiate GFP synthesis effectively. However, placement of the isolated JK domain into a new sequence context leads to occurrence of two uAUG codons which negatively affect translation initiation. This effect is abolished by two point mutations in the positions U65 and A73 and then the mutated JK domain drives translation more effectively. Finally, we showed that OspA, a protein from *Borrelia* spirochete with strong antigenic properties, is efficiently produced from RNAs with the 5'UTRs of laminin A2 and laminin B1, respectively. Therefore, the proposed 5'UTRs could be utilized in potential therapeutic RNAs.

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Smart Drug Delivery: Antibody Aggregation and Immunotargeted Doxorubicin for Breast Cancer Cells

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Targeted drug delivery to cancer cells remains a significant challenge in oncology. Nanocarriers offer a promising solution by enabling safe and efficient delivery of chemotherapeutics, such as doxorubicin (Dox), while minimizing off-target toxicity. In this study, Congo red-based supramolecular ribbon-like nanocarriers (SRN) were used due to their unique ability to bind proteins outside active sites, including structurally altered antibody regions formed upon antigen interaction. We demonstrated that this interaction between the SRN and Dox, facilitated by antibodies, allows for a reduced drug dose (0.1 nM) compared to free Dox while maintaining significant antiproliferative effects (MST, SRB) on breast cancer cells (MDA-MB231). Also, antibody aggregates – formed *via* thermal or covalent methods – were characterized and evaluated for their binding to these cells. We demonstrated that these aggregates can serve as targeting platforms for SRN-conjugated Dox. Complex formation and size were analyzed *via* electrophoresis, DLS, and microscopy. Hemagglutination confirmed the biological activity of these aggregates. These results support the feasibility of antibody- or antibody-aggregation-based immunotargeting for the selective delivery of low-dose drugs in breast cancer therapy.

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Adipose-derived stromal cells: molecular characteristics and applications in tissue engineering.

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Adipose-derived stromal cells (ASCs) are a promising cells widely used in regenerative medicine, owing to their accessibility, multipotency, and immunomodulatory properties. Due to their phenotypic similarity to dermal fibroblasts, distinguishing these cell types remains a challenge. Using single-cell RNA sequencing and qPCR validation, we identified a panel of differentially expressed genes- such as MMP1, MMP3, S100A4, CXCL1, PI16, and IGFBP5- that enable precise molecular distinction between ASCs and fibroblasts. These findings offer a foundation for improved cell-type identification in both experimental and clinical settings. In parallel, we utilized ASCs in two-dimensional (2D) culture systems to evaluate the biological response to selected biomaterials and bioactive peptides, focusing on regenerative potential. Additionally, we initiated the optimization of three-dimensional (3D) ASC-derived organoids to better replicate in vivo conditions and create more physiologically relevant models for future applications in drug screening and tissue engineering. Our work underscores the value of ASCs as a versatile platform for both molecular research and translational applications, including material testing, pharmacological evaluation, and personalized regenerative strategies.

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Generation of hydrogen peroxide in plant-based beverages and food

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Hydrogen peroxide is a reactive oxygen species, playing an important signaling role in the body. Exogenous sources of hydrogen peroxide are thus also of interest. It is known that hydrogen peroxide is generated in milk, honey, and such beverages as tea and coffee. We demonstrated that hydrogen peroxide is also generated in micromolar concentrations in infusions of medicinal herbs and spices, in cooked vegetables, and in alcoholic beverages such as beer, wine, brandy, whisky and fruit liqueurs exposed to air. Generation of hydrogen peroxide is due mainly to autoxidation of polyphenols and proceeds in two steps, the first being the formation of superoxide and phenoxyl radicals. Phenol-containing beverages and food both generate and scavenge hydrogen peroxide, so the measured concentration of hydrogen peroxide is a net result of processes of generation and scavenging.

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Light modulation of mitochondrial potassium channels activity isolated from Guinea Pig cardiomyocytes

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Ischemic heart disease remains one of the leading causes of mortality. Local blood flow restriction leads to hypoxia and nutrient deprivation in cells. Restoration of circulation after ischemia causes ischemia-reperfusion (I/R) injury. Mitochondria are key mediators of ischemic injury and the apoptotic response. Strategies that prevent mitochondrial damage represent a promising approach to mitigating cell death associated with I/R injury. The MitoK channel activators have cytoprotective properties, but they cause side effects. In our laboratory we demonstrated that infrared (IR) light influence the activity of a large-conductance calcium-activated potassium (mitoBK$_{Ca}$) channels. Mitochondrial photoreceptors for IR light are cytochrome c oxidase (COX) or complex IV, which contains metal centers, CuA and CuB. Studies suggested functional interaction between COX and the mitoBK$_{Ca}$ channel. We observed that mitoBK$_{Ca}$ channel located in the IMM from cardiomyocytes, is inhibited under conditions, oxidizing (K□ [Fe(CN)□]) and reducing (TMPD and ascorbate). Inhibition was reversed by IR light: 820 nm light under oxidizing conditions, and 760 nm light under reducing conditions. The impact of 820 nm IR light on the survival of isolated guinea pig cardiomyocytes subjected to I/R was evaluated. Findings suggest that 820 nm IR light, holds potential as a novel non-invasive strategy for cardioprotection in I/R injury.

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Assessment of IL-6 concentration in culture medium of CRC lines treated with bioactive compounds

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Introduction: Colorectal cancer (CRC) is a common gastrointestinal malignancy characterized by chronic inflammation, with interleukin-6 (IL-6) playing a pivotal role. IL-6 promotes tumor progression by activating signaling pathways that support cancer cell proliferation and survival. Betulin and its semi-synthetic derivatives, known for their anti-inflammatory and potential anticancer properties, are promising therapeutic agents.
Aim: To evaluate the effects of betulin and its semi-synthetic derivatives on IL-6 protein levels in the culture medium of colorectal cancer cells over different exposure times.
Materials and Methods: Supernatants from colorectal cancer cell cultures were collected and analyzed. The impact of tested compounds on secreted IL-6 protein levels was assessed using Proximity Ligation Assay. Cisplatin and 5-fluorouracil served as reference compounds.
Results and Conclusions: The tested compounds influenced IL-6 protein concentrations in the culture medium, with effects varying depending on the cell line (SW1116 and CCD-841CoN). Notably, IL-6 was undetectable in two out of four analyzed cell lines (HT-29 and RKO). This may result from low or silenced *IL-6* gene expression, differences in inflammatory signaling, mutations affecting cytokine production, or epigenetic changes contributing to transcriptional silencing.

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Ribosomes as zinc storage modules and their functional role during aging in *Caenorhabditis elegans*

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Zinc is an essential micronutrient required for numerous biological processes including enzymatic catalysis, gene regulation, and cellular stress responses. Emerging evidence suggests that ribosomal proteins act as major intracellular zinc reservoirs, yet the functional implications of this zinc storage during aging remain poorly defined. In this project, we investigated the molecular role of ribosome-bound zinc and its mobilization during aging using *Caenorhabditis elegans*. Our data showed that optimum zinc supplementation significantly improves lifespan, locomotion, and antioxidant activity (SOD assay) in aged *C. elegans*. We are characterizing zinc dynamics in wild-type worms and zinc transporter mutants and assessing healthspan and proteomic changes under zinc supplementation. Proteomic analysis reveals upregulation of ribosomal and stress-related proteins, suggesting a zinc-responsive adaptation in proteostasis pathways. This study aims to define ribosomal zinc storage and mobilization mechanisms, and how zinc availability influences translation, redox balance, and aging, with implications for micronutrient-based interventions in age-related decline.

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Microbial Composition of Urban Air: Seasonal Variation and Pathogenic Potential

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The airborne microbiome associated with particulate matter (PM) remains poorly characterized, despite its relevance to public health. Urban PM – particularly its fine fractions (PM2.5 and PM1) – poses respiratory risks and can act as a vector for pathogens and antibiotic resistance genes (ARGs).This study explores airborne microbial communities in two urban settings with contrasting emission profiles: industrial Zabrze (dominated by coal combustion) and a less industrialized area near an infectious disease hospital in Racibórz. A total of 291 PM samples were collected across seasons and analyzed using whole-metagenome sequencing to characterize microbial communities and detect ARGs. The results revealed diverse, spatiotemporally dynamic microbial communities strongly shaped by location, PM type, and season. Potentially pathogenic taxa – such as Pseudomonas spp. and Staphylococcus spp. – were more abundant in colder months. Other airborne microorganisms with pathogenic potential, including *Thermomyces vulgaris* and *Thermobifida fusca*, were also identified. Understanding the seasonal and compositional complexity of the airborne microbiome, along with ARG prevalence, is key to evaluating air quality and public health risks related to respiratory infections and antimicrobial resistance. Our findings underscore the importance of incorporating microbial indicators into air quality standards and urban health policies.

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SLC35A2-deficient HEK293T cells display hallmarks of the epithelial-to-mesenchymal transition (EMT)

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SLC35A2 is a multitransmembrane protein that delivers UDP-galactose to the ER and Golgi lumen of mammalian cells to enable galactosylation of macromolecules. Pathogenic mutations in the *SLC35A2* gene cause a subtype of congenital disorders of glycosylation (CDGs), namely SLC35A2-CDG. We have recently discovered that the loss of SLC35A2 activity triggers several hallmarks of the epithelial-to-mesenchymal transition (EMT) in Madin-Darby canine kidney (MDCK) cells. In this study, we sought for EMT traits in the SLC35A2-deficient epithelial-like human embryonic kidney (HEK) 293T cell line. Using immunoblotting we found that the levels of two proteins involved in cell-cell adhesion, namely occludin and beta-catenin, were significantly decreased comparing with the wild type cells. We also observed an increase in the levels of the EMT-inducing transcription factors ZEB1/ZEB2. RNA sequencing of the SLC35A2-deficient HEK293T cells also revealed a gene expression signature indicative of EMT. Finally, immunofluorescence staining of the *cis* Golgi marker GM130 revealed that SLC35A2 deficiency triggered Golgi compaction, a phenomenon that was previously observed in SLC35A2 knockout MDCK cells. To conclude, SLC35A2 depletion triggers EMT in HEK293T cells, which suggests that this multitransmembrane protein is one of the gatekeepers of the epithelial phenotype *in vitro*. It is tempting to speculate that EMT driven by the dysfunctional SLC35A2 may contribute to the pathogenesis of SLC35A2-CDG.

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Ion Transport Modulation by Modified Ionophores and Bioactive Compounds

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Cystic fibrosis (CF), the most common genetic rare disease, is caused by mutations in the *CFTR* gene, impairing ion and water transport across epithelial cells and resulting in thick mucus. CFTR function depends on ATP, so boosting ATP levels may enhance chloride secretion and improve epithelial hydration.

Cellular ATP can be increased by mitochondria-targeting ionophores, which transport ions across membranes, depolarize the mitochondrial membrane, and elevate respiration. These ionophores also influence epithelial electrophysiology through signaling pathways. Similar effects may come from natural compounds like flavonoids, which modulate ion channels and metabolism.

This study assessed ion transport by ionophores using Ussing chamber and Black Lipid Membrane techniques, respiration *via* the Oroboros system, and ATP levels using a bioluminescent assay. Additional assays evaluated cell migration, *via*bility, ROS, and transepithelial resistance in A549 and 16HBE14o- cell lines.

Results showed that ionophores increased respiration without altering chloride transport. In contrast, the flavonoid luteolin raised ATP levels, improved transepithelial resistance, influenced metabolism, proliferation, and modulated chloride secretion. These findings suggest luteolin may strengthen epithelial barriers and support hydration, offering therapeutic potential in CF.

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Unsymmetrical Bisacridine C-2028 Modulates Macrophage Polarization and Maintains Anticancer Activity in the Pancreatic Tumor Microenvironment

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Tumor-associated macrophages (TAMs) are a crucial element of the tumor microenvironment (TME). TAMs significantly impact tumor development and progression, acting either by supporting (M2-like macrophages, immunosuppressive) or against (M1-like macrophages, proinflammatory) the disease. Hence, therapies targeting or reprogramming TAMs toward an M1 phenotype are needed. Unsymmetrical bisacridines (UAs), synthesized at Gdańsk University of Technology, are highly cytotoxic and effective against many cancers. This study evaluated the impact of the UAs derivative C-2028 on macrophages' polarization during their differentiation from human monocytic THP-1 cells, as well as on their phenotype in co-culture with pancreatic cancer AsPC-1 cells, as determined by qPCR analysis. MTT assay assessed whether the macrophages affect the activity of C-2028. We showed that C-2028 changed the expression of key signaling molecules involved in inflammation and immune response during macrophages' polarization (CXCL10, TNFα) and in macrophages from co-culture (CXCL10, TNFα, and TGFβ). Furthermore, the presence of M1 and M2 macrophages decreased the *via*bility of AsPC-1 cells, but did not affect the activity of C-2028. Summing up, C-2028 modulates macrophages' polarization by influencing the expression of key immunomodulatory genes. These results indicate that UA not only retains its anticancer efficacy in the presence of macrophages but may also contribute to reprogramming the tumor microenvironment.

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Subcellular distribution of human ribonuclease Dicer and its truncated variant Dicer1e

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Human Dicer (hDicer) is a key endoribonuclease involved in microRNA biogenesis, playing a crucial role in post-transcriptional gene regulation. This canonical role of hDicer is associated with its cytoplasmic localization. However, hDicer can shuttle between the cytoplasm and the nucleus upon specific signals, and its translocation to the nucleus is attributed to a nuclear localization signal located in the dsRNA-binding domain. Moreover, phosphorylation of hDicer, upon induction of DNA damage, can also trigger hDicer movement to the nucleus. In addition to the full-length form, several alternatively spliced isoforms of hDicer have been identified, including hDicer1e, whose biological function is poorly understood. The hDicer1e variant consists of two RNase domains (IIIa and IIIb) and the dsRBD domain. It has been demonstrated that hDicer1e localizes in both the cytoplasm and the nucleus. hDicer1e is enriched in certain cancers and highly proliferative cells. Disturbed distribution of hDicer and hDicer1e in the cell can cause pathological conditions or diseases, including cancerogenesis. Therefore, methods that allow for the unambiguous determination of the subcellular localization of hDicer and its variants are of great importance for monitoring and assessing the well-being of cells. Here, we undertake such an effort by using high-resolution confocal microscopy of single cells. In the assay, we use HEK293T cells and derivative lines expressing GFP-tagged hDicer or hDicer1e.

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Tardigrade alternative oxidase expressed in yeast alleviates the oxidative stress

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Tardigrades are known as toughest animals on Earth as they can endure many adverse environmental conditions. Several mechanisms contribute to the resistance of tardigrades to harmful agents, including effective antioxidant systems. Levels and activity of many antioxidant proteins increase during exposure to stress factors. One such protein with antioxidant potential is alternative oxidase (AOX). AOX activity can be studied by examining in detail the impact of heterologous AOX expression on the physiological responses of model organisms under stressful environmental conditions. The effect of functionally expressed alternative oxidase derived from the tardigrade Milnesium inceptum in superoxide dismutase1 knockout (Δsod1) yeast on superoxide anion radical level, mitochondrial membrane potential, the redox status and survival of yeast cells were investigated. Based on experimental data, we conclude that AOX confers a function of the antioxidant system and mitigates the lack of SOD1 in yeast cells. Therefore, we propose that AOX may play a role in preventing the formation of reactive oxygen species result-ing from excessive reduction of electron transport chain components and in maintaining redox balance in cells of organisms exposed to environmental stress.

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Mitochondria distribution during oocyte maturation and the first cleavage divisions in cattle - from zygote to the 4/5 cell stage embryo

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High energy demand during preimplantation embryo development is fulfilled by mitochondria (mt). As mt biosynthesis resumes only at the blastocyst stage, the maternal mt pool gradually decreases with each cell division. Even distribution of mt among blastomeres is essential for proper embryo development. Mt move along microtubules, aided by transport and anchoring proteins, ensuring equal segregation. Studies also suggest that mt contribute to spindle stabilization and accurate chromosome segregation, supporting genomic stability.

We aimed to (1) characterize mt distribution from oocyte maturation (1st and 2nd polar body extrusion, n=50), through zygote (n=19), 2-cell (n=24), and 4/5-cell stages (n=18) using high-resolution confocal imaging, and (2) assess co-localization of mt (MitoTracker, VDAC antibody) with microtubules and mt-associated proteins (Dynein, Syntaphilin) to understand transport mechanisms.

Our findings show that in MII oocytes, mt migrate toward the dividing nucleus and associate with karyokinetic spindle elements, a pattern sustained through meiosis. In pronuclear-stage zygotes, mt are evenly distributed in the cytoplasm, a pattern preserved in the 2- to 5-cell stages. Across all stages, mt frequently associate with lipid droplets, forming distinct, ring-like fluorescent structures. These results provide new insights into the spatial organization and dynamics of mitochondria during early bovine embryo development.

