

XXVII Gliwice Scientific Meetings



Gliwice, November 16-17, 2023

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XXVII Gliwice Scientific Meetings, 2023

Scientific Program

70th anniversary of DNA helix: Nucleic acid structure meets function

Thursday, 16th November 2023

9.00 **Opening of the conference and the Professor Chorąży Lecture 2023**

Victor Ambros (*University of Massachusetts, Worcester*): Genetic regulatory networks involving noncoding RNA

10.00 – 14.15 Session: **Helical structures, molecular crowding, and nuclear processes**
tribute to Prof. Ronald Hancock

- **Thomas Cremer** (*Ludwig-Maximilians-University, Munich*): Major differences of chromatin compaction and accessibility between active and inactive nuclear compartments.
- **Marion Cremer** (*Ludwig-Maximilians-University, Munich*): Topography of replication domains in nuclear landscapes studied with super-resolution microscopy: local details and global context.
- **Tobias Aurelius Knoch** (*Erasmus University Medical Center, Rotterdam*): On the essence of existence: how the finalization of the 3D genome organization leads to a novel generalized/unified evolution theory.

11.30 – 11.45 **Coffee break**

- **Katrina Erenpreisa** (*University of Riga, Latvia*): Repair and misrepair of the fragile telomere repeats in pml bodies and their traffic with nuclear lamin b1 in cell nucleus.
- **Aleksandra Pękowska** (*Nencki Institute of Experimental Biology, Warszawa*): Mechanisms shaping the consolidation of chromatin architecture upon loss of pluripotency.
- **Yegor Vassetzky** (*CNRS, Gustave Roussy Institute, Villejuif*): Factors that affect the formation of chromosomal translocations.
- **Jerzy Dobrucki** (*Jagiellonian University, Kraków*): Imaging DNA Damage Response.
- **Yasmina Hadj-Sahraoui** (*Laval University, Quebec*): Nuclei isolation in media containing an inert polymer to mimic the crowded cytoplasm.
- **Tomasz Sarnowski** (*Institute of Biochemistry and Biophysics, Warszawa*): Impairment of SWI/SNF chromatin remodeling complex in cancer development and progression.

14.15 – 16.00 Lunch and Poster Session

16.00 – 18.30 Session: **Role of the helix in RNA**

- **Gunter Meister** (*University of Regensburg, Regensburg*): Mechanisms of m6A- and miRNA-guided gene regulation and their links to cancer progression.
- **Jan Barciszewski** (*Institute of Bioorganic Chemistry, Poznan*): Diagnosis and therapy of brain tumor.
- **Martin Simard** (*Laval University, Quebec*): Uncovering the regulation of the microRNA-mediated gene regulatory pathway in animals.
- **Antonio Monari** (*Universite de Paris, Paris*): How is DNA and RNA 3D structures shaping viral reproduction and immune system response? The answer comes from molecular modeling and simulation.
- **Alice Ghidini** (*Sixfold Bioscience, London*): Embedding natural RNA-protein binding mechanism in RNA-based delivery scaffolds.

19.30 - Social event and conference dinner

Friday, 17th November 2023

9.00 – 11.15 Session: **Artificial Intelligence and Modeling in Molecular Biology and Medicine**

- **Tibor Antal** (*Edinburgh University School of Mathematics*): Models of cancer initiation, progression, and metastasis formation.
- **Marek Kimmel** (*Rice University, Houston*): Site frequency spectra of tumor genomes: estimating past dynamics of growth and mutation.
- **Julia Debik** (*NTNU, Trondheim*): Exploring sources of variation in the female serum metabolome in light of breast cancer risk factors, in healthy participants of the HUNT2 study.
- **Joanna Polańska** (*Silesian University of Technology, Gliwice*): Radiomics in lung cancer diagnosis.
- **Anna Karpukhina** (*CNRS, Gustave Roussy Institute, Villejuif*): Super-enhancers in Mantle Cell Lymphoma.

11.15 – 11.30 **Coffee break**

11.30 – 14.15 Session: **Cancer Proteomics and Metabolomics**

- **Malcolm R. Clench** (*Sheffield Hallam University, Sheffield*): Mass Spectrometry Imaging of metabolic processes in 3D tissue models.