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Enhanced Cytotoxicity of Bromine- and Chlorine- Substituted Flavonoids in Colon Cancer Cells

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Flavonoids, compounds naturally found in plants, possess anti-inflammatory, cytotoxic, and bactericidal properties. The addition of a halogen bond to their structure may enhance these qualities and also improve drug-target interactions, metabolism, and therapeutic efficacy. Due to the growing incidence of colon cancer, halogenated flavonoid derivatives – especially those containing bromine and chlorine – have gained interest due to their anticancer properties.

This study focuses on three such compounds: 3'-bromo-5'-chloro-2'-hydroxychalcone, 8-bromo-6-chloroflavanone, and 8-bromo-6-chloroflavone. Their physicochemical properties: melting point, molecular weight, polarity, and log P were evaluated using laboratory methods and SwissADME modeling. Examination showed that although the compounds have identical molar masses, they differ in structure and bond flexibility – features potentially influencing biological activity. Next, cytotoxicity was assessed *via* sulforhodamine B (SRB) assay on HCT-116 and HT-29 colon cancer cell lines, as well as the healthy FHC line. Halogenated flavonoids demonstrated significantly greater cytotoxicity than their non-halogenated counterparts, surpassing quercetin in efficacy, a flavonoid well-known for its cytotoxic properties.

Our findings highlight the potential of halogenated flavonoids as promising anticancer agents. Ongoing studies aim to further examine their mechanisms of action, including evaluations of Caco-2 permeability and bioavailability.

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KEAP1-driven Dysfunction of Aging Endothelial Cells

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Cardiovascular diseases (CVDs) are the leading cause of death globally. Endothelial cells (ECs) that line the inner surface of blood and lymph vessels pay critical role in maintaining healthy phenotype of vasculature. Aging dysregulates function of ECs which contributes to a higher risk of CVDs in the elderly. Our data suggest that NRF2 and KEAP1 can be involved in this process.

KEAP1 (Kelch-like ECH-associated protein 1) is a redox-sensitive repressor of NRF2 (*NFE2L2* – nuclear factor (erythroid-derived 2)-like 2) – a transcription factor that mediates the protective response against oxidative stress. But, as we recently proposed, KEAP1 can act independently from NRF2 and along with nitric oxide synthase (NOS) and transnitrosating protein GAPDH be involved in the formation of S-nitrosothiols, leading to changes in protein function. In NRF2-deficient ECs this leads to the accumulation of protein aggregates, loss of function and premature senescence.

Our data show that with age NRF2 level decreases in ECs, which suggests that the same dysfunctional phenotype of ECs can be occurring in physiological aging. Therefore, we study the relation between protein aggregation and cell function in young and aged human-derived primary endothelial cells with the possible involvement of S-nitrosation mediated by KEAP1/NOS/GAPDH SNO complex. Our results indicate that a higher level of protein aggregates appears with age along with the impairment of function, which can be restored by the modulation of KEAP1.

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Investigating the role of NPAS4 in endoplasmic reticulum under hypoxia and oxidative stress conditions

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NPAS4 (Neuronal PAS domain containing protein 4) is a transcription factor from the bHLH-PAS family. NPAS4 was discovered in neurons, where increased expression protected neuronal cells during ischemia. Later, its expression was documented in pancreatic β-cells, where NPAS4 was protective in type II diabetes, likely *via* modulation of endoplasmatic reticulum stress. The mechanism by which NPAS4 plays a protective role in hypoxia and oxidative stress remains to be elucidated. In our study, we analysed Npas4 expression in N2a neuronal cells using RT-qPCR. To track its localisation, we overexpressed YFP-tagged NPAS4 using confocal microscopy. Hypoxia was induced both chemically (DFO/CoCl□) and physically (hypoxia chamber), while oxidative stress was induced using H□ O□. We observed increased *Npas4* expression under physiological hypoxia, but no increase under chemically induced hypoxia. Under hypoxia, NPAS4 localized in the cytoplasm in a punctate pattern that partially colocalized with stress granules, which are membraneless organelles. This observation suggest a propensity of NPAS4 to phase separation. *Npas4* expression in hypoxia resulted in increased expression of *Perk*, *Atf6*, and to a lesser extent *Ire1*, while the expression of *Sod1* and *Nrf2* seems to be dependent on the expression of NPAS4 under oxidative stress. The results of our study suggest that NPAS4 has the ability to function in a non-genomic way, similarly to other representatives of the bHLH-PAS family, such as AhR.

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Cell membrane modifications as a determinant in research on the anticancer potential of venom proteins

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Proteins in animal venoms are increasingly recognized as valuable sources of potential therapeutic agents. The dual nature of snake venom fractions—their ability to exert both toxic and therapeutic effects—has sparked growing interest in their mechanisms of action. A key factor in evaluating their anticancer potential is understanding how venom proteins interact with cell membranes, particularly those of cancer cells. This study analyzes both model systems (Langmuir monolayers) and native membranes. Using a three-finger toxin fraction from spitting cobra (*Naja ashei*) venom, we investigated its interactions with promyelocytic leukemia cell line (HL-60). The experiments included cytotoxicity and oxidative stress assays in native cells, as well as the determination of physicochemical and thermodynamic parameters for single- and multi-component Langmuir monolayers mimicking HL-60 cell membranes. Modeling studies demonstrated the potential for toxin localization within membranes, a decrease in membrane stiffness, and mechanical interactions with lipid components. Biochemical analyses revealed cytotoxic effects and supported the model findings, indicating that the toxins disrupt membrane integrity. These results support the role of three-finger toxins in mechanisms leading to cancer cell death and suggest their potential as selective membrane modulators in future anticancer strategies.

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Analysis of Age-Related Differences in Human Serum Levels of Mitochondrial and Nuclear Circulating Cell-Free DNA

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Cell-free DNA (ccf-DNA), suggested to be released during cell damage, has gained recognition as a potential biomarker in neurodegenerative disorders. A recent study by Wojtkowska *et al*. (2024) reported increased levels of mitochondrial and nuclear ccf-DNA in the serum of patients with Parkinson's disease (PD). However, it remains unclear whether these changes reflect disease-specific mechanisms or are secondary to age-related processes.

Our study aims to address this question by analyzing the levels of both types of ccf-DNA in healthy individuals stratified by age. Specifically, we compared two control groups with distinct median ages: a younger group (median age = 27.5 years) and an older group (median age = 46 years). Preliminary quantitative PCR (qPCR) analysis showed no statistically significant differences in either mitochondrial or nuclear ccf-DNA levels between these age-defined groups.

These findings suggest that, in the absence of disease, age alone may not substantially influence circulating ccf-DNA levels. This supports the hypothesis that elevated ccf-DNA observed in PD patients is more likely to be disease-related rather than age-dependent. Further validation is currently ongoing using droplet digital PCR (ddPCR) to enhance sensitivity and quantification accuracy.

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Garlic under the heat lens: *Blanching* – the best way to preserve its antioxidant and anticancer power

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The study evaluated the impact of various thermal processing on the antioxidant properties and antiproliferative activity of garlic against two human cancer cell lines (PEO1 and SKOV-3), and its cytotoxicity for normal lung fibroblasts (MRC-5). Both garlic homogenates heated between 40–100 °C and whole garlic cloves subjected to blanching, roasting, and steaming were analysed. Antioxidant activity was assessed using five standard methods: ABTS$^{\bullet}$ and DPPH$^{\bullet}$ reduction, FRAP, CUPRAC, and ORAC. Results showed that blanching was the most favourable thermal treatment in terms of preserving the biological activity of garlic. Compared to roasting and steaming, blanched garlic retained significantly higher levels of phenolic compounds and total antioxidant capacity (TAC), with only a slight reduction in alliinase activity and thiosulfinate content. As a result, it maintained strong antiproliferative effects while exhibiting limited cytotoxicity toward normal MRC-5 cells. In contrast, roasting and steaming caused substantial losses of bioactive compounds, marked inactivation of alliinase, and a significant decrease in antiproliferative activity. Garlic homogenates heated up to 60 °C showed increased antioxidant capacity, but further heating led to the degradation of thiosulfinates and reduced cytotoxic effects. In summary, blanching was identified as the most beneficial thermal treatment, minimally affecting the garlic's health-promoting properties.

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From Genes to Defense: Transcriptomic Exploration of Major latex proteins and Glycine-rich proteins in Viral Infection

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Climate change, intensified agriculture, and emerging phytopathogens threaten global crop productivity, highlighting the need for resilient, sustainable plant varieties. Among proteins involved in plant defense, Major Latex Proteins (MLPs) and Glycine-Rich Proteins (GRPs) are notable. These protein families participate in responses to abiotic stresses (e.g., salinity, cold, drought), with gene expression levels modulated under such conditions, implying a role in adaptation. MLPs, members of the PR-10 family, possess a Bet v1-like domain capable of binding hydrophobic molecules such as phytohormones. GRPs, particularly class IV, often contain RNA recognition motifs or cold-shock domains and contribute to post-transcriptional regulation. Both protein families have been identified in *Chelidonium majus*, a non-model plant with ethnomedicinal relevance and limited genomic resources, historically constraining molecular studies. However, advances in sequencing now allow genome annotation and identification of MLP and GRP genes in *C. majus*. Our current work investigates transcriptomic changes in *Arabidopsis thaliana* during viral infection in transgenic lines overexpressing *C. majus* MLPs and GRPs. The heterologous expression of these proteins, coupled with their functional characterization, represents a novel approach to understanding their roles in plant antiviral defense. Ultimately, these findings could support the development of new plant protection strategies that integrate insights from phytotherapy and molecular plant defense.

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Profiling of N-Glycans in Head and Neck Cancer Cells Treated with Protein Kinase Inhibitors – A Pilot Study

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Introduction: The incidence of head and neck cancer (HNC) is rising globally, including in Hungary and Poland. HNC encompasses malignancies that are often difficult to diagnose and treat. Repurposing drugs with established mechanisms of action and efficacy in other cancer types is a promising therapeutic strategy. This pilot study investigated the effects of two protein kinase inhibitors, trametinib and dasatinib, on the glycan profiles of selected HNC cells.
Methods: In the study Detroit 562, FaDu and SCC25 cells were used. Following drug treatment, MTT assay and WB were performed. For glycomic analysis, the glycoproteins in the cell lysates were deglycosylated, and the released glycans were derivatized. The glycans were analyzed using MALDI-MS.
Results: MTT assay demonstrated a dose-dependent reduction in cell *via*bility after treatment. WB confirmed the effective inhibition of target signalling pathways. MALDI-MS analysis revealed remodeling of the cellular glycome of treated HNC cells.
Conclusions: Combining the MTT assay and WB results with the glycomic data suggests that the drugs used not only affect cell *via*bility and signaling but also modulate glycosylation. Glycan profiling revealed differences in the composition of glycans in HNC cells following treatment. Thus, glycans may serve as novel biomarkers or therapeutic targets in HNC.

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Interaction of red cabbage antioxidants with exogenous antioxidants

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Interactions between antioxidants are of interest. A combined action of two or more antioxidants can produce an effect greater or smaller than the sum of effects of individual antioxidants (synergism or antagonism). This study aimed to examine the effects of adding exogenous antioxidants (ascorbate, glutathione, gallate, Trolox and TEMPOL) on the antioxidant activity (AO) of anthocyanin-rich aqueous red cabbage extract in antioxidant assays of ABTS• reduction and FRAP, and the degradation of anthocyanins in the extract. The interaction coefficient IC = (AO of extract with antioxidant)/[(AO of extract) + (AO of antioxidant)] determined from the slope of dependence on AO on the concentration, was mostly <1 (0.74-1.07) in the ABTS reduction assay and decreased with increasing reaction time for most antioxidants, but gallate for equimolar concentrations of extract anthocyanins and exogenous antioxidants (2.5-25 μM). In the FRAP assay, so determined IC was > 1 for glutathione and TEMPOL (1.09-1.13) and < 1 (0.82-0.93) for other antioxidants, its values being higher for 30 min than for 5 min reaction. Ascorbate (2 mM) augmented the spontaneous degradation and degradation induced by 1 mM H_2O_2 of anthocyanins in the extract. Glutathione and Trolox (2 mM) augmented the spontaneous anthocyanin degradation but inhibited the H_2O_2-induced degradation while gallate (2 mM) was protective in both cases.

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From expression to cellular context: searching for regulatory lncRNAs in the mouse pituitary gland cells

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The pituitary gland (PG) is a central hub in the endocrine system, which controls the peripheral glands and plays a key role in the organism's homeostasis. It contains six endocrine cell types, each secreting distinct peptide hormones, as well as non-endocrine cells such as pituicytes and stem cells. The cell-type specificity and the impact of lncRNAs on the endocrine functions of PG remain unclear. In this study, we profiled lncRNA expression in male mouse PG at three postnatal stages of maturation (P1, P30, adult) and in two cell line models: TαT1 (thyrotropes) and AtT-20 (corticotropes), using RNA-seq. Based on multiple criteria, including expression profiles and genomic context, we chose 4 candidate lncRNAs for further functional studies. We analyzed cell-type specific expression in a few additional pituitary cell lines, a response to hormonal stimulation, subcellular localization and tissue specificity. These preliminary results reveal dynamic regulation of lncRNAs in PG, with many lncRNAs being upregulated during postnatal maturation. Additionally, we shed light on specific lncRNA in the cellular context.

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The efficacy of resistance induction against Cucumber mosaic virus (CMV) and Potato virus Y (PVY) by BTH and chitosan is dependent on the proper functioning of the DCL2 protein in *Nicotiana benthamiana* plants

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The lack of effective plant protection products against plant viruses necessitates alternative control strategies in field and greenhouse crops. These include removal of infected plants, vector elimination, and transmission prevention. Increasing interest is directed toward biostimulants—natural (e.g., chitosan) and synthetic (e.g., BTH: benzo[1,2,3]thiadiazole-7-carboxylic acid S-methyl ester) – which enhance plant physiology and activate defense responses, including post-transcriptional gene silencing (PTGS).

Our previous work showed that BTH induces defense responses in tomato plants infected with tomato mosaic virus (ToMV), including PTGS-related gene expression. The current study uses *Nicotiana benthamiana* mutants lacking functional *dcl2, dcl4*, or both genes. These plants were infected with cucumber mosaic virus (CMV) or potato virus Y (PVY) following treatment with salicylic acid, BTH, or chitosan.

Using GFP as a reporter, the compounds' influence on PTGS was assessed in the presence of viral silencing suppressors (2b, HC-Pro, p19). Viral RNA accumulation and PTGS-related gene expression were also analyzed. The antiviral effects of the biostimulants were dependent on functional DCL2, with the strongest response against CMV, where viral accumulation was significantly reduced.

Understanding these mechanisms may aid in developing alternative antiviral crop protection strategies.

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Microbiome profiling and abundance of *Fusobacterium nucleatum* in colorectal cancer tissues – pilot study

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Introduction: There is growing evidence that the gut microbiome plays a major part in the development of colorectal cancer (CRC). Certain members of the gut microbiota, such as *Fusobacterium* spp., *Escherichia coli* and *Bacteroides fragilis*, are suspected to play a key role in CRC, causing infection and carcinogenesis processes.

Aim: The purpose of the study was to compare the microbiome composition of colorectal cancer tumour tissue (G) and adjacent normal tissue (M) to evaluate the relationship between bacteria and carcinogenesis.

Materials and methods: Five patients (n=5) diagnosed with colorectal cancer participated in the study. Genomic DNA was isolated from tumour tissue and adjacent normal tissues. qPCR analysis was performed to assess the presence of *F. nucleatum* followed by next-generation sequencing (NGS) of the V3-V4 region of the 16S rDNA.

Results: Analysis by qPCR screening showed a trend towards higher abundance of *F. nucleatum* in G versus M ($p=0.0679$, Wilcoxon test). This is confirmed by the results obtained with NGS profiling, which also showed differences in the relative abundance of *Fusobacterium*.

Conclusions: Our results support a significant role for *Fusobacterium nucleatum* as bacterium associated with CRC. However, further studies on a larger number of patients are needed to confirm its potential as a causative agent or biomarker.

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Characterization and PCR application of a novel DNA polymerase from the metagenome of extreme environments

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This study reports the discovery, characterization, and PCR application of a novel DNA polymerase identified from the metagenome of extreme environments. Bioinformatic analysis revealed that the enzyme shares low sequence identity (<37%) with members of the B-family DNA polymerases, except for one uncharacterized DNA polymerase from a *Thermoprotei archaeon*, showing 90% identity. The gene encodes an open reading frame of 2,382 bp, corresponding to a protein of 793 amino acids with a predicted molecular weight of 91.18 kDa. The enzyme contains a nucleotidyltransferase domain and a 3'–5' exonuclease domain responsible for proofreading.

The recombinant enzyme was overexpressed in *Escherichia coli* BL21(DE3), purified by heat treatment followed by two steps of column chromatography, and subsequently biochemically characterized. The optimal pH for DNA polymerase activity was 8.0, and the optimal temperature was 80°C. Enzyme activity was dependent on the presence of magnesium ions. Notably, the enzyme exhibited exceptional thermostability, with a melting temperature (T_m) of 94.5 ± 0.03°C, and half-lives of approximately 120 and 45 minutes at 95°C and 99°C, respectively. Optimal PCR conditions were established, confirming the enzyme's suitability for high-temperature PCR applications.

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The sensitivity of the Horvath's epigenetic clock to simulated global methylation offset

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Introduction: Methylation clocks predict biological (epigenetic) age, which highly correlates with chronologic age. The broadly applied Horvath's clock also works across tissues and is thus considered insensitive to global methylation. We hypothesized that Horvath's methylation age is sensitive to simulated changes in global methylation levels.
Methods: Whole blood was obtained from 82 children with inflammatory bowel disease (37 female, 45%) aged 6–18 years, mean 14.3 ± 3.1 years. After bisulfite conversion, DNA was hybridized onto Illumina EPICv2 arrays. Data analysis was carried out with minfi (1.54.1; noob correction) and methylclock (1.14.0). We simulated increases and decreases of global methylation by altering M value matrices with an offset (0.1 yielded <2% beta value change).
Results: Samples' mean M values ranged from 0.25 (beta=54% methylation) to 0.80 (64%). Epigenetic age depended on the simulated global M value offset: mean age 36.7 years at M value offset -2; 26.6 years at -1; 20.4 years at -0.5; 15.8 years at -0.1; 14.8 years at no offset; 13.8 years at +0.1; 10.7 years at +0.5; 8.1 years at +1; and 6.0 years at +2. Linear correlation between epigenetic and chronologic age also changed with the offset: r=0.70 at -2; r=0.74 at -1; r=0.70 at no offset; r=0.67 at +1; and r=0.59 at +2.
Conclusion: The Horvath's methylation clock can be sensitive to biologically relevant offset in global methylation levels. (Bioethics: Poznań Univ. Med. Sci. 960/15+amendments).

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Expression of programmed cell death (PCD) markers in colorectal cancer cells DLD1 and HCT116 treated with mesalazine

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Programmed cell death (PCD) is essential for preventing tumor development and progression. In addition to apoptosis, several PCD subtypes—such as necroptosis, pyroptosis, ferroptosis, panoptosis, autophagy, cuproptosis, and alkaliptosis—have been identified, each with distinct molecular mechanisms. These pathways influence tumor initiation, immune evasion, and therapy resistance. Understanding the activation context of these pathways is critical for developing targeted therapies, though detecting specific PCD markers remains challenging. Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, highlighting the need for novel therapeutic strategies. One emerging approach is drug repurposing—using existing drugs for new oncological indications.
This study aimed to evaluate the effect of mesalazine on colorectal cancer (CRC) cells and its role in modulating PCD-related pathways. Two CRC cell lines, DLD-1 and HCT116, were treated with mesalazine. Gene expression profiling of PCD-related markers was performed using the Affymetrix HG U133A 2.0 microarray platform.
Mesalazine induced distinct transcriptional responses in the two cell lines, with differential regulation of genes associated with various PCD pathways. These findings suggest cell-type-specific activation of molecular mechanisms by mesalazine, which may modulate multiple forms of PCD in a cell-specific manner, offering new insights into its potential as a repurposed anticancer agent in CRC therapy.

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Host factors shaping the human gut mycobiota

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Recent studies have highlighted the important role of gut fungi in human health. In this study, we analyzed the composition and diversity of the gut mycobiota in 923 individuals from the European population and explored its associations with 53 host-related sociodemographic, lifestyle, health, and dietary factors. Fecal DNA was analyzed using whole-metagenome high-throughput sequencing to obtain taxonomic profiles of fungal species, followed by computational and statistical analyses.
The gut mycobiota was dominated by *Saccharomyces*, *Candida*, and *Sporisorium*. Ten factors, mainly dietary, showed significant associations with overall mycobiota variation. These included the consumption of chips, meat, sodas, sweeteners, processed foods, and alcohol, as well as age and marital status. Differences in fungal diversity and composition were also associated with body mass index, occupation, autoimmune diseases, and probiotic use. Differential abundance analysis identified fungal species that varied in relation to specific host conditions.
Our findings confirm that yeast species such as *Saccharomyces*, *Malassezia*, and *Candida* dominate the human gut mycobiota. Despite high inter-individual variability, certain species were consistently detected across most samples, suggesting the presence of a core gut mycobiota. This study presents the first comprehensive, large-scale analysis of gut fungal communities in a European population and highlights the influence of host-related factors on mycobiota composition and diversity.

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Epigallocatechin gallate (EGCG) corrects behavior in a mouse model of mucopolysaccharidosis I (MPS I)

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Epigallocatechin gallate (EGCG) is a catechin derived from green tea. It has been shown to possess numerous health-promoting properties. Moreover, it is known to cross the blood-brain barrier. Mucopolysaccharidoses (MPS) are a group of hereditary diseases in which the degradation of glycosaminoglycans (GAGs) by lysosomal enzymes is impaired, resulting in cellular and tissue dysfunction and severe, multi-organ symptoms. MPS I subtype can be assigned to neuronopathic forms of MPS due to the manifestation of symptoms related to the central nervous system (CNS). In the mouse model of MPS I, we can also observe several of these symptoms, including impaired long-term and short-term memory, increased anxiety, and decreased locomotor activity. To assess the effects of EGCG supplementation on MPS I mice behavior, we performed four behavioral tests: the measurement of locomotor activity in actometers, the open-field test, the novel object recognition test, and the elevated plus-maze test. We have observed that supplementation *via* oral gavage with EGCG at two doses: 75 mg/kg/day and 150 mg/kg/day for 3 months, not only normalized the level of GAGs but also corrected the behavioral pattern in animals. It is important to notice that we did not observe any adverse effects of this treatment. The positive influence of EGCG can be due to reduced neuroinflammation, particularly in regions of the brain associated with memory and anxiety, such as the hippocampus, medial septal nucleus, or amygdala.

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Single-cell profiling of mesothelial differentiation reveals stage-specific markers and potential regulators of WT1$^+$ fate

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During early embryogenesis, mesothelial Wilms Tumor 1+ (WT1$^+$) cells arise from the splanchnic mesoderm (SM), which contributes to the formation of digestive and respiratory organs as well as the heart. While studies in model organisms show SM is abundant and heterogeneous, the molecular mechanisms driving its differentiation into mesothelial cells remain poorly understood. To investigate this process, I performed single-cell RNA sequencing (scRNA-seq) on human pluripotent stem cells (hPSCs) undergoing stepwise *in vitro* differentiation into mesothelial (ME) cells. Analyzed stages included: hPSC, middle primitive streak (Mid.PS), lateral plate mesoderm (LPM), SM, and ME. Bioinformatic analysis – including differential gene expression and pseudotime trajectory mapping – revealed novel candidate marker genes for specific stages. Notably, NFIB, ROBO2, and PDE3A emerged as potential regulators of ME development. To explore NFIB's role, I used an hPSC line with inducible NFIB overexpression. Induction at the LPM stage significantly increased WT1$^+$ cell numbers by day 7, supporting scRNA-seq predictions. NFIB overexpression also reduced total cell numbers and the thickness of the resulting layer. These findings enhance our understanding of mesothelial development and its broader implications, for instance, in organogenesis and cancer research.

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Influence of hypoxia on the expression of L-cysteine-metabolizing enzymes and the proliferation of human neuroblastoma cells

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Neuroblastoma (NB) is a cancer of peripheral nerve tissue that typically arises outside the central nervous system. It is one of the most common cancers in children and is often linked to MYCN oncogene amplification, which correlates with poor prognosis and therapy resistance. Non-oxidative L-cysteine metabolism, which is associated with production of hydrogen sulfide and sulfane sulfur-containing compounds, may play a role in redox regulation in NB cells. The main enzymes involved in L-cysteine metabolism are: cystathionine β-synthase (CBS), cystathionine γ-lyase (CTH), 3-mercaptopyruvate sulfurtransferase (MPST), and thiosulfate sulfurtransferase (TST). The study was performed on two human neuroblastoma cell lines - SH-SY5Y (non-MYCN-amplified) and LAN-1 (MYCN-amplified) cells. LAN-1 cells showed significantly higher expression of the MYCN gene as well as CBS under normoxia. We also studied the influence of low oxygen environment (using Modular Incubator Chamber) on SH-SY5Y and LAN-1 cell proliferation and selected genes expression. Hypoxia led to decreased proliferation in LAN-1 cells, while no such effect was observed in SH-SY5Y cells. Expression of phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 (PFKFB4) was induced in both cell lines under hypoxic conditions. In addition, significant changes in CBS and MPST expression were observed. The obtained results show that changes in sulfur metabolism may have significant implications for the proliferation of NB cells.

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IGH enhancer RNAs - new players in B-cell lymphoma

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Chromosomal translocations in non-Hodgkin lymphoma (NHL) result in activation of oncogenes by placing them under the regulation of immunoglobulin heavy chain (IGH) super-enhancers. Aberrant expression of translocated oncogenes induced by enhancer activity can contribute to lymphomagenesis. The role of the IGH enhancers in normal B-cell development is well established, but knowledge regarding the precise mechanisms of their involvement in control of the translocated oncogenes is limited. We performed a tiling CRISPR interference screen in three NHL cell lines and identified three regions crucial for NHL cell growth. With chromatin-enriched RNA sequencing we showed transcription from the core enhancer regions and subsequently validated expression of the enhancer RNAs (eRNA) in a panel of NHL cell lines and tissue samples. Inhibition of the essential IGH enhancer regions decreased expression of eRNA and translocated oncogenes in several NHL cell lines. Furthermore, silencing of the eRNA from the Eμ region also inhibited NHL cell growth, indicating the role of the eRNA transcript itself.

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Innovative CPL976H-MMAE: A Humanized Bifunctional Conjugate for Superior Cancer Treatment Targeting PD-L1 and AXL.

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Immunotherapy combined with chemotherapy shows promising results but may cause significant toxicity. Antibody-drug conjugates (ADCs) offer an alternative by enabling targeted drug delivery that enhances efficacy while minimizing off-target effects. The CPL976H-MMAE is bispecific ADC based on an anti-PD-L1/AXL antibody conjugated with the cytotoxic monomethyl auristatin E (MMAE). This approach enables precise therapy targeting immune evasion and tumor growth.

CPL976H-MMAE was engineered using site-specific Fc-glycan remodeling and click-chemistry. Surface Plasmon Resonance (SPR) confirmed target binding. *In vitro* and *in vivo* studies assessed efficacy, cytotoxicity, internalization and selectivity across cancer cell lines and xenograft mouse models.

CPL976H-MMAE shows strong, selective cytotoxicity against targets-expressing cancer cells by enhancing uptake, inducing dose-dependent cell cycle arrest and apoptosis. *In vivo* studies showed tumor regression across all tested doses, with efficacy exceeding that of free MMAE. CPL976H-MMAE was well-tolerated, showed no toxicity, induced durable tumor regression with efficient accumulation and sustained retention in tumor tissue.

CPL976H-MMAE represents a dual-action strategy, combining bispecific antibody-guided MMAE delivery with degradation of targets driving tumor growth and immune evasion. It offers a option for patients with primary or acquired resistance to PD-1/PD-L1 therapies.

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Functional Screening of Small-Molecule TMPRSS2 Inhibitors Using a Fluorogenic Substrate Assay: Implications for Host Protease Targeting in Antiviral Therapy

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TMPRSS2 is a membrane-anchored serine protease that facilitates viral entry by activating surface glycoproteins, including the SARS-CoV-2 spike. Its inhibition blocks membrane fusion, offering a host-targeted antiviral strategy.

In this study, we performed a structure-based screening of a small-molecule library to identify TMPRSS2 inhibitors using a quantitative, fluorogenic assay. Recombinant TMPRSS2 and a synthetic peptide substrate were used in a microplate-based system to monitor enzymatic activity in real-time *via* a fluorescent signal (λ_{ex} = 383 nm / λ_{em} = 455 nm). The assay was validated with camostat, a known TMPRSS2 inhibitor (IC_{50} ~1.9 nM). Compounds showing ≥50% inhibition in preliminary screens were subjected to IC_{50} determination.

A total of 41 compounds exceeded the 50% inhibition threshold. Among these, 28 showed sub- to low-micromolar potency ($IC_{50} \leq 5 \pm 0.5$ μM). The reproducible dose-response profiles and sharp inhibition curves indicate direct binding to the catalytic domain. No cytotoxicity was observed in DMSO-matched controls.

Although viral variants may bypass TMPRSS2 dependency, this host protease remains a biologically validated target for future pandemics and infections caused by epithelial viral entry. The enzymatic assay workflow developed here provides a robust and scalable platform for discovering serine protease inhibitors with potential antiviral or anticancer applications.

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Distinct expression patterns of H_2S-metabolizing enzymes in human L-363 multiple myeloma cells

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Multiple myeloma (MM) remains a largely incurable malignancy of plasma cells. The transsulfuration and L-cysteine desulfuration pathways, central to endogenous H□ S and sulfane-sulfur metabolism, are implicated in MM cell proliferation and survival and have emerged as attractive targets. Cystathionine β-synthase (CBS), cystathionine γ-lyase (CTH), 3-mercaptopyruvate sulfurtransferase (MPST), thiosulfate sulfurtransferase (TST), and thiosulfate sulfurtransferase-like domain-containing protein 1 (TSTD1) are crucial enzymes that participate in L-cysteine, H□ S, and sulfane-sulfur metabolism, maintain redox and mitochondrial homeostasis, and participate in cyanide detoxification. In this study, human L-363 myeloma cells were analyzed for expression of TST, MPST, CTH, CBS, and TSTD1. The effect of varying concentrations of sodium thiosulfate (STS; a substrate for TST) on L-363 cell proliferation was assessed. In L-363 cells, expression of CBS and MPST significantly exceeded that of mitochondrial TST. In turn, CTH expression in these cells was negligible. Interestingly, expression of cytosolic TSTD1 was the highest among all examined enzymes. In the presence of sodium thiosulfate, we observed a concentration-dependent decrease in L-363 cell numbers.The high expression of TSTD1, CBS, and MPST in L-363 cells, along with STS-mediated inhibition of proliferation, suggests that modulation of expression/activity these enzymes may play a significant role in myeloma cell function.

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The Effect of PKR Inhibitor 2-Aminopurine on Influenza A Virus Replication and Segment 8 mRNA Splicing

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Influenza A virus (IAV) segment 8 encodes NS1 and NS2 proteins, whose expression depends on alternative mRNA splicing. Recent studies suggest that structured RNA elements, such as pseudoknots, may modulate splicing *via* PKR activation. Here, we investigated whether inhibiting PKR with 2-aminopurine (2-AP) affects IAV replication and segment 8 mRNA processing. Using HEK-293T, MDCK 2, and A549 cells infected with IAV (H1N1), we found that 2-AP reduced viral replication in a cell-specific manner and significantly altered NS1 and NS2 expression levels. In A549 cells, 2-AP increased the NS2/NS1 mRNA ratio, suggesting splicing modulation. Our findings indicate that 2-AP not only inhibits PKR but also exerts antiviral effects and may influence IAV mRNA splicing, highlighting its potential as a research tool and therapeutic candidate.

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Antioxidant properties of fresh and fermented white and red cabbage

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Antioxidant properties and multiple beneficial health effects of anthocyanins have been convincingly demonstrated, so consumption of anthocyanin-rich vegetables and fruits should be recommended. Red cabbage is the most popular anthocyanin-rich vegetable in Poland. This study aimed to compare the antioxidant properties of white and red cabbage, both fresh and fermented (sauerkraut). The phenolic content and total antioxidant capacity (TAC) estimated by the ABTS$^{\bullet}$ decolorization assay, DPPH$^{\bullet}$ decolorization assay, FRAP, and ORAC of red cabbage were significantly higher than those of white cabbage (6 915 ± 91 vs 821 ± 10 mg gallic acid equivalents/kg, 87.9 ± 3.3 vs 5.8 ± 0.2, 63.9 ± 2.0 vs 2.4 ± 0.1, 36.2 ± 0.9 vs 1.2 ± 0.1 and 110.3 ± 17.2 vs 5.1 ± 0.2 Trolox equivalents/kg, respectively. Cabbage subjected to 15-day fermentation showed lowered content of anthocyanins and phenolics, and lowered TAC, due to the extraction of antioxidant components to the juice, which also had a significant antioxidant capacity, comparable to that of the solid fermented cabbage; therefore, consumption of juice of fermented red cabbage can also be encouraged. In red cabbage, fermentation caused a hypsochromic shift in the absorption spectra and changes in the fluorescence spectra of anthocyanins.