- **Laura Cole** (*Sheffield Hallam University, Sheffield*): Multimodal Mass Spectrometry Imaging of ocular disease.
- **Guro F. Giskeødegård** (*NTNU, Trondheim*): Changes in circulating metabolites and lipoprotein subfractions after breast cancer treatment.
- **Piotr Mlynarz** (*Wrocław University of Science and Technology, Wrocław*): Kidney cancer recognition in men and women population.
- **Sachin Kote** (*University of Gdańsk, Gdańsk*): Serum Peptidomics: novel approaches to cancer diagnostics, prognosis, and monitoring.
- **Anna Wojakowska** (*Institute of Bioorganic Chemistry, Poznań*): Molecular predictors of colorectal cancer metastasis.
- **Monika Drobna-Śledzińska** (*Institute of Human Genetics, Poznań*): Proteomics unravels novel miR-363-3p targets implicated in JAK-STAT pathway upregulation in T-cell acute lymphoblastic leukemia.

14.15 – 15.00 **Poster Session** – Presentations of Awarded Posters

15.00 – Closing remarks, coffee, and free discussions



Ronald Hancock (1933-2022)

- PhD in Microbiology, Cambridge, UK; postdoc, Harvard Medical School
- 1966-1985: Institut Suisse de Recherches Expérimentales sur le Cancer, Lausanne; Staff Scientist
- 1986-2015: Laval University, Centre de Recherche en Cancérologie, Québec, Canada; Full Professor
- 2005-2020: Biotechnology Centre, Silesian University of Technology, Gliwice, Poland; Visiting Scientist

- Member of the International Committee of the Wilhelm Bernhard's International Workshop on the Cell Nucleus (for Canada)
- 2019: Wilhelm Bernard medal awarded during the 26th Workshop in Dijon, France

Most important contributions:

- Hancock R. Conservation of histones in chromatin during growth and mitosis in vitro. *J Mol Biol.* 1969;40:457-66
- Fakan S, Turner GN, Pagano JS, Hancock R. Sites of replication of chromosomal DNA in a eukaryotic cell. *Proc Natl Acad Sci U S A.* 1972; 69:2300-5
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- Hancock R. A new look at the nuclear matrix. *Chromosoma.* 2000; 109:219-25
- Hancock R. Packing of the polynucleosome chain in interphase chromosomes: evidence for a contribution of crowding and entropic forces. *Semin Cell Dev Biol.* 2007; 18:668-75
- Hancock R. The crowded nucleus. *Int Rev Cell Mol Biol.* 2014; 307:15-26
- Hancock R. Editor: *Methods in Molecular Biology 463.* The Nucleus Vol. 1: Nuclei and Subnuclear Components. *Humana Press*, 2008
- Hancock R. Editor: *Methods in Molecular Biology 464.* The Nucleus Vol. 2: Chromatin, Transcription, Envelope, Proteins, Dynamics, and Imaging. *Humana Press*, 2008
- Hancock R. Editor: *Methods in Molecular Biology 2175.* The Nucleus. Springer Protocols, Third Edition. *Humana Press*, 2020

Selection by Piotr Widlak and Katarzyna Lisowska



Mieczysław Chorąży (1925-2021)

- World War II soldier, wounded in the 1944 Warsaw Uprising
- Graduated from Warsaw Medical Academy (1951), PhD in medical science (1958)
- Researcher at the Institute of Oncology in Gliwice (1953-2021) and chairman of the Department of Tumor Biology (1961-1995)
- Postdoctoral researcher at Wisconsin University McArdle Memorial Laboratory for Cancer Research (1959-1960) and Sloan-Kettering Institute for Cancer Research, NY (1961-1963)
- Member of the Polish Academy of Sciences (since 1986); Professor (since 1970)
- Member of the International Committee of the Wilhelm Bernhard's International Workshop on the Cell Nucleus (for Poland)
- Decorated with the Order of the White Eagle, the highest Polish state distinction (2017)

Most important contributions:

- Chorąży M, Gettlich A, Góral L, Kołoczek B, Molawka E, Penar B, Szweda Z. Experimental chemotherapy of tumours with hydrogen peroxide. *Nature* 1958; 182:395-6
- Chorąży M. Effect of chlorpromazine on Croker sarcoma and Ehrlich ascites carcinoma. *Nature* 1959; 184:200-201.
- Chorąży M, Bendich A, Borenfreund E, Hutchinson DJ. Studies on the isolation of metaphase chromosomes. *J Cell Biol* 1963; 19:59-69
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- Rzeszowska-Wolny J, Filipski J, Grobner S, Chorąży M. Complex of DNA with chromatin proteins investigated by isopycnic centrifugation in metrizamide. *Nucl. Acids Res.* 1978; 5: 4905-4917
- Chorąży M. Sequence rearrangements and genome instability. A possible step in carcinogenesis. *J. Cancer Res. Clin. Oncol.* 1985; 109: 159-112
- Hemminki K, Grzybowska E, Chorąży M, et al. DNA adducts in humans related to occupational and environmental exposure to aromatic compounds. *IARC Sci Publ* 1990; 104:181-92
- Perera FP, et al., Chorąży M. Molecular and genetic damage in humans from environmental pollution in Poland. *Nature* 1992; 360:256-8
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- Chorąży M, Hancock R. Translational research: lost in complexity. (in) *Lost in Translation*" (eds W. Lopaczynski, R. Srivastava, W. Maksymowicz), World Scientific Publishing. 2014