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The cytoskeletal protein AMOTL2 regulates metabolic control of germ layer specification in human pluripotent stem cells

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Emerging evidence shows that metabolic processes act as guiding signals during cell development, but their specific function in germ layer determination in human is not fully clear. It is also uncertain whether metabolism directly drives cell fate choices or simply refines decisions that are already predetermined. In this study, we discover that AMOTL2, a cortex protein, acts as a previously unknown controller of germ layer development in human. When this protein is removed, cells preferentially develop into ectoderm. We demonstrate that AMOTL2 regulates proliferation, colony morphology, F-actin polymerization, and Hippo signaling in human pluripotent stem cells. We further exclude F-actin polymerization and YAP1 activity as direct cues for human germ layer specification, instead identifying a distinct mechanism that relies on AMOTL2-mediated metabolic reprogramming. AMOTL2 normally limits OXPHOS and glycolysis, and when depleted, it increases OXPHOS and promotes alternative glutamine processing - ultimately promoting ectoderm development. Our research reveals an unexpected metabolic function for AMOTL2 in determining cell fate and suggests that cytosolic proteins can link metabolic processes to developmental outcomes.

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Evaluation of Histone Lysine Demethylase Inhibitors in a Head and Neck Cancer Model

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Histone lysine demethylases (KDMs) have recently gained attention as promising therapeutic targets due to their role in the epigenetic regulation of gene expression in cancer. In head and neck squamous cell carcinoma (HNSCC), the overexpression of LSD1, KDM4, KDM5, and KDM6 has been linked to poor prognosis and increased metastatic potential. In particular, KDM4A, KDM5B, and KDM6B have been implicated in the regulation of stemness-related genes, suggesting their involvement in therapy resistance and tumor recurrence.

Our study evaluated the anticancer efficacy of selected KDM inhibitors – GSK-J4, ML324, and JIB-04—as well as their combinations with cisplatin. HNSCC cell lines, cultured as monolayers and 3D spheroids, were treated with the compounds, and cell *via*bility was assessed using the resazurin assay. Changes in gene expression were analyzed by qPCR.

All tested compounds were able to reduce cell *via*bility, with JIB-04 showing the highest potency. Also, treatment of the tested cell lines with KDM inhibitors resulted in the down-regulation of stemness-associated genes. Notably, combining KDM inhibitors with cisplatin enhanced cytotoxicity in 3D cultures.

Our findings support the potential of KDM inhibitors as therapeutic agents in HNSCC.

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BbKI improves insulin sensitivity in palmitate-treated C2C12 myotubes by targeting Akt signaling pathway

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Type 2 diabetes (T2D) is a chronic metabolic and endocrine disorder marked by persistent hyperglycemia. The development of T2D involves multiple pathogenic mechanisms, including peripheral insulin resistance and progressive dysfunction of pancreatic β-cells. A wide range of natural compounds have demonstrated antidiabetic properties and are increasingly explored as adjunct therapies to manage T2D and its associated metabolic disorders. Among these, peptide derived from the *Bauhinia bauhinoides* plant - such as BbKI - have shown promising therapeutic potential. In this study, we aimed to investigate the molecular mechanisms underlying the hypoglycemic effects of BbKI. Experiments were performed using differentiated C2C12 myotubes treated with BbKI, palmitate (16:0), or their combination. To assess the effects on endoplasmic reticulum (ER) stress and insulin signaling, we analyzed the expression of key genes and proteins using RT-PCR and Western blotting. Our results demonstrate that BbKI significantly enhanced insulin signaling in C2C12 myotubes subjected to lipotoxic stress. Specifically, we observed increased phosphorylation of major signaling proteins, including Akt (Ser473), AS160 (Thr642). Under lipotoxic conditions BbKI also reduced the expression of ER stress markers – CHOP, BiP, and phospho-PERK. Together, these findings underscore the therapeutic potential of the BbKI peptide in mitigating lipotoxicity by enhancing insulin signaling.

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Altered expression of mitochondrial proteins in astrocytes isolated from a rat model of Parkinson's disease. Changes due to neuron and/or astrocyte loss

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Astrocytes are neuronal supporters in energy function. Their loss is harmful for nervous system in Parkinson's disease. Partial degeneration of dopaminergic neurons can be compensated when astrocytic support is effective. How it affects cell metabolism is unknown.

Aim: to check how neuron vs astrocyte degeneration affected remaining astrocyte mitochondrial proteome.

7-day infusion of fluorocitrate into substantia nigra (SN) killed 30% astrocytes (10.1016/j.mito.2018.12.002). 6-OHDA caused dopaminergic neuron degeneration. Astrocytes sorted from dissociated brain SN tissue by FACS were analyzed for mass spectrometry proteome on timsTOF Pro 2.

Neuron death decreased expression of CxI proteins (Ndufs2/3, Ndufv1, Ndufa6/9/10), CxIII (Cyc1) and Vdac1, Slc25a4. Astrocytic death decreased the same CxI and CxIII and Vdac1 but also Aco2, Ldhb, Hsp90aa1, Atp1a1 and 3, Cnp, Ckb.

When both astrocytes and neurons were affected all mitochondrial complexes markers decreased: CxI (Ndufs2/3, Ndufv1/2, Ndufa6/9/10), CxII (Sdha), Cx III (Cyc1, Uqcrc2), CxIV (Cox5a), CxV (Atp5f1a/b/c) and Vdac3. Main difference between neuron vs astrocyte loss were observed in increased Dlst, Ahcyl1 and Rap1gds1 vs Arg1.

The current data indicate that dying astrocytes become activated and shift their energy metabolism from oxidative respiration to other sources.

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Lymphocyte-activation gene 3 and Galectin-3 in non-small cell lung cancer

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Lymphocyte-activation gene 3 (LAG-3) is an inhibitory receptor expressed, among others, on T cells, NK cells, and Tregs. LAG-3, along with TIM-3 and TIGIT, belongs to the next generation of immune checkpoints and has been linked to immunosuppression in the tumor microenvironment (TME). We analysed LAG-3 and GAL-3 (one of the LAG-3 ligands) in NSCLC TME and its two main histological subtypes, adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC), at the mRNA (ddPCR) and protein levels (immunohistochemistry and/or multiparametric flow cytometry). LAG-3 expression on TILs was assessed by IHC in 68 tumor tissues (TTs), 34 of which were negative. Expression above 5% was found in 28 TTs (41.18%). GAL-3 expression was assessed in 65 TTs. One TT (1.54%) was GAL-3 negative, and the remaining 64 TTs (98.46%) presented the H-score above 10. We observed high correlations at the mRNA level ($p<0.0001$) between 1) LAG3 and PDCD1, 2) HAVCR2 and LAG3, 3) TIGIT and LAG3, as well as between CD274 and LGALS3 ($p=0.0029$). These correlations were also observed at the protein level ($p < 0.001$) in CD3+ cells, specifically between PD-1 and LAG-3, as well as between TIM-3 and LAG-3. The expression of LAG-3 did not differ between LUSC and LUAD; however, we observed higher GAL-3 expression in LUAD than in LUSC. In summary, our results demonstrate a high correlation between LAG-3, PD-1, and TIM-3 expression. Additionally, our results suggest that GAL-3 may be differentially expressed in LUSC and LUAD.

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Selective inhibition of β_2-adrenergic receptors impairs the viability of papillary renal cell carcinoma (pRCC) cells

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The β2-adrenergic receptor (β2-AR), activated by epinephrine and norepinephrine, is widely expressed in tissues and frequently overexpressed in tumours. β2-AR activation promotes cancer cell proliferation, survival, and metastasis. β2-blockers exhibit antitumor effects, though efficacy varies by tumour type. Renal cell carcinoma (RCC), a heterogeneous group of kidney tumours, is mainly represented by clear cell (ccRCC) and papillary (pRCC) subtypes, which differ molecularly and genetically. Comprehensive data on β2-AR's role in RCC are lacking, with only limited evidence pointing to therapeutic benefits of β2-AR targeting in von Hippel-Lindau-related ccRCC. Therefore, we investigated the impact of β2-AR modulation on pRCC cell *via*bility. We have shown that *ADRB2* (encoding β2-AR) expression is higher in pRCC cell lines (Caki-2, ACHN) than in ccRCC lines (Caki-1, 786-O), what is consistent with the data from pRCC and ccRCC clinical samples deposited in The Cancer Genome Atlas. The selective inverse agonist/antagonist ICI-118,551 reduced *via*bility in pRCC cell lines (Caki-2 and ACHN) while the agonist salbutamol had no effect. Moreover, β2-AR blockade with ICI-118,551 enhanced the inhibitory effect of sunitinib, a tyrosine kinase inhibitor, on *via*bility of both pRCC lines. While sunitinib is used to treat RCC, it is notably less effective against pRCC than against ccRCC when administered as monotherapy. The combination of both drugs may offer therapeutic benefits in the treatment of pRCC.

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Mitochondrial dysfunction caused by the SARS-CoV-2 main protease in a yeast model

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Yeasts have been used as model organisms in multiple studies, which have led to key discoveries in the fields of cell and molecular biology, genetics and medicine. As essential biological processes are functionally conserved, studies in yeast models have direct implications for medical research. In this study we used a yeast model to investigate the effect of SARS-CoV-2 main protease (Mpro) on cells, focusing on its effects under both fermentative and respiratory conditions. Both fully active Mpro and less active Mpro were expressed in yeast. We show that active Mpro caused growth arrest on nonfermentable carbon sources. What is more, severe impairment of mitochondrial function was observed. Basal respiration, as well as spare respiratory capacity and ATP-linked respiration were significantly reduced in active Mpro expressing cells. while a strain expressing a proteolytically less active Mpro variant remained largely unaffected and retained normal mitochondrial function. These findings were supported by the observation of severe abnormalities in mitochondrial morphology and mitochondrial membrane potential in active Mpro expressing strain.
Our findings suggest that SARS-CoV-2 Mpro targets yeast mitochondrial pathways that are critical for respiratory metabolism. As many aspects of mitochondrial physiology are highly conserved among eukaryotes these findings may be relevant for pathophysiological mechanisms underlying COVID-19.

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Optimization of beige adipogenic differentiation of Adipose-Derived Stem Cells

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In recent years, brown adipose tissue has attracted increasing interest as a potential target for treating metabolic diseases such as type 2 diabetes and obesity. Developing a standardized *in vitro* model for studying adipogenesis and beige fat metabolism is essential to advance this research.
The aim of this study was to optimize the differentiation of Adipose-Derived Stem Cells into beige adipocytes and to evaluate the effectiveness of the optimized conditions on primary ADSC lines obtained from patients. Protocol optimization was performed using a commercial ADSC line, testing over 30 variants of adipogenic conditions. Differentiation was assessed by microscopy, Oil Red O staining, and UCP-1 expression analysis *via* RT-qPCR and Western blot. Generated cells exhibited lipid droplets and UCP-1 expression. The findings demonstrated that enriching the adipogenic medium with 1 μM dexamethasone throughout the culture period, along with extending the induction phase from the standard 4 days to 8 days, significantly increased differentiation efficiency. The medium was most effective when supplemented with 5 μM rosiglitazone and 20 μg/ml insulin.
This study successfully optimized a method for differentiating ADSCs into beige adipocytes, effective for both commercial and patient-derived cell lines. However, differentiation efficiency varied significantly among patient samples, likely due to individual variability or environmental factors, which requires further investigation.

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Liraglutide Modulates microRNA Expression in Ovarian Cancer Cells

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Ovarian cancer (OC) is a leading gynecologic malignancy with poor prognosis and high mortality. Current screening methods are insufficient, while microRNAs (miRNAs) have emerged as promising biomarkers for cancer diagnosis and prognosis.
This study investigates the impact of liraglutide, a novel antidiabetic drug, on miRNA expression and metabolic pathways in SKOV-3 and OVCAR-3 ovarian cancer cell lines. SKOV-3 and OVCAR-3 cells were treated with liraglutide, followed by RNA extraction and miRNA profiling using GeneChip miRNA 4.0 microarrays. Selected miRNAs were validated by qPCR; analysis involved RMA normalization and one-way ANOVA with $p < 0.05$ and ≥2-fold change as cutoffs. Liraglutide treatment led to differential expression of 21 miRNAs in SKOV-3 (13 up-regulated, 8 down-regulated) and 3 up-regulated miRNAs in OVCAR-3 cells. These results show liraglutide may modulate regulatory networks in ovarian cancer *via* miRNA-mediated mechanisms, with cell line–specific responses. Liraglutide could impact cancer pathophysiology beyond glycemic control. Further studies are needed to define the biological roles of these altered miRNAs and their therapeutic implications. miRNA profiling may ultimately improve early OC diagnosis, prognosis, and treatment, especially for patients with diabetes.

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A novel, protein-truncating variant of *CFAP221* is a cause of a mild form of primary ciliary dyskinesia

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Primary ciliary dyskinesia (PCD) is a rare, genetically and clinically heterogeneous disease characterized by the dysfunction of motile cilia, organelles protruding from the surface of many eukaryotic cells. One gene whose role in the PCD pathogenesis requires further evidence is *CFAP221* (cilia and flagella associated protein 221).

Using whole-exome sequencing we identified a novel, homozygous variant in *CFAP221* in a PCD patient and we investigated its effect on the motile cilia structure and function in the patient's nasal epithelial cells using immunofluorescence, high-speed videomicroscopy, and mucociliary transport assay. Obtained results indicated subtle abnormalities in the proteins composition of the ciliary central apparatus, which were consistent with the asynchronous, circular motion of cilia and reduced ciliary beat frequency.

To confirm the evolutionary conserved role of *CFAP221* in motile cilia function, RNA interference (RNAi)-mediated knockdown of *CFAP221* homolog (*Smed-cfap221*) was performed in a ciliated flatworm, *Schmidtea mediterranea*. Knockdown of *Smed-cfap221* led to a slight change in the worm's locomotion speed by impairing motile cilia function.

Our study provided an independent confirmation of the involvement of *CFAP221* in the pathogenesis of PCD. The subtle effect of *Smed-cfap221* knockdown was consistent with the mild course of PCD in the patient.

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Cytokine profiling in non-small cell lung cancer (NSCLC) patients to discover novel diagnostic approaches

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Lung cancer is one of the leading causes of mortality worldwide. According to the World Cancer Research Fund, 1,817,469 people died from lung cancer globally in 2022. Non-small cell lung cancer (NSCLC) represents 87% of lung cancers and involves a number of different lung cancers, particularly adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. The most frequent symptom of the disease is cough, which poses a difficulty in diagnosis in the early stages. In the advanced phase, hemoptysis, indigestion, and chest pain additionally appear. Detection of NSCLC in patients is mainly based on computed tomography. Consequently, new diagnostic approaches are being sought for NSCLC.

Therefore, we screened various cytokines in 76 NSCLC patients looking for early metastasis markers and novel diagnosis pathways. Plasma samples were collected at the Medical University of Gdańsk. Patients were divided into a control (NSCLC patients without metastasis), pleural metastasis, non-pleural metastasis, and secondary NSCLC groups. Received samples were used to analyze cytokine levels using Luminex® xMAP® technology. The appearance of e.g., MMP2, MMP9, osteonectin, periostin, tweak, OPG, MIP1α, IGFBP-3, Dkk1, IL-4, IL-6, and IL-13 was studied. An increased level of IGFBP-3 was observed in patients with non-pleural metastasis.

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Remodelling of the NANOS1 proteome by a variant linked to the absence of germ cells in the patient's testes

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The NANOS morphogen is essential for maintaining germ cell identity across various species by preventing somatic fate. Its highly conserved CCHC zinc-finger domain allows interactions with diverse RNAs and proteins. We previously identified a NANOS1 variant (vNANOS1) linked to the absence of germ cells in patients' testes. Recently, we found that vNANOS1 disrupts canonical WNT signalling during primordial germ cell (PGC) differentiation, leading to a significant reduction or loss of PGCs, alongside a notable increase in RNA targets. The current study aimed to assess whether protein-protein interactions of vNANOS1 were remodelled. The PiggyBac doxycycline-inducible transgenic stable cell lines, expressing both wt and vNANOS1, were used as previously described. The study was performed at the pre-mesodermal (preME) and PGC stages. NANOS1-bound proteins were co-immunoprecipitated, identified, and quantified using tandem mass tag mass spectrometry. In both the preME stage and PGCs, among the most enriched proteins for wt and vNANOS1, approximately half were RNA-binding proteins. Of these, only two proteins in the preME and one in the PGC stage were common to both wt and vNANOS1. We are currently analysing the significance of this extensive NANOS1 ribonucleoprotein interactome remodelling to understand the mechanism behind the loss of PGCs in patients carrying vNANOS1.

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Membrane Dynamics in the Epithelial-to-Mesenchymal Transition of Breast Cancer Cells

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Epithelial-to-mesenchymal transition (EMT) enables epithelial cancer cells to acquire a motile, invasive phenotype, driving metastasis and therapy resistance. While EMT-associated signalling has been widely studied, plasma membrane remodelling in this process remains poorly understood. The membrane organizes lipids and proteins, including cholesterol-rich domains and scaffolding proteins such as flotillins, to coordinate key signalling events. We propose that EMT is linked to plasma membrane reorganization, altering its lipid composition and physical properties. Our study uses a breast cancer model cantered on MCF-7 cells. EMT was induced transiently by TGF-β1 or stably by Snail overexpression, alongside vector-only and inhibitor controls, and compared to the mesenchymal-like MDA-MB-231 line. This system enables comparison of reversible and stable EMT states and their impact on membrane features. Our analyses showed that TGF-β1 treatment increased cellular cholesterol, while GM1 ganglioside levels decreased in both TGF-β1-treated and mesenchymal cells, pointing to shifts in lipid balance during EMT. Importantly, fluorescence lifetime imaging with a tension-sensitive probe revealed changes indicating reduced membrane order and increased fluidity in TGF-β1 and Snail cells. Together, these findings suggest that EMT is accompanied by notable alterations in plasma membrane organisation, offering a fresh angle to explore how lipid changes may contribute to tumour cell plasticity.

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Role of the FGFR1 C-terminal region in protein stability and maturation

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FGFR1 is a transmembrane tyrosine kinase receptor belonging to the FGFR family, which mediates cell signaling in response to specific FGFs ligands. We postulate that its unstructured C- terminus constitutes a switchable protein-protein interaction hub that enables precise regulation of FGF/FGFR signaling and trafficking, thereby determining cell fate. Here, we analyzed the role of the C-terminal region in the integrity of FGFR1. Transient expression of FGFR1 lacking the C-tail resulted in rapid degradation of the receptor and, consequently, reduced protein levels compared to the full-length receptor. Based on this, we hypothesized that the C-tail is essential for proper receptor folding and stability. Our proteomic analysis identified Hsp90 as one of the potential proteins binding to the C-terminus of FGFR1. Therefore, we decided to investigate whether there is a relationship between the C-terminal fragment of FGFR1 and Hsp90 activity in the context of receptor stability. Inhibition of Hsp90 using the specific inhibitor 17-AAG led to a reduction in the level of full- length FGFR1, indicating that Hsp90 is involved in receptor maturation. The observed receptor pattern was similar to that observed for FGFR1 lacking the C-tail. This suggests that the C-terminal fragment may influence the folding and stability of FGFR1 in an Hsp90-dependent manner.

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HAND2 Enhances NR5A1-Driven *SOX9* Expression Critical for Testis Development: Insights from a *HAND2* variant associated with 46,XY Gonadal Dysgenesis

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The expression of *SOX9* is essential for male gonadal sex determination, directing bipotential gonads toward testis development during early embryogenesis. This expression is driven by NR5A1, a transcription factor whose activity is modulated by its cofactor GATA4. Pathogenic variants in any of these key genes – *SOX9*, *NR5A1*, or *GATA4* – disrupt testis development, leading to 46,XY disorders of sexual development, where individuals with a 46,XY karyotype develop a female or ambiguous phenotype. Beyond its role in gonadal development, GATA4 is essential in heart development, where it cooperates with HAND2. Although *HAND2* is also expressed in developing gonads, its role in testis development has not been previously characterized. Notably, through whole-exome sequencing of a patient with 46,XY gonadal dysgenesis, we identified heterozygous rare variants of uncertain significance in both *GATA4* and *HAND2*. This study aimed to (1) investigate the role of HAND2 in NR5A1-mediated *SOX9* activation and (2) assess the impact of the patient-specific *GATA4* and *HAND2* variants. Using luciferase transactivation assays with the *SOX9* testis-specific enhancer, we found that HAND2 significantly enhances NR5A1-mediated *SOX9* expression. Importantly, the *HAND2* variant identified in the patient abolished this enhancement, implicating HAND2 as a previously unrecognized factor in testis development. In contrast, the patient-derived GATA4 variant did not affect SOX9 activation. To our knowledge, it is the first report implicating HAND2 in testicular development.

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Therapeutic Insights from Conformational Dynamics of Human 5S RNP Assembly and MDM2 Binding

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The 5S ribonucleoprotein (RNP) complex, consisting of 5S rRNA, ribosomal proteins RPL5 and RPL11, is integral to the large ribosomal subunit and pivotal in p53-mediated stress signaling through its interaction with MDM2. This interaction is essential for cellular regulation, with potential implications for cancer and ribosomopathies. To explore the dynamic principles governing 5S RNP assembly and its interaction with MDM2, we used all-atom molecular dynamics simulations. Our combinatorial approach provides insights that RPL5 establishes a stable binding on 5S rRNA and RPL11 provides a more flexible interface, allowing greater 5S rRNA flexibility. Crucially, within the full RPL5–RPL11–5S rRNA complex, RPL5 and RPL11 cooperatively interact, jointly stabilizing 5S rRNA, yielding a highly stable assembly. Building on this, we simulate the interaction of the 5S RNP complex with MDM2 to understand how conformational dynamics mediate this critical regulatory axis. By elucidating these conformational interactions, this work aims to provide insights into the 5S RNP–MDM2 regulatory axis, laying a foundation for future structural studies and novel therapeutic strategies targeting RNP-MDM2 dysfunction.

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Hypermethylation of the *BRCA1* promoter increases the risk of ovarian cancer: insights from high-resolution profiling of homologous recombination genes

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While germline mutations in *BRCA1* and other homologous recombination genes are established ovarian cancer (OC) risk factors, promoter hypermethylation (PHM)—either constitutional or acquired somatic—may serve as an alternative gene inactivation mechanism, acting as one of the two hits in Knudson's model of tumorigenesis. Using targeted deep bisulfite sequencing, we analyzed PHM in OC-related genes (*ATM*, *BRCA1/2*, *BRIP1*, *CHEK2*, *NBN*, *RAD51C/D*) in blood from a large group of 229 OC patients and 149 healthy controls. We also examined DNA methylation and its effect on gene expression in ovarian tumors (n=1107) and normal tissues (n=285) using GEO and TCGA datasets. In blood, *BRCA1* PHM was substantially higher in OC than control samples (5.24% vs. 0.67%; OR_{adj}=9.56, p=0.03). The lower frequencies of PHM were also detected for *RAD51C* and *BRIP1*. PHM typically showed low-level mosaic patterns with consistent methylation across CpG sites. Consistently—but more pronounced and frequent in tumors compared to normal tissue—PHM of *BRCA1* and *RAD51C* was observed and associated with decreased gene expression, supporting its functional impact. Our findings highlight PHM, particularly of *BRCA1* and *RAD51C*, as a complementary mechanism to genetic alterations in OC. While most tumor-associated methylation changes appear somatic, the presence of low-level *BRCA1* PHM in blood suggests a potential early-developmental or constitutional factor that may contribute to OC risk in a subset of individuals.

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The Landscape of *MIR142* Mutations in Diffuse Large B-Cell Lymphoma: From Genetic Insights to Clinical Implications

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MIR142 is the most frequently mutated miRNA gene in cancer. It is specifically mutated in hematologic malignancies. Although the frequency of MIR142 mutations appears to be highest in diffuse large B-cell lymphoma (DLBCL), few DLBCL samples have been analyzed to date. To better characterize MIR142 alterations, we analyzed 110 DLBCL samples. Using Sanger sequencing, we identified 16 mutations in 13 (11.8%) cases and one novel promoter mutation (0.9%), including 14 substitutions and 2 insertions/duplications, that were not reported before. Mutations were located in various regions of MIR142, including the miRNA seed. All mutations were validated by bidirectional sequencing, and selected ones were confirmed by pyrosequencing. Additionally, we developed an MLPA assay for chromosome 17q and identified 2 focal MIR142 deletions. miRNA-seq of 8 mutated and 7 wild-type samples confirmed the presence of mutations at the RNA level and revealed decreased miR-142-3p levels and an altered miR-142-5p/3p ratio. Finally, genotype/phenotype analysis showed that MIR142 mutations were enriched in the more aggressive non-GCB subtype, supporting their potential as biomarkers. This is the most comprehensive study of MIR142 in DLBCL, both in sample size and number of the identified alterations, and the first to link these mutations to clinical characteristics.

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Polyunsaturated fatty acids (PUFA) augment lipids peroxidation in bovine *in vitro* embryos indicating hallmarks of ferroptosis

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Ferroptosis is a recently discovered mechanism of programmed cell death. Induction of this process includes an increase of reactive oxygen species driven by accumulated intracellular iron and extensive lipid peroxidation. The group of lipids indispensable for the ferroptosis to occur are polyunsaturated fatty acids (PUFA). We hypothesized, that the excess of PUFA in the culture medium triggers ferroptosis in bovine embryos. The aim of the study was to analyze the ferroptosis-related mechanisms in preimplantation, blastocyst stage embryos.

In vitro produced embryos were divided into a control (n=12) and experimental group (n=8) supplemented with a 3:1 ratio of linoleic acid and alpha-linolenic acid mixture at final (physiological) concentration of 100 μM. On day 7 of development, live embryos were stained for lipid peroxidation and Fe^{2} levels and captured using confocal microscopy. Calculations of fluorescence intensity were performed using ImageJ Fiji.

Fatty acid supplemented group had significantly higher levels of oxidized lipids than control (2.06 +/- 0.58 vs 0.20 +/- 0.06; $P<0.001$). It shows, that supplementation of PUFA during IVC alters the lipid composition of bovine blastocysts and suggests the susceptibility of embryos to potential ferroptosis activation under elevated level of intracellular Fe^{2} (under investigation).

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Assessment of the impact of low-temperature plasma on morphological alterations and physiological irregularities in cells of phytopathogenic fungi

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Food loss refers to the reduction in mass or deterioration in quality of food products, excluding feed and inedible parts. Food loss occurs throughout the entire supply chain—from production and harvest at the farm, through transportation, storage, processing, packaging, and distribution—to the point of consumption.
In this study, the efficacy of inactivating agriculturally significant fungi from the genera *Fusarium* and *Alternaria* on the surfaces of packaging materials commonly used in the agri-food industry was evaluated using atmospheric-pressure air plasma. Variability in susceptibility to plasma treatment was observed among the tested fungal strains, with a 90% pathogen mortality rate achieved within no more than 11 minutes of exposure. The results revealed that repeated plasma treatments induced significant morphological alterations in hyphal structures, as well as physiological disruptions, such as changes in the specific activity of hydrolytic enzymes. Furthermore, multiple exposures to cold plasma appeared to enhance fungal sensitivity to fungicides and hydrogen peroxide, suggesting a potential oxidative stress response. This phenomenon of sensitization warrants further investigation due to its potential application in managing resistant strains. The use of plasma for controlling fungal pathogens is a promising approach, particularly given that no development of cellular resistance or tolerance to non-thermal plasma has been observed.

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The effect of alternative splicing and alternative polyadenylation of primary miRNA precursors (pri-miRNAs) on miRNA biogenesis.

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There are two major steps in biogenesis of mammalian microRNAs (miRNA). In the first, pre-miRNA precursors are excised in the nucleus from long primary miRNA precursors - pri-miRNAs. Pri-miRNAs can be subject to splicing and/or alternative polyadenylation, whose effects on pre-miRNA excision by ribonuclease DROSHA is not well understood. Moreover, information on full length pri-miRNA sequences and their positions within the genome is sparse. To answer these questions we performed RNA sequencing using Oxford Nanopore Technology (ONT). The total RNA was isolated from HeLa cells with siRNA induced DROSHA silencing (siDROSHA) and from control cells (siCTRL). RNA was reverse transcribed with either random hexamers for measurement of DROSHA silencing efficacy, or with cDNA-RT adapters from cDNA-PCR Barcoding Kit 24 V14 for ONT sequencing. Treatment with siDROSHA significantly enriched pri-miRNAs. The ONT reads were aligned to reference human genome with splice aware aligner. Putative pri-miRNA sequences were identified by searching for genomic regions with significantly higher mapping depth in siDROSHA samples. Then, we focused on two human pri-miRNAs: pri-miR-21 and pri-miR-23b-27b-24 cluster. We enriched studied pri-miRNAs from the total RNA using complementary biotinylated oligonucleotides and streptavidin coated beads and sequenced them. Transcript abundance was quantified from cDNA ONT reads pseudoaligned to reference transcriptome. Finally, we estimated the effect of alternative splicing/polyadenylation on biogenesis of miRNAs encoded by miR-21 and miR-23b-27b-24 genes.

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Lithium's Modulatory Role in the Cholinergic System: Insights from *Tenebrio molitor* beetle

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Lithium (Li) is a well-established therapeutic agent in the treatment of bipolar disorder and has gained attention for its neuroprotective potential in various neurological and neurodegenerative conditions. While its mechanisms of action include inhibition of GSK-3β and regulation of neurotrophic factors, less attention has been given to its effects on the cholinergic system. In the nervous system, acetylcholine (ACh) is the main excitatory neurotransmitter; within cholinergic synapses, it regulates neuromuscular transmission, cognitive functions, as well as mood modulation. ACh is degraded by acetylcholinesterase (AChE), which, beyond its catalytic role, also participates in synaptogenesis, regulation of inflammatory and oxidative responses—processes frequently altered in neurodegenerative and affective conditions. To better understand Li's potential modulatory effect on the cholinergic system, we conducted an experimental study using *Tenebrio molitor* larvae, an emerging model in neurotoxicological research.
Here, we present the effect of Li salt on the cholinergic system of *T.* molitor. Larvae were orally exposed to Li_2CO_3 at 0.01% and 0.1% concentrations for 7 days under controlled conditions. After exposure, insect heads and ventral nerve cords were dissected. We measured the AChE activity spectrophotometrically, while *AChE* gene expression was assessed using RT-qPCR.

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Acrolein Stress in Yeast with Impaired Pentose Phosphate Pathway: When the NADPH Rescue Strategy Backfires

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Acrolein, a highly electrophilic aldehyde formed intracellularly from allyl alcohol, severely perturbs redox homeostasis by rapidly depleting reduced glutathione (GSH) and triggering oxidative stress. To maintain redox balance, cells use the glutathione system, the thioredoxin system, and redox-active cofactors, mainly NADPH. The study analysed the response of yeast cells, defective in PPP-dependent NADPH production, to allyl alcohol exposure. The investigation assessed growth capacity, redox parameters, mitochondrial activity, and redox-related gene expression. The results show that after short-term exposure to allyl alcohol, the Δ*gnd1* mutant cells showed the most pronounced sensitivity, because, unlike other strains, they were unable to activate sufficient stress responses as an attempt to restore glutathione levels or maintain NADPH/$NADP^+$ ratios. The heightened sensitivity to allyl alcohol in Δ*gnd1* cells was also the result of an ineffective compensatory strategy based on ALD6-dependent NADPH synthesis and elevated expression of the *ADH1* gene, likely leading to enhanced acrolein formation. The findings demonstrate that tolerance to acrolein requires coordinated glutathione and NADPH-dependent systems, and that compensatory metabolic rewiring may sometimes paradoxically intensify stress.

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Gluten proteins-polyphenols interactions studied with application of fluorescence spectroscopy

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Gluten is a viscoelastic network formed within dough during dough mixing process. Gluten is composed of two proteins – gliadins and glutenins. Structure of gluten proteins directly affect quality of bread dough as well as bread. Addition of some additives e.g. dietary fibre or polyphenols extracts to the dough disturbs structure of the gluten network and hence changes bread quality.
Fluorescence spectroscopy can be applied to determine changes in proteins structure. In the case of gluten, fluorescence is observed only from tryptophan residues. The aim of the presented studies was to determine whether and how phenolic compounds, added to the wheat dough, interact with tryptophan residues. Two types of fluorescence spectroscopy have been used: steady-state and time-resolved fluorescence.
Results obtained from both fluorescence techniques showed that mechanism of interaction depends on the number and type of functional groups present at the aromatic ring of the polyphenol. If the polyphenol has only one OH group at the aromatic ring, e.g. coumaric acid, did not interact with tryptophan. Whereas if it has more than one functional group, like caffeic acid, form complexes with gluten proteins through hydrophobic interactions and/or H bonds. It can be observed as considerable fluorescence quenching and/or shortening of the fluorescence lifetime (from 2.7 ns (control) to 0.6 ns (modified sample)).

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PiggyBac System for Sustained Production and Functional Studies of DP71 Splice Variants in Duchenne Muscular Dystrophy Cell Models

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Duchenne muscular dystrophy is a neuromuscular disease that causes progressive muscle weakness, cognitive and neuropsychiatric symptoms. It arises from mutations in the *DMD* gene, which encodes multiple dystrophin isoforms, including DP427 and the ubiquitous short isoform DP71. Alternative splicing generates several DP71 variants, each differing in level, subcellular localizations, and functions. Although all patients lack DP427, about 10 % also lack DP71, worsening disease severity; this observation suggests that individual DP71 splice variants may perform significant roles, including in neurons and glia.
To investigate different functions of DP71 splice variants, we first constructed PiggyBac transposon vectors encoding the human *DP71* coding sequence with all exons, fused to Flag or Flag-GFP tags. Co-transfection with a transposase helper plasmid into *DMD* knockout HeLa cells yielded stable genomic integration confirmed by genomic PCR. Western blotting showed single bands at the expected molecular weights, and fluorescence microscopy demonstrated *GFP* expression, indicating efficient, long-term transgene synthesis. We are currently generating *DMD* knockout HeLa cells with stable synthesis of other DP71 splice variants.
These preliminary data show that PiggyBac vectors provide a reliable, long-term delivery system for DP71 in cell culture and can be applied to study the functions and therapeutic potential of DP71 variants in other cell types, including neurons and glial cells.