Selection by Piotr Widlak and Katarzyna Lisowska

Professor Mieczysław Choraży Lecture 2023

GENETIC REGULATORY NETWORKS INVOLVING NONCODING RNA

Victor Ambros, Charles Nelson

University of Massachusetts Chan Medical School

The proper form and function of a multicellular animal requires the flawless execution of an elaborate temporal and spatial choreography of cellular proliferation, morphogenesis, and differentiation -- all driven by developmentally dynamic programs of gene activity. Developmental regulation of gene expression involves transcriptional and post-transcriptional modes -- including the regulation of mRNA stability and translatability by noncoding RNAs such as the microRNA class of small noncoding RNAs. MicroRNAs are encoded by genes that have evolved to regulate other genes by sequence complementarity, and most animal genomes contain hundreds of distinct microRNAs. MicroRNAs associate with Argonaute proteins, and recognize their mRNA targets by direct base-pairing to complementary sequences in 3 untranslated regions (3 UTRs), thereby determining the target-specificity of Argonaute-mediated post-transcriptional regulation. The precise nature of the complementarity between a microRNA and an mRNA UTR sequence can determine the nature and potency of the regulatory outcome of the interaction, and can exhibit deep evolutionary conservation for certain ancient microRNA-target pairs. At the same time, the overall repertoire of targets for a typical microRNA can be evolutionarily fluid, indicating important roles for microRNAs in adapting gene regulatory networks for the particular developmental and physiological challenges faced by an organism. MicroRNAs play prominent roles in the genetic regulation of developmental progression and cell fate specification in the nematode *C. elegans*, situating *C. elegans* as an effective experimental model for exploring the principles and mechanisms underlying microRNA function in animals. Interestingly, microRNA-mediated regulation of key protein determinants of cell fate in *C. elegans* can be highly conditional important under certain physiological conditions or life history trajectories, yet dispensable under other conditions. This conditionality is consistent with a current view of broad role for microRNAs and other noncoding RNAs in conferring developmental robustness under variable environmental and physiological conditions.

LECTURE ABSTRACTS

Session

**“Helical structures, molecular crowding,
and nuclear processes”**

tribute to Prof. Ronald Hancock

[L-1] MAJOR DIFFERENCES OF CHROMATIN COMPACTION AND ACCESSIBILITY BETWEEN ACTIVE AND INACTIVE NUCLEAR COMPARTMENTS

Thomas Cremer

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The cell nucleus with its compartmentalized chromatin architecture has evolved over a period of several billion years and enabled workable and rather robust solutions for the necessary functions of all cell types in a multicellular organism. In this lecture, I will consider challenges to develop an integrated multidisciplinary approach for a better understanding of how cell nuclei fulfill their functional tasks during expression, replication and repair of genomes. Proteins required for these functions are synthesized in the cytoplasm and enter the nucleus through nuclear pores. Where and how individual proteins, such as RNA- and DNA polymerases, form functional machines with other proteins and how individual macromolecules, such as transcription factors, find their target sites within the crowded nuclear interior is a matter of active research, attempting to solve two interrelated key problems: the problem of physical constraints of chromatin for the accessibility of individual macromolecules and macromolecular machines, and the problem of dynamic requirements that allow cells to rapidly change the transcription status of genes and gene networks as a result of environmental conditions. I dedicate this lecture to the memory of two pioneering researchers of the functional nuclear architecture, Ron Hancock (1933 - 2022) and Stan Fakan (1941 - 2023).