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Non-infectious SARS-CoV-2 replicon as a model for testing novel antiviral inhibitors

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RNA viruses are dangerous threats to human and animal health Several of them are significantly hazardous because of their high epidemic or pandemic potential, e.g., influenza, SARS (including SARS-CoV-2), Ebola, Zika, and MERS viruses. Constant changes occurring within the genomes of RNA viruses, including the SARS virus, lead to the emergence of new virus variants and strains. Hence, a better understanding of the SARS-CoV-2 virus biology could help develop more effective drugs against COVID-19. The presented research aims to better understand the role of RNA structure in the replication process of SARS-CoV-2 and to use this knowledge to design potential inhibitory tools. Based on the RNA secondary structure of SARS-CoV-2, we then designed and synthesized a group of modified antisense oligonucleotides targeting the sequences within the N protein coding region of SARS-CoV-2 genomic RNA. To test their inhibitory effect, we generated a non-infectious SARS-CoV-2 replicon containing green fluorescent protein (eGFP) that allowed us to validate the oligonucleotide antiviral effect in the HEK293T cell line. Our research demonstrated that the most prominent inhibitory effect was obtained for seven of the nineteen tested oligonucleotides. In addition, four of the best performing ones were tested on a simplified plasmid system and remain their effectiveness on a non-infectious SARS-CoV-2 replicon. Overall, our results suggest that presented here new, modified oligonucleotides designed based on the revealed secondary structure can be potential therapeutic tools against SARS-CoV-2.

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Linking Ribosome-Inactivating Protein Cytotoxicity with Cellular Stress Pathways

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Ribosome-inactivating proteins (RIPs) are cytotoxic proteins that are mainly produced by plants and bacteria. Based on their structural and functional features, they are classified as Type I, Type II or Type III. Type I RIPs, such as trichosanthin (TCS) and pokeweed antiviral protein (PAP), consist of a single polypeptide chain. Type II RIPs, including ricin, are heterodimeric proteins consisting of an enzymatically active A chain and a lectin-like B chain linked *via* a disulfide bond. The B chain facilitates cellular entry. Type III RIPs, such as the maize protein MOD, are synthesised as inactive precursors that require proteolytic activation. Despite extensive study, the precise molecular mechanisms of RIP-induced cytotoxicity remain unclear. The prevailing model proposes that RIPs depurinate the sarcin-ricin loop (SRL) of ribosomal RNA *via* their RNA N-glycosidase activity. The SRL is located within the GTPase-associated centre (GAC) of the ribosome and is critical for stimulating GTP hydrolysis. Damage to the SRL disrupts ribosomal function, leading to the inhibition of protein synthesis and cell death.In this study, we demonstrate that depurination of the SRL activates stress response pathways in mammalian cells using representatives from different RIP classes. Our findings suggest that ribosome depurination and translation arrest are not the primary drivers of RIP-induced cell death. Rather, RIPs trigger a widespread ribotoxic stress response (RSR), which activates signalling pathways that culminate in apoptosis.

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Targeting segment 8 of Influenza A Virus vRNA with Antisense Oligonucleotides

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RNA viruses pose a serious threat to public health due to their high genetic variability and ability to spread rapidly. The influenza A virus (IAV), one of the most commonly occurring RNA viruses, causes seasonal epidemics and influenza pandemics. Finding effective therapeutic strategies to combat the influenza A virus (IAV) is crucial in growing resistance to available antiviral drugs. This study evaluated the efficacy of antisense oligonucleotides targeting conserved motifs within vRNA segment 8 in inhibiting IAV replication. The antisense oligonucleotides used were conjugated to compounds such as cholesterol, GalNac, and palmitate to increase intracellular delivery efficiency. This modification allowed their autonomous uptake into cells. In addition, the antisense oligonucleotides were labeled with cyanine (Cy3), enabling analysis by confocal microscopy. The results showed effective entry of the antisense oligonucleotides into cells, confirming their ability to be independently internalized. Based on the obtained images, it is possible to determine the location of the applied antisense oligonucleotides within the cells. The Immunofluorescence Focus Formation Assay (IFFA) results showed significant inhibition of IAV replication. The study confirms the potential of the used antisense oligonucleotides as effective inhibitors of influenza virus replication, which may represent a promising therapeutic strategy for treating and preventing influenza infections.

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Effects of CKD and CVD serum on the endothelial cells

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Chronic Kidney Disease (CKD) is defined as abnormalities in the structure and function of the kidneys persisting for more than three months, and can lead to progressive loss of renal function. Interestingly, the leading causes of mortality in CKD patients are cardiovascular-related complications following accelerated atherosclerosis. Atherogenic processes are driven by systemic inflammation, the recruitment of inflammatory cells from the circulation, and their infiltration into the subendothelial space. Here, we have investigated the effects of the CKD and CVD serum on the proteome of endothelial cells in vitro and in vivo, utilising mass spectrometry-based proteomics and physiological experiments. Short-term results of serum exposure on endothelial cells (HUVECs) in vitro revealed dysregulation of proteins involved in LDL clearance and cell-surface lipoprotein metabolism. Long-term effects of serum exposure on endothelial cells were examined in aorta samples derived from patients with or without renal dysfunction. Revealed effects demonstrated alterations of proteins involved in chromatin organisation. In coherence with the long-term effects, in vitro short-term exposure of adult aortic cells (HAECs) to patient-derived serum indicated dysregulation of proteins responsible for DNA damage response. Our results revealed that CKD-derived uremic toxins in the serum altered the cell-surface lipoprotein metabolism, cell-signalling, and caused DNA damage in endothelial cells.

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Evaluating methods to eliminate artifacts in NGS Data from FFPE samples.

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Formalin-fixed, paraffin-embedded (FFPE) samples are widely used in both clinical and research settings, particularly for genomic applications like next-generation sequencing (NGS). However, the formalin fixation process can introduce DNA artifacts - most notably cytosine deamination resulting in C>T and G>A substitutions - which reduces the accuracy of variant calling methods. To address this, we assessed the performance of five computational tools - Ideafix, microSEC, FFPolish, Deepomics and SOBdetector - for removing such artifacts. Our evaluation was based on three datasets: (1) 36 matched WES FFPE and fresh-frozen samples from TCGA, (2) 21 matched WGS samples from CGCI, and (3) our custom WES dataset comprising fresh-frozen and formalin-treated samples, with and without enzymatic repair using NEBNext FFPE DNA Repair v2. Variant filtering was followed by comparison to fresh-frozen reference samples using som.py, considering only variants with DP>10. For WGS data, Deepomics showed the best performance. In WES data, Deepomics, FFPolish, and SOBdetector were most effective, depending on the dataset. The choice of the best method depends on data type and tumor context. Importantly, enzymatic repair with NEBNext outperformed all computational methods. These findings underscore the value of integrating enzymatic and computational approaches to enhance the reliability of FFPE-based genomic analyses in cancer research.

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Structural architecture of the human long non-coding RNA-PAAN, as a potential target for anti-influenza drug development

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Influenza A virus (IAV) is considered one of the most dangerous pathogens in the world because of its great variability. Available anti-influenza drugs suffer from the rapid emergence of drug resistance. Thus, there is an urgent need to develop new antiviral strategies with new mechanisms of action and reduced drug resistance potential. Host lncRNAs are much less variable than influenza RNA/protein; therefore, it can be a new and universal aim of antiviral therapy. Recently, studies have revealed the interferon-independent host lncRNAs-PAAN that interacts with influenza virus PA protein to promote viral replication. Until now, data concerning the lncRNA-PAAN structure *in vitro* and in biological environments has not been discovered. Here, for the first time, we used chemical mapping and the SHAPE method to propose the secondary structure of lncRNA-PAAN *in vitro* and in the cellular environment. We discuss the experimental and computational approaches that have led to distinct structural models. Finally, we defined the structural motifs of lncRNA-PAAN of potential functionality forming during influenza A infection and designed lncRNA-PAAN structure-specific antisense oligonucleotides (ASOs). Several of these ASOs significantly lowered the level of lncRNA-PAAN and inhibited IAV infection. Our findings not only advance our understanding of the complexity of the IAV-host interactions but also could be used for designing a new anti-influenza A strategy targeting the host lncRNAs.

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Cytoskeletal structure and cell junctions: obstacles or drivers of β cell differentiation?

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Pancreatic endocrine development depends on branching morphogenesis, a process in which progenitor cells delaminate and reorganize to form the islets of Langerhans. This morphogenetic remodeling requires tight coordination between transcriptional networks and cytoskeletal dynamics. Here, we use human pluripotent stem cells (hPSCs) and two morphogenesis models – gastruloids and directed pancreatic differentiation – to identify ETS Variant Transcription Factors (ETVs) as key regulators of biophysical properties and lineage specification. Genetic ablation of *ETV1*, or combined loss of *ETV1/ETV4/ETV5*, enhances cell-cell and cell-ECM adhesion, resulting in aberrant multilineage differentiation, disrupted germ layer organization, loss of ectoderm, and overgrowth of extraembryonic cells in gastruloids. In pancreatic differentiation, *ETV1* deletion abolishes pancreatic progenitor formation. Single-cell RNA sequencing and follow-up assays reveal dysregulated mechanotransduction *via* the PI3K/AKT pathway and upregulation of genes involved in cytoskeletal regulation, including the microtubule-associated protein Neuron Navigator 3 (*NAV3*). During pancreatic differentiation of *NAV3* knockout hPSCs, spheroids disintegrate at the pancreatic progenitor stage and fail to generate endocrine progenitors. These findings suggest that *ETV1* and its downstream effector *NAV3* regulate key morphogenetic events during endocrine progenitor specification by modulating cell adhesion and cytoskeletal remodeling.

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Role of Polycomb complexes in keratinocyte differentiation

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The epidermis, a stratified epithelial tissue forming the skin's outermost layer, acts as dynamic barrier essential for protecting the body and maintaining homeostasis. This barrier is maintained through tightly regulated transcriptional program that governs keratinocyte (KC) differentiation. Key chromatin modifiers, Polycomb Repressive Complexes (PRCs), are known to mediate transcriptional repression through histone modifications, yet their precise roles in epidermal gene regulation remain incompletely understood.

To clarify contribution of PRC-mediated transcriptional repression to epidermal differentiation, I used bioinformatics to analyze transcriptional and epigenetic landscape within KC-specific gene clusters (EDC, Ker I and II) during mouse epidermal development. My findings reveal dynamic transcriptional changes and enhanced deposition of repressive mark H3K27me3 in differentiated KCs, suggesting increased PRC2 activity in terminal differentiation.

Moreover, I established 3D culture system by human N/TERT KCs to model epidermal differentiation in vitro to be able to genetically modify KCs. This platform enables investigation of polycomb-regulated transcriptional networks and enhancer-promoter architecture underlying KC maturation.

To functionally test the roles of PRCs, I aim to generate knockouts of *EZH2*, catalytic subunit of PRC2, and *RING1A/B*, core components of PRC1. The impact of PRC1/2 depletion on transcriptional regulation will be tested in both 2D/3D cultures.

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Strategies to enhance the effectiveness of 5-aminolevulinic acid-based photodynamic inactivation of bacteria

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Photodynamic inactivation (PDI) offers a promising alternative to antibiotics amid rising resistance. The method uses light-activated photosensitizers to generate reactive oxygen species (ROS), leading to bacterial cell damage. 5-aminolevulinic acid (5-ALA) induces endogenous synthesis of protoporphyrin IX (PpIX), a compound with photosensitizing properties. However, hydrophilic and zwitterionic nature of 5-ALA limits cellular uptake and reduces intracellular PpIX accumulation, thereby constraining its antimicrobial efficacy.

This research aimed to enhance the efficiency of 5-ALA-PDI toward *Proteus mirabilis*. To improve 5-ALA uptake in bacterial cells, organophosphorus compounds acting as divalent cation chelators were used. Additionally, intracellular accumulation of PpIX was promoted by inhibiting ferrochelatase, the enzyme converting PpIX to heme. Iron chelators, including deferiprone and 2,2'-bipyridyl, were used to reduce ferrochelatase activity by limiting iron availability, thus further enhancing PDI effectiveness.

The results highlight the effectiveness of the investigated strategies in enhancing 5-ALA-PDI. These findings point to the potential of photodynamic approaches as promising alternatives to conventional antibiotic therapies, offering a novel direction for the development of light-activated antimicrobial treatments.

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The effect of metformin and kifunensine on intestinal epithelial barrier integrity in an *in vitro* model

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The functionality of the intestinal barrier depends on the quality of intercellular connections, where adhesive junctions responsible for epithelial integrity and tight junctions controlling permeability and transport of substances play a key role. It is also known that metformin increases the integrity of the intestinal barrier by reducing its permeability. In addition, changes in *N*-glycosylation can regulate cell adhesion. In this study, the *in vitro* effect of metformin and kifunensin (an *N*-glycosylation inhibitor) on cell junction proteins in the intestinal epithelial barrier was investigated. The research model was based on the co-culture of CaCo-2 and HT29-MTX cell lines, which mimic human intestinal epithelium *in vitro*. The state of the formed cell junctions was examined using qPCR and WB analysis. The integrity of the epithelia was checked by measuring TEER. Metformin was shown to affect permeability of the barrier by upregulating TEER and regulating tight junction proteins, including claudin-1, claudin-2, and Zonulin Occludens, in differentiated intestinal epithelium. In addition, changes in the JAM-A glycosylation status was observed after the use of kifunensin, indicating the involvement of *N*-glycans in the regulation of this adhesion protein. Our results therefore indicate the influence of metformin and *N*-glycosylation on the modulation of intestinal barrier integrity.

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Unveiling the Secrets of Human High-Molecular-Weight Kininogen Using Cryo-EM and Computational Modeling

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High-molecular-weight kininogen (HK) is a plasma glycoprotein essential as a coagulation cofactor and bradykinin precursor. Despite its physiological importance – regulating vascular permeability, blood pressure, and inflammation – its full 3D atomic structure remains unresolved.
This study explores HK's molecular architecture and interaction sites using an integrative approach combining cryo-electron microscopy (Cryo-EM), AI-based modeling (AlphaFold, I-TASSER), and molecular dynamics (ChimeraX, ISOLDE, Foldit, GROMACS). Commercial HK (Calbiochem) was analyzed *via* Glacios and Krios G3i microscopes at SOLARIS. Data were processed in CryoSPARC using PLGrid infrastructure.
Cryo-EM revealed ordered domains (D1–D3) and flexible regions (D5–D6). Models suggest a "beads-on-a-string" conformation stabilized by a Cys10–Cys596 disulfide bridge. Structural changes were observed after treatment with Mg^{2+}, Zn^{2+}, and glutaraldehyde, likely due to Zn^{2+} complexation and cross-linking. Compact and extended conformations indicate the structural plasticity of HK and its importance for interactions with prekallikrein and factor XI. This study demonstrates the synergy of cryo-EM, AI, and HPC in uncovering structural insights and guiding future therapeutic strategies.

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Differential influence of selected antidepressants on the *in vitro via*bility of breast cancer cells and their sensitivity to doxorubicin

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The need for improving efficacy and tolerability of anticancer therapy regimens remains consistently high. Following the idea of drug repurposing, the presented study aimed to evaluate the influence of three selected representatives of FDA-approved, clinically exploited antidepressants (ADs). The chosen pharmaceutics cover a range of different AD structural and functional classes: imipramine as a tricyclic AD, mirtazapine as a tetracyclic AD, and sertraline as a selective serotonin reuptake inhibitor. Although some ADs do exhibit anticancer potency towards various cancer types, they are poorly evaluated in context of BC, pointing at a considerable knowledge gap.
Therefore, the aim of this pilot study was to assess the effect of mirtazapine, imipramine and sertraline on two different breast cancer cell lines (MDA-MB-231 and MCF-7), alone or in combination with doxorubicin (DOX) and in various concentrations of both agents. To achieve this aim, *via*bility assays (MTT), real time cell behavior evaluation upon treatment through impedance measurement, and apoptosis induction ability of all compounds have been assessed. The results suggest highly pronounced anticancer activity of sertraline and a rationale of its combining with DOX, while two other pharmaceutics, interestingly, seem to decrease basal sensitivity to DOX. The molecular bases of these findings, suggesting some metabolic priming of cancer cells to endure DOX-induced stress, are yet to be evaluated.

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Role of tunnel entrance mutations in ABCG46-mediated plant stress response: From molecular mechanisms to biological function

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Our research investigates determinants of membrane transport in ATP-binding cassette transporters subfamily G (ABCG). Plant ABCG proteins function as molecular pumps, transporting compounds across cellular membranes for homeostasis and environmental responses. ABCG46 plays a key role in plant biotic stress response by transporting deoxy(iso)flavonoids - secondary metabolites essential for plant defense. Understanding structure-function relationships provides insights into plant transport system evolution and may inform crop stress tolerance strategies. Previous investigations of ABCG46 uncovered the first molecular insights into its tight multispecificity, while postulating further research questions.

Building on our laboratory findings identifying F562, in the central part of the ABCG46 tunnel, as crucial for substrate transport. This follow-up study investigates how specific mutations at the transporter's tunnel entrance inactivate transport function. Using molecular dynamics simulations, we examine alanine mutations (A667T, A1319G, and A1319V) where substrates first interact with the protein. The study involves ABCG46 protein AlphaFold3 model embedded in membrane, which enables tracking substrate translocation pathways. Mutation of small, nonpolar alanine residue could interrupt conformational changes or even cause collapse of the open tunnel, affecting transport efficiency.

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Chromatin Looping as a Determinant of Transcriptional Termination

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Three-dimensional genome organization has emerged as a critical regulatory layer influencing gene expression. However, its role in transcriptional termination remains poorly understood. Premature termination of transcription acts as a checkpoint that impacts RNA polymerase II activity, adjusts RNA levels, and contributes to the diversity of transcript isoforms. Genome-wide studies reveal frequent colocalization between termination sites and binding sites for CTCF and the cohesin complex, key mediators of chromatin loop formation and extrusion. These observations suggest that the three-dimensional chromatin organization might actively regulate transcriptional attenuation. We have integrated nascent transcriptome data with available chromatin folding data and shown that, in many cases, the choice of alternative polyadenylation sites is affected by the local epigenetic landscape.

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Vault RNAs and TEP1: Modulators of Drug Response in Breast Cancer Cells

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Vault RNAs are small non-coding RNAs transcribed by RNA polymerase III and form part of large eukaryotic ribonucleoprotein complexes called vaults, which also include the proteins TEP1, MVP, and vPARP. Emerging evidence suggests that vtRNAs are involved in various highly relevant cellular processes.
In this study, we investigated the expression and functional role of four vtRNA paralogs across a panel of breast cancer cell lines and a non-cancerous breast epithelial cell line (MCF-12A). Basal vtRNA levels were quantified *via* qPCR, and cells with high expression were subjected to targeted silencing using siRNA. The impact on cell *via*bility and proliferation in response to doxorubicin and cisplatin treatment was assessed through MTT assay and western blot analysis of proliferation markers.
Additionally, we examined the role of TEP1, an RNA-binding protein, by silencing its expression to assess its influence on vtRNA levels and drug responsiveness. In MDA-MB-231 cells, doxorubicin treatment induced upregulation of vtRNAs in a dose- and time-dependent manner. Notably, TEP1 knockdown sensitized cells to chemotherapeutic agents and was associated with altered cell cycle regulation and proliferation dynamics.
These findings support a functional role for vtRNAs and TEP1 in modulating breast cancer cell responses to treatment and highlight their potential relevance in the development of targeted cancer therapies.

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Reproductive capacity of various background yeast strains in relation to cell size changes

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The ability of cells to proliferate is a fundamental feature of all organisms, ensuring growth, regeneration, or reproduction. This process is tightly regulated at both the organismal level, to maintain homeostasis of cell numbers, and at the cellular level, where coordination of extracellular and intracellular signals is necessary for regulating the cell cycle. Different cell types vary in their proliferative capacity, and the irreversible loss of a cell's ability to divide is known as replicative senescence.
The yeast *Saccharomyces cerevisiae*, a model organism used to study many aspects of eukaryotic cell function, is characterised by limited reproductive capacity influenced by various factors, including genetic, environmental, and pharmacological ones.
The aim of our research was to conduct an physiological and morphological analysis of wild-type yeast strains to identify the factors most significantly affecting cellular reproductive capacity. We examined several parameters, including the glucose uptake rate, gene expression related to glycolysis and the pentose phosphate pathway, mitochondrial morphology and activity, the content of essential macromolecules, as well as cell size and its changes during the reproductive phase of cell life.
The results suggest that the analysed strains adopt specific life strategies that may directly or indirectly influence changes in cell size, which is an important aspect in explaining differences in their reproductive capacity.

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Molecular Characterization and Supramolecular Targeting of Bladder Cancer: A Study on the CP-673451:Congo Red Complex Integrating Gene Expression, micro-RNA Profiling and *In Silico* Modeling

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The development of targeted therapies has transformed oncological treatment strategies, with precision drug delivery becoming a key focus. Among novel carrier systems, supramolecular self-assembling platforms such as Congo red (CR) offer non-covalent encapsulation and selective transport of therapeutic agents.This study investigates the biological effects of a supramolecular complex formed between the selective PDGFR inhibitor CP-673451 and CR, aiming to enhance targeted bladder cancer therapy. Spectroscopic and thermodynamic analyses confirmed complex formation and optimal CP-673451:CR stoichiometry. The CP-673451:CR complex showed improved cellular uptake and stronger antiproliferative effects compared to the free drug in bladder cancer cell lines.Functional assays demonstrated dose- and time-dependent inhibition of cell proliferation and migration, apoptosis induction (FACS), and cytoskeletal reorganization. Gene and micro-RNA expression profiling revealed modulation of pathways involved in receptor tyrosine kinase signaling and tumor progression. *In silico* modeling supported the specificity and stability of the CP-673451:CR interaction.These results highlight the potential of supramolecular platforms for advancing targeted oncologic therapies.

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Proteomic approach to study the influence of natural/synthetic protectors on kinase base signalling in UVB irradiated keratinocytes

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Human skin as the most external organ of the body is constantly exposed to environmental factors, including the harmful effects of UVB radiation. Since UVB radiation is an inevitable factor that reaches skin with the sun rays, there is a constant search for compounds with a protective effect on skin cells, including epidermal keratinocytes.
Therefore, in this study the effect of natural phytocannabinoid - cannabigerol (CBG) and a synthetic ascorbic acid derivative - 3-O-ethyl ascorbic acid (EAA) added separately or together to the medium of UVB-irradiated keratinocytes, was analyzed using a proteomic approach (SDS-PAGE/LC-MS/MS).
Obtained results indicated that CBG or/and EAA protects cells against UVB induced changes in proteins expression and structure. This was particularly evident in the context of kinase-dependent signaling, based on protein structure modifications, *via* adduct formation with the lipid peroxidation product 4-hydroxynonenal (4-HNE). Mitogen-activated protein kinases were most frequently modified by 4-HNE, but also IKK and NLK, whose UVB-induced expression/modification was reduced by CBG and EAA, especially when used together. Therefore, CBG and EAA were able to inhibit autophagy as well as pro-inflammatory/pro-apoptotic signaling in UVB-irradiated keratinocytes.
In conclusion, it can be suggested that the combined cytoprotective effect of CBG and EAA in skin cells, especially previously exposed to UVB radiation, is based on 4-HNE-related signaling.

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Inhibitory Effects of Tea-Derived Phenolics on Aflatoxin B1 Gene Expression and hyphae growth in *Aspergillus flavus*

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Due to climate change, the contamination of crops by *Aspergillus flavus* and its immunotoxic metabolite, aflatoxin B1 poses a significant threat in Europe. Recent studies suggest that phenolic compounds can inhibit aflatoxin biosynthesis by molds, however, the genetic mechanisms of this process are poorly understood. The following research hypothesis was formulated: the black tea extract exerts a concentration-dependent inhibitory effect on both the hyphal growth of *A. flavus* and the expression of genes involved in aflatoxin B1 biosynthesis under in vitro conditions.
Commercially available black tea extracts were applied in the experiment in the following dilutions: 100%, 75%, 50%, 25%. The total phenolic compound concentration was quantified using the Folin-Ciocalteu method. The effect of tea extract on mold hyphae growth was assessed using the modified disk-plate method. The relative expression of the aflR, aflS, aflD, and aflQ genes was measured using the RT-qPCR method after 20 hours of incubation of the mold plate with tea extract.
In vitro studies on *A. flavus* demonstrated that tea extract (85±5mg GAE) reduced the expression of aflS, aflD, and aflQ genes in a dose-dependent manner. Interestingly, the undiluted extract (100%) stimulated biomass growth by 65%, a phenomenon not observed at the lower dilutions. These findings suggest a potential application for improving food safety during storage by reducing aflatoxin risk without promoting fungicide resistance.

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Modulation of the endogenous *MBNL1* expression *via* RNAa mechanism as an alternative therapeutic approach towards myotonic dystrophy type 1 (DM1)

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In myotonic dystrophy type 1 (DM1), *DMPK* (*DM1 Protein Kinase*) transcripts carrying expanded CUG-triplet repeats sequester Muscleblind-like (MBNL) proteins, impairing their function and shifting the alternative splicing of target RNAs from adult to fetal splice isoforms. This spliceopathy leads to a plethora of multiorgan defects including myotonia, muscle atrophy and wasting. To overcome MBNL proteins insufficiency in DM cell models, we harnessed an evolutionarily conserved mechanism of RNA activation (RNAa) *via* small activating RNA (saRNA) targeted to the most active promoter of *MBNL1* gene, the major paralog of the *MBNL* gene family. We identified saRNA duplexes that stimulated *MBNL1* transcription *via* an on-site mechanism that involves AGO2-mediated loading of the antisense strand onto target sequence, and dissected the underlying mechanism, including the role of the lncRNA *MBNL1-AS1* overlapping *MBNL1* promoter. We show that RNA activation enhances MBNL1 protein content and corrects the alternative splicing defects of MBNL1 target pre-mRNAs in distinct cellular models of DM1. This is the first report that site-specific augmentation of the endogenous *MBNL1* transcription mitigates disease-associated defects, hence it offers new perspectives in DM1 therapeutic options as well as in *MBNL* expression regulation.

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Lamin-Mediated Chromatin Organization and Its Impact on Transcriptional Programs During Epidermal Differentiation

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The mammalian genome consists of 2m long DNA which is packed into a 10μm diameter nucleus. This requires a high level of organization balancing between compaction and maintenance of transcriptional activity determining cell identity. It is achieved through hierarchical chromatin folding, one of which is the lamina-associated domains (LADs), regulated by the nuclear lamina. Lamins A/C and B form a meshwork beneath the inner nuclear membrane, anchoring heterochromatin, regulating promoter–enhancer interaction, and maintaining transcriptional boundaries. Altering Lamin expression disrupts nuclear architecture and lineage-specific transcription programs.

Epidermal differentiation occurs in a well-ordered sequence from basal progenitors to suprabasal layers. This transition is driven by activation of gene clusters (*EDC*, *KRT* I/II), tightly regulated at the transcriptional level through dynamic chromatin looping and spatial repositioning. While RNA-seq studies revealed transcriptional changes during keratinocyte differentiation, the role of lamin-mediated genome organization in shaping these RNA expression profiles remains underexplored.

To address this, I mapped lamin B, histone modifications, and chromatin accessibility in basal and suprabasal murine keratinocytes. The outcome showed how changes in LaminB1 occupancy correlate with transcriptional activity and chromatin accessibility suggesting a critical role for LAD repositioning in regulating key epidermal gene programs.

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AuNP:OSI-774 Supramolecular Conjugates as a Novel Anticancer Platform

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Pancreatic cancer is a highly aggressive malignancy with one of the poorest prognoses among solid tumors. Despite ongoing research, treatment outcomes remain unsatisfactory, underscoring the need for more effective strategies. Targeted therapies, especially those using biomedical nanotechnology, show great potential. Gold nanoparticles (AuNPs), due to their small size, high surface reactivity, and large surface-to-mass ratio, are promising drug carriers. This study aimed to examine the formation of complexes between AuNPs and a tyrosine kinase inhibitor (OSI-774), and to evaluate their *in vitro* effects on pancreatic cancer cell lines (PANC-1, BxPC3) of varying malignancy.

The methods included biophysical analysis of AuNP:OSI-774 complexes, MTT cell *via*bility assay, IC_{50} determination, Annexin V/PI staining for apoptosis, and assessment of cell migration and invasion.

Biophysical analyses confirmed stable interactions. DLS identified the optimal AuNP-to-drug molar ratio. All tested compounds inhibited cancer cell proliferation in a dose- and time-dependent manner. FACS confirmed apoptosis induction and reduced migration and invasion of cancer cells.

These findings support further exploration of AuNP-drug complexes as promising tools in targeted anticancer therapy.

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Streptomyces tritrimontium sp. nov. – characterization of actinomycetes isolated from moonmilk in Szczelina Chochołowska cave of Tatra Mountains in Poland

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The taxonomic position of two streptomycete strains, isolated from moonmilk in Szczelina Chocholowska cave located in Tatra Mountains in Poland, was established using a polyphasic taxonomic approach. Strains 2.9^T and 4.24 have morphological characteristics and chemotaxonomic properties consistent with their classification in the genus *Streptomyces*. They are Gram-stain-positive filamentous bacteria which formed an extensively branched substrate mycelium and straight long chains of smooth surfaced spores. They contain *LL*-diaminopimelic acid, glucose and ribose in whole-organism hydrolysates, produce major proportions of straight, *iso*- and *anteiso*- fatty acids, hexa- and octahydrogenated menaquinones with nine isoprene units and have a polar lipid pattern with phosphatidylethanolamine (diagnostic lipid). Based on the genomic, phylogenetic and associated phenotypic data it is proposed that strains 2.9^T and 4.24 be assigned to the genus *Streptomyces* as *Streptomyces tritrimontium* sp. nov. with strain 2.9^T (DSM 119353^T =PCM 3548^T) as the type strain. The genomes of strains 2.9^T and 4.24 contain relatively high number of biosynthetic gene clusters (28 and 27, respectively), some of which were discontinuously distributed, indicating possible expression of genes coding for enzymes involved in synthesis of previously unknown, specialized metabolites.

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Supramolecular Targeting in Pancreatic Cancer: Functional Evaluation of Congo Red–OSI-774 Complexes

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Pancreatic cancer remains one of the deadliest malignancies, marked by rapid progression, chemoresistance, and limited therapeutic options. Hyperactivation of receptor tyrosine kinases (TKIs) plays a crucial role in its pathogenesis, positioning TKIs as a promising therapeutic avenue. Supramolecular drug delivery systems, such as those based on Congo red (CR), offer potential to improve treatment precision and efficacy through targeted drug transport.

In this study, we explored the formation and functional properties of supramolecular complexes composed of CR and OSI-774, evaluating their in vitro effects on pancreatic cancer cell lines. Biophysical analyses established the optimal CR:OSI molar ratio and confirmed complex formation. The complexes were assessed using MTT assays, IC□□ calculations, flow cytometry and transwell migration and invasion assays.

The CR:OSI complexes demonstrated enhanced antiproliferative activity in a dose- and time-dependent manner. FACS analysis revealed increased apoptosis, while functional assays showed a significant reduction in the migratory and invasive of cancer cells.

These findings indicate that supramolecular encapsulation of OSI in CR may represent a promising strategy for enhancing the efficacy of pancreatic cancer therapies.

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Comparison of the epigenetic regulation between cultured and primary keratinocytes

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The epidermis is a multilayered, self-renewing epithelium composed mainly of keratinocytes, which form a protective barrier against dehydration, infection, and pathogens. Epidermal development and regeneration are tightly regulated by genetic and epigenetic programs. Both cultured and primary keratinocytes are widely used as models for skin biology, but the systematic comparison of differences between them is still missing at the epigenetic level. Cultured cells may not fully mimic *in vivo* differentiation due to the absence of native extracellular matrix, physiological signaling, and cell–cell interactions.

My project aims to compare epigenetic gene regulation during differentiation of primary (*ex vivo*) and cultured keratinocytes. Reverse transcription qPCR (RT-qPCR) and Western blotting will assess expression of key differentiation markers at mRNA and protein levels. Chromatin immunoprecipitation qPCR (ChIP-qPCR) will evaluate histone modifications (H3K4me3 and H3K4me1) at regulatory regions of epidermal genes. By comparing transcriptional activity and chromatin states, this study will highlight how well *in vitro* models mimic natural differentiation and inform their use in skin biology and regenerative medicine.

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The Role of Interleukin-17 in Biofilm Formation by Uropathogenic *Escherichia coli* Strains in the Urine of Kidney Transplant Recipients

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Interleukin-17 (IL17) is a pro-inflammatory cytokine synthesized by numerous cell types, archetypically by Th17. It is responsible for the accumulation and migration of neutrophils to the place of bacterial invasion. Kidney transplant recipients are at an increased risk of urinary tract infections (UTI). Bacterial biofilm determines the risk of UTI recurrences. The aim of the study was to investigate the direct influence of IL17 on bacterial biofilm formation. The model of biofilm formation by reference Escherichia coli strain in urine samples from kidney transplant recipients was created. The total number of 46 urine samples from kidney recipients was used. Absolute concentration of IL-17 in urine was measured with ELISA and IL-17-to-creatinine ratio was calculated for each sample. Biofilm biomass and *via*bility were measured with spectrophotometry. Statistical analyses were conducted using Statistica 13 software. Biomass and *via*bility parameters were insignificantly lower and less diverse in samples where IL-17 levels were undetectable. In samples where IL-17 was detected, its concentration negatively correlated with the *via*bility and biomass parameters of the biofilm produced by the uropathogenic strain of E. coli. Our findings suggest that IL-17 plays a role in biofilm dynamics, providing insights into potential therapeutic implications.