References:

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Cremer T., Cremer M., Cremer C (2018) *Biochemistry (Moscow)* 83, 313-325
Cremer T., Cremer M. et al. (2020) *BioEssays* 42 (2), 1900132
Cremer M., Brandstetter K. et al. (2020) *Nature Communications* 11 (1), 6146
Gellri M. et al. (2023) *Cell Reports* 42, 112567

[L-2] TOPOGRAPHY OF REPLICATION DOMAINS IN NUCLEAR LANDSCAPES STUDIED WITH 3D STRUCTURED ILLUMINATION MICROSCOPY: GLOBAL CONTEXT AND LOCAL DETAILS

**Marion Cremer⁴, Marton Gelleri¹, Michael Sterr², Hilmar Strickfaden³,
Thomas Cremer⁴**

¹Institute of Molecular Biology (IMB), 55128 Mainz, Germany

²Helmholtz Center Munich, Institute of Diabetes and Regeneration Research (IDR), Neuherberg, Germany

³Cell Imaging Core Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada

⁴Biocenter, Department Biology II, Ludwig Maximilian University (LMU), 82152 Martinsried, Germany

The replication of about 10x10⁹ nucleotides in a mammalian nucleus together with their respective nucleosomes and epigenetic signatures requires the spatial interaction of macromolecular complexes with replicating chromatin. We and others have recently described major chromatin compaction differences between the decondensed DNA of the active nuclear compartment (ANC) lining an interchromatin (IC) channel system and the highly compacted DNA of the inactive nuclear compartment (INC), indicating that the accessibility of macromolecular machines into the INC is precluded. Accordingly, access to replication machines should either become possible by local decompaction of entire chromatin domains or DNA segments localized in the INC should move into the ANC prior to replication (and relocated into the INC thereafter).

Using 3D-SIM, we studied the topography of replication domains (RDs) that persist as stable units of chromatin organization. Following pulse-replication labeling of RDs with EdU or incorporation of fluorophore-labeled dUTPs, cultured cells were fixed in formaldehyde either ~10 min after labeling or after a chase of ~80 min and counterstained with DAPI or SYTOX. In 3D image stacks seven DAPI or SYTOX intensity classes were determined as proxies for DNA compaction. Allocation of labeled replication sites on the respective classes allowed their relative signal distribution over these classes.

At the resolution level of 3D SIM (~120 nm lateral, ~300 nm axial), our quantitative assessment revealed a distribution of newly replicated chromatin in both low and high DNA intensity classes. After an 80 min chase the distribution of early/mid replicating chromatin showed only minor shifts towards higher intensity classes. In contrast, in late replicating heterochromatin blocks we observed newly replicated DNA within channels pervading these blocks, followed by a re-location of replicated DNA lining these channels after the 80 min chase.

The dynamic spatial organization of replicated DNA was studied by high-resolution imaging snap-shots within a time window of ~80 min after pulse-labeling of RDs. As a preliminary explanation, we consider a model of RDs with a compact chromatin core (attributed to the INC) and a periphery of decondensed chromatin (attributed to the ANC). In line with this model, the required relocation of DNA segments from the compact domain core to the decondensed periphery requires nanoscale movements smaller than the resolution limit of 3D SIM. In contrast, the replication of big heterochromatic blocks involves much larger and thus detectable

DNA movements. Further investigation with higher resolution as well as live cell observations

including the visualization of replication-associated proteins are needed to further evaluate this dynamic interpretation.

[L-3] ON THE ESSENCE OF EXISTENCE: HOW THE FINALIZATION OF THE 3D GENOME ORGANIZATION LEADS TO A NOVEL GENERALIZED/UNIFIED EVOLUTION THEORY

Tobias Aurelius Knoch

Erasmus University Medical Center, Rotterdam

What existence in general is has been an epic question as well as what life is, how it emerges, functions, evolves, and as it manifests in its most central organization unit - genomes. Their 3D architecture and dynamics, including the interaction networks of regulatory elements, obviously co-evolved as inseparable systems allowing the physical storage, expression, and replication of genetic information. Recently, we were able to fill finally the much debated centennial gaps in genome 3D architecture and dynamics, which is also completely consistent with the entire history of the field of the last 170 years.

Interestingly, this leads instantly to a novel consistent and cross-proven systems statistical mechanics genomics framework elucidating genome intrinsic function and regulation. It balances stability/flexibility ensuring genome integrity, enabling expression/regulation of genetic information, as well as genome replication/spread. Furthermore, genotype and phenotype are multiplicatively entangled being evolutionarily the outcome of both Darwinian natural selection AND Lamarckian self-referenced manipulation - all embedded in even broader unifying genome ecology (autopoietic) in- and environmental scopes. This allows formulating new meta-level functional semantics of genomics, i.e. notions as communication of genes, genomes, and information networks, architectural and dynamic spaces for creativity and innovation, or genomes as central geno-/phenotype entanglements. Beyond and most fundamentally, the paradoxical-seeming hierarchical local equilibrium substance stability in its entity though far from a universal heat-death-like equilibrium is solved, and system irreversibility, time directionality, and thus the emergence of existence are clarified! This is nothing less than a - by the upper experiments and thus already partially proven - novel general and unified theory of evolution. Consequently, real deep understandings of genomes, life, and complex systems in general appear in evolutionary perspectives as well as from systems analyses, via system damage/disease (its repair/cure and manipulation) as far as the understanding of extraterrestrial life, the de novo creation and thus artificial life, and even the *raison d'être*.

Keywords: Biophysical Genomics; Human Ecology and Complex Systems; TAK Renewable Energy UG;

References:

Knoch T. A. How genomes emerge, function and evolve: living systems emergence, genotype-phenotype-multilism, genome/systems ecology. *Results and Problems in Cell Differentiation* 70, Springer, ISBN 978-3-031-06572-9, DOI 10.1007/978-3-031-06573-6_4, 103-156, 2022.

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Wachsmuth M., Knoch T. A. & Rippe K. Dynamic properties of independent chromatin domains measured by correlation spectroscopy in living cells. *Epigenetics & Chromatin* 9:57, 1-20, 2016.

[L-4] REPAIR AND MISREPAIR OF THE FRAGILE TELOMERE REPEATS IN PML BODIES AND THEIR TRAFFIC WITH NUCLEAR LAMIN B1 IN CELL NUCLEUS

Katrina Erenpreisa

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The data are accumulating that cell nucleus envelope, in particular its underlying fibrillar meshwork lamin B1 interacting with the marginal chromatin is involved in the genome integrity regulation, which is compromised during cellular senescence but many aspects remain obscure. Cell nucleus is normally slowly rotating its peripheral compartment by microtubules (MT) and ATP-dynein motor, driven from a centrosome (CS) and contractility of concentrically-chiral actomyosin fibrils. CS also senses the DNA damage. The cell nucleus has two fractal boundaries to move the densely packed heterochromatin (HR) around – the nuclear envelope (NE) with lamina-associated heterochromatin underlined by the epichromatin granular layer interacting with lamin B1 and round central nucleolus bordered by NORs. Our study on cancer cell lines using immunocytochemistry for telomere shelterin TRF2, PML, RAD51, γ H2AX, lamin B1, Tubulin, Vimentin, counterstained with DAPI, Acridine orange DNA structural test, and electron microscopy showed that after genotoxic stress (anticancer drugs or ionizing irradiation), the concentric movement of MT and the HR arrays around NE and nucleolus accompanied by rigid-elastic lamina B1 as part of the nuclear-envelope chromatin sheets (ELCS) are enhanced by a clockwise gear-wheel-like mechanism. It likely favours the homology search and telomere DNA repair revealed at the same time in PML bodies by transient recombinative alternative telomere lengthening (ALT), while removing DNA waste into the nuclear autophagic pockets. Contrary to that, at high DNA damage, the cell undergoes deep senescence, PML form threads of dimeric rods end-joined by the unrepaired telomeres and tending to dash into the loops of nuclear lamin B1, which finally degrades and collapses towards the nucleolus. Lamin B1 undergoes the anti-chiral rotation around the damaged nucleolar DNA convoluting into the large PML body(ies) and ending in nuclear destruction. Anti-chiral rotation is likely performed by vimentin acting as a brake for the dynein motor. We hypothesize that the gear-wheel chiral traffic is moving the envelope-limited chromatin sheets enclosing two apposing arrays of supra nucleosomal epichromatin beads of the mobilised, stacked ALU-flanked interstitial telomere fragments, for search of homology and their repair by ALT, emergently safeguarding the genome integrity.

Keywords: Cancer, PBL, telomere repair, lamin B1, nuclear traffic

References:

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- Salmina et al., 2020. “Mitotic Slippage” and Extranuclear DNA in Cancer Chemoresistance: A Focus on Telomeres doi:10.3390/ijms21082779.