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Bridging the missing links in the Nexin-Dynein Regulatory Complex

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Motile cilia drive fluid, mucus, or cell movement across epithelial surfaces in the human respiratory tract, brain ventricles, and reproductive system. Ciliary beating depends upon coordinated activity of ciliary multiprotein complexes including the nexin-dynein regulatory complex (N-DRC). Using *Tetrahymena thermophila* as a model we investigate N-DRC protein composition and the role of specific subunits in ciliary motility. We identified at least ten additional lineage- or species-specific N-DRC proteins alongside the twelve conserved subunits (DRC1-DRC12).

Phenotypic analysis of *Tetrahymena* DRC mutants confirmed that DRC4, together with DRC1 and DRC2, are key structural N-DRC components. We found that *Tetrahymena* has two DRC4 paralogs, DRC4A and DRC4B, which preferentially form a heterodimer and interact with other DRC proteins.

Loss of DRC4B dramatically impairs motility and alters ciliary beating. Comparative proteomics of wild-type and DRC4B-KO cilia revealed either complete elimination or dramatic reduction of all DRC proteins, suggesting destabilization of the entire complex.

Our work led to the identification of other N-DRC subunits and highlighted the central role of DRC4 in N-DRC assembly and function. Ongoing work aims to map these new subunits within the complex and elucidate their functional roles in ciliary regulation.

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Noncanonical functions of telomerase affect efficacy of cancer therapy

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One of the most characteristic features of cancer cells is a high proliferative potential. It is partially associated with telomerase expression and activity of its key enzymatic subunit – telomerase reverse transcriptase - TERT. This enzyme is capable of crossing the Hayflick limit that consequently leads to cells immortality. However, telomere length restoration is not the only role of telomerase. It is expressed (and/or active) also in some normal cells that suggests potential side effects after telomerase targeting-based therapy. Our results provide new evidence to support the broad spectrum of human TERT (hTERT) functions. The study revealed that hTERT was involved in the response of breast cancer cells to doxorubicin which was demonstrated in MTT and clonogenic assays. During a long-term doubling time assessment, a decreased population doubling level was observed. Interestingly, it did not dramatically affect cell cycle distribution. hTERT downregulation was accompanied by an alteration in β1-integrin- and by focal adhesion kinase (FAK)-driven pathways together with the reduction of target proteins phosphorylation, i.e., paxillin and c-Src. Additionally, autophagy activation was observed in MDA-MB-231 cells manifested by alternations in Atg5, Beclin 1, LC3II/I ratio, and p62. These results provide new evidence supporting the possible therapeutic potential of telomerase downregulation leading to induction of autophagy and cancer cells elimination.

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Discovery of mitoBK$_{Ca}$ channel-associated proteins through proximity labeling

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Mitochondria are crucial for cell function, particularly in ATP production driven by the inner membrane potential. Potassium channels regulate mitochondrial activity by modulating membrane potential or ROS production and are linked to cytoprotection. One well-characterized example is the mitochondrial large-conductance calcium-activated potassium (mitoBK$_{Ca}$) channel, similar to its plasma membrane counterpart.

We aimed to identify interactors of BK$_{Ca}$/mitoBK$_{Ca}$, including mitochondrial partners. Using TurboID, a proximity labeling technique, we created a construct with the β4 subunit fused to TurboID ligase.

Mass spectrometry of biotinylated proteins from mitochondria and whole-cell lysates showed labeling of proteins from the ER, cytosol, PM, nucleus, and mitochondria. Among mitochondrial targets, we found MICOS complex components and proteins involved in complex IV assembly. However, co-immunoprecipitation did not confirm direct interactions.

TMX1, an ER protein enriched in mitochondrial-associated membranes, was a confirmed interactor, binding both β4 and α subunits. As an oxidoreductase, TMX1 may modulate BK$_{Ca}$ *via* redox regulation. Prior studies showed TMX1 influences calcium transfer between ER and mitochondria, affecting metabolism suggesting TMX1–BK$_{Ca}$ interactions may play a similar role. Further studies are necessary to confirm this hypothesis.

Acknowledgements
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Homocysteine, its metabolites and silencing of bleomycin hydrolase, impact amyloid β precursor protein processing in mouse neuroblastoma N2A-APPswe cells

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Background Homocysteine (Hcy) is a sulfur amino acid, which can be converted to homocysteine thiolactone (HTL). HTL modifies proteins in to form *N*-Hcy-protein. Bleomycin hydrolase (BLMH) is an enzyme with the ability to detoxify HTL. Hcy, its metabolites and dysfunction of BLMH are linked to Alzheimer disease (AD), characterized by the accumulation of amyloid β (Aβ). Aβ is formed from amyloid β precursor protein (APP), involving BACE1, Nicastrin, PSEN1, PEN2 and APH-1. The impact of Hcy, its metabolites and BLMH dysfunction on AD is not fully understood.

Objective To test the hypothesis that Hcy, its metabolites and *Blmh* silencing affect APP processing in mouse neuroblastoma N2A-APPswe cells.

Design Neuroblastoma N2A-APPswe cells were treated with Hcy, HTL, *N*-Hcy-protein. Expression of *Blmh* gene was silenced using a *Blmh*-specific siRNA. Proteins involved in APP processing were quantified by Western blotting and mRNA by RT-qPCR.

Results Hcy increased APP, pAPP, BACE1 and PSEN1 and lowered expression of *Psen1* gene. HTL increased APP, BACE1, PSEN1, Nicastrin and decreased mRNA of *Psenen* gene (encoding PEN2). *N*-Hcy-protein increased PSEN1 and downregulated the expression of *Psenen* gene. *Blmh* silencing resulted in upregulation of APP, Nicastrin, PEN2 protein, expression of *APP*, *Psenen* mRNA and downregulated BACE1 protein.

Conclusion Hcy, its metabolites and *Blmh* gene silencing resulted in dysregulated APP processing in N2A-APPswe cells, suggesting their association with AD.

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Extracellular polymers produced by green microalgae as a tool for lead bioremediation.

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Lead existing in the environment is a serious contamination. Although leaded gasoline has not been used for many years, the problem of lead contamination still is still persists. The current sources of lead in the environment come from: mining, plant protection products, chemicals, batteries, plastics, etc. Most heavy metals, in minimal concentrations, perform various functions in living organisms. But lead and cadmium are toxic in any amount.
Extracellular polymers (EPS) produced by microorganisms perform protective functions and exhibit numerous physicochemical, including sorption. Thanks to its sorption properties, EPS protects cells from the effects of toxic substances. This can also be used in bioremediation processes or when cultivating algae in wastewater.
There are several factors influencing sorption efficiency, eg. metal and EPS concentration, and characteristic, contact time, the presence of other substances. One of the key factors influencing the sorption process is the pH value which may be specific, depends on the chemical characteristics of the EPS.
The aim of our research was to determine the lead sorption capacity of EPS synthesised by unicellular green algae.
The sorption properties of the studied EPS were evaluated using spectroscopic techniques: optical emission spectrometry with inductively coupled plasma (ICP-OES) and Fourier transform infrared spectroscopy (FTIR).

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ATP reduces the motility of dystrophic (mdx) but not dystrophin-positive mice myoblasts

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Duchenne muscular dystrophy (DMD) is a severe X-linked neuromuscular disorder caused by mutations in the dystrophin gene, resulting in progressive muscle degeneration and premature death. While muscle regeneration initially occurs through the activation of satellite cells, this capacity diminishes over time due to their progressive depletion. Among potential therapeutic approaches, satellite cell transplantation offers promise but is complicated by immune responses against dystrophin. Dystrophic (mdx) myoblasts exhibit abnormal migratory behavior and adhesion, which may compromise their regenerative efficacy. In this study, we observed that mdx myoblasts exhibit significantly faster migration compared to wild-type (w/t) cells. Extracellular ATP and its derivatives suppress motility in mdx myoblasts through the activation of the P2RX7 purinergic receptor and subsequent calcium signaling. Inhibition of P2RX7 abrogates ATP-induced motility reduction in mdx but not in w/t cells.
Furthermore, inhibition of Rho kinase increases motility in w/t cells but does not affect mdx cells, whereas MLCK inhibition reduces motility in mdx cells only. Morphological analyses suggest that these functional differences may arise from distinct migration mechanisms – lamellipodia-based movement in w/t myoblasts versus bleb-driven motility in mdx cells. These findings provide new insights into the altered behavior of dystrophic myoblasts and offer implications for cell-based therapies in DMD.

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Genetic predisposition to cancer therapy-related cardiac dysfunction

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Cancer therapy-related cardiac dysfunction (CTRCD) encompasses a broad range of cardiovascular complications arising from cytotoxic chemotherapy, targeted agents, and immunotherapies. CTRCD includes left ventricular dysfunction, arrhythmias, vascular injury, or long-term cardiomyopathy, which can significantly impact morbidity and mortality in cancer survivors. The pathogenesis of CTRCD is multifactorial, involving oxidative stress, mitochondrial damage, DNA injury, ferroptosis, calcium dysregulation, and immune-mediated inflammation. Recent evidence has highlighted genetic predisposition to individual susceptibility to CTRCD. Variants in genes encoding drug transporters (e.g., *ABCB1*, *SLC28A3*), metabolizing enzymes (e.g., *GSTM1*, *CBR3*), oxidative stress regulators (e.g., *HAS3, TOP2B*), and sarcomeric proteins (e.g., *TNNT2*, *TTN*) contribute to interpatient variability in cardiac response to chemotherapeutic agents. Despite emerging insights, clinical implementation of pharmacogenetic screening remains limited. Integration of genomic data with clinical risk models may enable precision cardioprotection strategies to optimize cancer therapy while minimizing cardiovascular complications. This review poster summarizes the genetic landscape of chemotherapy-induced cardiotoxicity and discusses its potential role in advancing personalized cardio-oncology.

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CD3$^+$ Plasma-Derived Small Extracellular Vesicles Reveal Functional and Proteomic Differences Between Cancer and Health

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Small extracellular vesicles (sEV) secreted by T cells regulate immune responses. In melanoma, T cells undergo tumor-driven reprogramming, releasing immunosuppressive CD3(+) sEV that reflect parental cell features. Such vesicles may serve as a "liquid T cell biopsy" to monitor anti-tumor immunity. Using immune capture, we show that CD3(+) sEV from melanoma patients (MPs) differ in proteome and function from those of healthy donors (HDs).
Blood plasma from 10 MPs and 10 HDs was processed by ultrafiltration/SEC. CD3(+) sEV were isolated with anti-CD3 antibodies, analyzed by LC-MS/MS, and tested for effects on CD8(+) Jurkat T cells and melanoma Mel526 cells (apoptosis, mitochondrial potential, ATP production). CD3(+) sEV from MPs displayed pro-tumor activities resembling melanoma-derived sEV (MTEX). Proteomics revealed 294 sEV proteins, 226 shared with parent T cells. Sixty-six were differentially expressed between MPs and HDs, enriched in cancer-related pathways. In MPs, upregulated proteins included ITGB3 and YWHAB, linked to BRAF-driven signaling and correlating with BRAF mutations.
Our results suggest that melanoma T cells release CD3(+) sEV with altered functional and proteomic signatures, partly mimicking tumor vesicles. CD3(+) sEV may serve as a novel liquid biopsy for tumor-reprogrammed T cells.may therefore represent a promising liquid biopsy marker for tumor-reprogrammed T cells in cancer.

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Cell wall biogenesis as a key ffactor in the replicative aging of budding yeast *Saccharomyces cerevisiae*

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Aging in Saccharomyces cerevisiae is a multifactorial process influenced by both conserved and species-specific mechanisms. While the yeast cell wall is known to play critical roles in growth and stress response, its impact on replicative aging remains underexplored. In this study, we investigated five non-essential genes involved in cell wall biogenesis—CRH2, CWP1, FLO11, GAS1, and HSP12—using deletion mutants. We observed that disruptions in these genes significantly affect replicative lifespan, with gas1Δ and cwp1Δ mutants showing accelerated aging, while flo11Δ, crh2Δ, and hsp12Δ exhibited lifespan extension. Raman spectroscopy revealed distinct biochemical fingerprints, particularly in protein and RNA content, correlating with aging phenotypes. Notably, gas1Δ mutants displayed altered cell wall composition, increased sensitivity to environmental stressors, and unique metabolic profiles. Our findings highlight the importance of cell wall integrity in yeast aging and suggest that cell wall-related factors must be carefully considered when using yeast as a model for aging research [1].

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Spatial transcriptomic insights into gonadal dysgenesis in cryptorchid dogs

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Cryptorchidism is the most common disorder of sex development in dogs, affecting fertility and increasing the risk of cancer in undescended testes. In this study, we performed a comparative spatial transcriptomic analysis of descended and undescended testes from two dogs with unilateral cryptorchidism. The Visium Spatial Gene Expression protocol (10x Genomics), followed by cDNA sequencing on the Illumina NextSeq 2000 platform, was used. Cell types were annotated by module scoring against a curated list of canine testis markers. The study revealed altered mRNA expression mainly in Leydig and Sertoli cells of undescended testes. Elevated mRNA levels of candidate genes associated with cryptorchidism risk (INSL3, CYP17A1, WT1, AMH) and genes belonging to the heat shock protein families (HSPA8, HSPB1, HSPD1, HSPA5) were observed in undescended testes. For genes linked to testicular cancer (KIT, KLF4, DMRT1, SALL4, GATA4, INHA, CLPTM1L), expression levels varied significantly between descended and undescended testes, with changes depending on gene function. This study provides spatially resolved transcriptomic profiles, offering novel insights into the pathogenesis of canine cryptorchidism. However, certain limitations should be noted, as the Visium platform aggregates transcripts from multiple neighboring cells within each spot and thus does not achieve single-cell resolution.

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MOSAIC 3D – personalized disease models from iPSCs to digital twins

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Cell-based preclinical models are essential tools for understanding human diseases and developing patient-specific therapies. Compared to traditional animal models, they offer superior relevance for personalized medicine while also mitigating certain ethical concerns.
As part of the ECBG – European Centre for Bioinformatics and Genomics – MOSAIC 3D project (2025-2028), we are developing the MOSAIC 3D platform to integrate in vitro and in silico approaches for preclinical disease modelling. This initiative involves establishing the first Polish biobank of patient-derived induced pluripotent stem cells (iPSCs). These cells retain the donor's genetic background and can be differentiated into various cell types, facilitating the study of disease mechanisms in a patient-specific context. Three-dimensional organoids derived from iPSCs replicate essential tissue features, enabling the analysis of organ-specific pathologies, drug responses, and regenerative processes. Digital twins are computational models built from multiomic, bioimaging, and clinical data. They will expand the MOSAIC 3D platform's capabilities by allowing for the simulation of disease developmental trajectories and the prediction of patient-specific therapeutic responses. Together, these resources will constitute a comprehensive infrastructure designed to bridge laboratory discoveries with clinical translation.
The MOSAIC 3D platform will leverage advanced research technologies, including high-throughput genomics, spatial transcriptomics, and AI-driven data integration and modelling. This ecosystem will support functional studies, personalized therapeutic development, preclinical drug screening, and validation of new targets. By fostering partnerships across academia and industry, the MOSAIC 3D platform aims to enhance translational research and promote the development of innovative solutions for personalized medicine.

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Preliminary physicochemical studies of extracellular polymeric substances obtained from fresh sediments of upland dam reservoir

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Bacteria and algae synthesize extracellular polymeric substances (EPS) that exhibit a wide range of biologically important properties in aquatic systems. EPS can interact with different ions and particles in the processes of flocculation, biomineralization and sorption. The basic functions of exopolymers in natural systems are their abilities to form flocs and biofilms, aggregate bacterial cells, and adhere to surfaces. In addition, EPS establish a protective barrier for cells in harmful conditions. Many of these properties can be applied in industrial processes, particularly due to non-toxicity and biodegradability of EPS.
In the present study, samples of fresh sediments were collected from dam reservoir of Zalew Zemborzycki and the EPS were extracted by ethanol precipitation. The chemical components of the studied EPS were estimated by series of spectrophotometric analyses. Obtained exopolymers were also characterised by the X-ray photoelectron spectroscopy and the Fourier transformation infrared spectra. The morphological studies of extracted exopolymers were performed by scanning electron microscopy (SEM). Additionally, the crude EPS samples were examined for the flocculating activity in the presence of kaolin suspension.

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