[L-5] MECHANISMS SHAPING THE CONSOLIDATION OF CHROMATIN ARCHITECTURE UPON LOSS OF PLURIPOTENCY

Aleksandra Pękowska

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Nencki Institute of Experimental Biology
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Topologically associating domains (TADs) are genomic intervals of strong self-contact that demarcate cognate promoter-enhancer pairs. TAD boundaries often interact, forming architectural loops. Binding of an eleven-zinc finger protein CTCF defines the positions of TAD borders and loop anchors. Despite a remarkable preservation of the genomic coordinates of TADs, the strengths of TAD boundaries and architectural loops increase during mammalian development. However, the mechanisms orchestrating structural consolidation of chromatin remain elusive. Here, using embryonic stem (ES) to neural stem (NS) differentiation, we reveal the transcriptional imprint of the enhanced insulatory role of CTCF during lineage commitment. Taking advantage of Selective Isolation of Chromatin Associated Proteins (SICAP-ChIP), we define the CTCF-bound proteome in ES and NS cell chromatin. We uncover that differentiation is mirrored by a pervasive gain of interactions between CTCF and RNA binding proteins (RBPs). We show that, while having no impact on CTCF in the ES cells, the genetic ablation of chosen RBPs results in an attenuated CTCF binding and leads to weakening of architectural loop formation in the NS cells. Altogether, we reveal the dynamic changes of CTCF-protein interactome contributing to consolidation of chromatin structure during mammalian development.

[L-6] FACTORS THAT AFFECT THE FORMATION OF CHROMOSOMAL TRANSLOCATIONS

Anna Shmakova, Anna Karpukhina, Yegor Vassetzky

CNRS UMR 9018, Institut Gustave Roussy, Villejuif

Most cancer-related chromosomal translocations appear to be cell type-specific. It is currently unknown whether this is due to the cell type-specific spontaneous translocations between different loci or to the post-translocation selection of translocations that confer adaptive survival advantages (enhanced growth, resistance to apoptosis, etc.) to the cell. We experimentally addressed this question by simultaneous induction of DSBs at several specific loci in the same cell to generate chromosomal translocations. The relative frequency of the generated chromosomal translocations in different cell types was then correlated with the transcriptional activity, nuclear radial position and spatial proximity of the gene loci, before and after DSB induction. Only the spatial proximity between gene loci after the DSB induction correlated with the resulting translocation frequency, supporting the breakage-first model. Furthermore, long term culture of cells with the generated chromosomal translocations displayed varying translocation persistence in different cell types, implying that chromosomal translocations result from a combination of initial non-random translocation events and the following selection. Overall, the results suggest that chromosomal translocation can be generated after DSB induction in any type of cell, but as to whether the cell with the translocation would persist depends on the cell type-specific selective survival advantage that the chromosomal translocation confers to the cell.

[L-7] IMAGING DNA DAMAGE RESPONSE

Jerzy W. Dobrucki

*Department of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology,
Jagiellonian University, Kraków, Poland*

Recent advances in fluorescence imaging techniques and methods of labelling of DNA breaks opened new research avenues in the field of DNA damage response. I will describe applications of advanced optical microscopy methods, including fluorescence correlation spectroscopy (FCS), fluorescence lifetime imaging (FLIM) [1], and super-resolution imaging, in studies of molecular crowding in the chromatin regions surrounding DNA breaks, dynamics of repair factors recruited to DNA lesions, and mobility of chromatin fibres in repair foci. I will also discuss recent data on the number of endogenous DSBs in cells in mammalian tissues based on direct imaging approaches (STRIDE labelling technology [2]) (0~50) and compare them with the data provided by some new genome-wide DSB mapping methods (tens of thousands, e.g. [3]).

Keywords: DNA damage response, DNA repair foci, fluorescence imaging

References:

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[L-8] NUCLEI ISOLATION IN MEDIA CONTAINING AN INERT POLYMER TO MIMIC THE CROWDED CYTOPLASM

Yasmina Hadj Sahraoui

Cégep Garneau, Québec, Canada

As cations aren't free in cytoplasm, the aim of our work was to verify if ionic conditions, generally used to extract cell nuclei to preserve chromatin compaction, represent the environment of nuclei *in vivo*. Commonly, small scale motion and chromatin conformation *in vivo* are not reproduced in isolated nuclei using cations. Ions are in fact not soluble and free in the cytoplasm but bound to macromolecules as reported by theory and experiments which estimated these molecules concentration in the range of 130mg/ml. The aim of my work in Ronald Hancock's laboratory was to study if cytoplasmic crowding or osmotic effects could have an impact in preserving structure and nuclei functions. I will present the examples of our studies on effects of molecular crowding induced *in vitro* by inert media.

We worked on K562 (human erythroleukemia) cells grown in suspension culture in DMEM, U2OS cells stably expressing GFP-PML isoform IV grown in DMEM and HeLa cells inducibly expressing GFP-coilin, grown as monolayers with transfer to tetracycline-free medium for 4-6h to allow GFP coilin expression. Cell nuclei were isolated at room temperature using 50% (w/v) Ficoll (70kDA) or 35% (w/v) dextran (70kDA) or PEG (8kDA) in 100mM K-Hepes buffer, pH 7.4, containing 100mg/ml digitonin.

As seen by electron microscopy, nuclei isolated in the presence of inert polymer conserved their volume and their ultrastructure (nucleoli, PML and coiled bodies, foci of RNA polymerase II and nascent RNA transcripts). The maintenance of ultrastructure elements depended on the concentration of inert polymers. The results of the experiments in conditions which mimic the crowded cytoplasm supported the hypothesis that crowding conditions are essential to maintain the correct structure of nuclei and their ultrastructure components.

[L-9] IMPAIRMENT OF SWI/SNF CHROMATIN REMODELING COMPLEX IN CANCER DEVELOPMENT AND PROGRESSION

Tomasz J. Sarnowski

Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

The SWI/SNF chromatin remodeling complex (CRC) is involved in numerous processes including transcriptional control of gene expression, cell cycle, DNA repair and maintenance of higher order chromatin structure. Genes encoding SWI/SNF subunits are mutated in about 20% of all cancer types. The overexpression of some SWI/SNF subunits like central BRM ATPase, was associated with cancer resistance for chemotherapy and metastasis in ovarian and pancreatic cancers. The Pan-cancer analysis of expression of main SWI/SNF subunits revealed the differences between expression of BRM and BRG1 ATPases as well as for other subunits.

We found numerous changes in the expression of genes encoding the main BRM, BRG1, BAF155 and BAF170 subunits of SWI/SNF CRCs. The observed changes were cancer-specific indicative of the existence of various effects on the SWI/SNF function in studied tumours. Loss or overexpression of SWI/SNF was correlated to tumour progression and chemoresistance dependent on tumour tissue origin and type. We subsequently found that the SWI/SNF complex plays an important role in the T CD4+ lymphocyte exhaustion by cancer cells expressing PD-L1 on their surface. Collectively, our results indicate numerous distinct regulatory roles of SWI/SNF CRCs not only in carcinogenesis but also during the action of immune system (i.e. tumour infiltrating lymphocytes) fighting with cancer. Therefore, the subunits of SWI/SNF CRCs represent attractive targets for the development of new compounds which may be used in the future as epidrugs in various treatments.

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LECTURE ABSTRACTS

Session

“Role of the helix in RNA”

[L-10] MECHANISMS OF M6A- AND MIRNA-GUIDED GENE REGULATION AND THEIR LINKS TO CANCER PROGRESSION

Gunter Meister

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Gene expression is regulated at many post-transcriptional steps of RNA maturation. Various classes of non-coding RNAs have been identified that affect gene expression including microRNAs, lncRNAs or circular RNAs. In many cases, these non-coding RNAs use their sequence information and form double stranded (ds) RNA with their target RNAs. Furthermore, many non-coding RNAs are generated from dsRNA precursors or fold themselves into complex secondary structures to become functional. Such structured RNAs, however, do not act as sole RNA molecules but are instead incorporated into RNA-protein complexes (RNPs), in which at least one RNA-binding protein (RBP) contacts the RNA directly. Consequently, RBPs are essential for many RNA-mediated processes including miRNA-guided gene regulation, miRNA biogenesis or RNA modification. Thus, RBPs serve as regulatory hubs to coordinate RNA-guided processes and numerous studies unraveled that such processes are malfunctioning in disease.

Our work aims at a detailed mechanistic understanding of RNP function in healthy and disease tissues. For example, we use biochemical strategies to unravel the mechanisms of ds pre-miRNA processing and regulation by the Lin28a/b-TUTase-Dis3L2 system and recently extended this work to other dsRNA precursors. Furthermore, RBPs such as the Lupus autoantigen La can function as RNA chaperones and help generating dsRNA structures. La is part of a larger protein family termed La-related proteins and our work assigns additional exciting roles to these RBPs.

[L11] DIAGNOSIS AND THERAPY OF BRAIN TUMORS

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Brain malignancies are a significant source of morbidity and mortality. They form a group of neoplasia with several of histological characteristics and different malignancy grades. Their precise description is important because of its strong prognostic and therapeutically implications. There are many molecular (genetic and epigenetic) alterations occurred in brain tumors which may have diagnostic and predictive values as they are linked with histological determined tumor types and malignancy grades.

We have developed a new, simple and reliable method for diagnosis of brain tumours. It is based on a thin layer chromatography (TLC) quantitative determination of 5-methylcytosine (m^5C) in relation to its damage products of DNA from tumour tissue. The m^5C level in DNA of different brain tumor tissues overlap with that of blood and negative correlates with grades of malignancy. In addition to that, DNA global methylation (m^5C) analysis allows an easy differentiation of low and high grade gliomas and is also specific for various diseases. So, genomic methylation (m^5C) can be a good marker for the early detection of the relapse of brain tumors and other diseases.

Malignant gliomas express preferentially a number of surface markers that may be exploited as therapeutic targets, such as tenascin-C, an extracellular matrix glycoprotein contributes to tumor cell adhesion, invasion, migration and proliferation. TN-C is a dominant epitope in GBM.

We used double stranded interfering RNAs (dsRNAs) to reduce tenascin-C expression in brain tumor cells. Patients suffering from brain tumor were resected and treated with dsRNA (ATN-RNA) complementary to the sequence of tenascin-C mRNA. MRI and CT follow up studies showed growth tumor delay or lack of its recurrence symptoms, due to inhibition of TN-C synthesis. A significant improvement in overall survival (OS) was observed without losing of the quality of life (QOL) of patients. This novel therapy based on RNA interference shows a big therapeutical potential. To our knowledge intervention with RNAi (iRNAi) is the first protocol of application of RNAi in human disease treatment. The technology we called interference RNA intervention (iRNAi).

[L-12] UNCOVERING THE REGULATION OF THE MICRORNA-MEDIATED GENE REGULATORY PATHWAY IN ANIMALS

Martin Simard

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Systematic studies with animal models have helped us uncover different ways microRNAs use to regulate gene expression. For example, using the roundworm *Caenorhabditis elegans* as an animal model, we recently discovered the existence of different miRNA-induced Silencing complexes (or miRISC) in animals that affect targeted mRNAs distinctively. In our quest to understand what modulates microRNA functions, we focus on identifying genetic and molecular partners as well as post-translational modifications of the Argonaute proteins, the core component of the silencing complex. During this talk, we will present our recent discoveries that identify new phosphorylation sites onto Argonaute that regulate the microRNA function during animal development.

[L-13] HOW IS DNA AND RNA 3D STRUCTURES SHAPING VIRAL REPRODUCTION AND IMMUNE SYSTEM RESPONSE? THE ANSWER COMES FROM MOLECULAR MODELING AND SIMULATION

Antonio Monari

Université Paris Cité and CNRS, ITODYS

Nucleic acid, and RNA in particular, assume a rather complex polymorphisms, which ultimately results in the coexistence of different secondary and tertiary structures. Non-canonical secondary structures, including guanine quadruplexes and RNA hairpins are, indeed, crucial in regulating different biological functions, such as gene expression and replication. Yet their role is often overlooked and should be precisely taken into account.

Even if the organization of the genome in viruses is much simpler than in eukaryotic organisms RNA viruses are characterized by a complex landscape and the secondary structures of RNA are fundamental in assuring, on the one side the virus correct reproduction and regulation, and on the other side the recognition by the host immune system.

In this talk we will show how long-scale all-atom molecular simulation may help in understanding the complex and dynamic organization of the viral genome and its interplay with its life cycle and/or the triggering of the immune response. These results may also pave the way to the development of original therapeutic strategies targeting the viral genome organization and its interaction with human or viral proteins.

[L-14] EMBEDDING NATURAL RNA-PROTEIN BINDING MECHANISM IN RNA-BASED DELIVERY SCAFFOLDS

Alice Ghidini

Sixfold Bioscience, London

RNA serves a dual purpose: encoding information through its sequence and controlling processes via its structure. Advances in biophysical and biochemical techniques over the past decade, especially next-generation sequencing, enable comprehensive exploration of RNA folding in cells and the design of RNA-therapeutics. A deeper understanding of RNA's 2D and 3D structures is crucial for comprehending its functions, designing synthetic RNAs, and developing RNA-targeted medications. Currently, RNA structure analysis methods provide only partial information, but direct measurement of intact RNA structures could enhance studies of function, regulation, and druggability. Understanding the interaction between RNA-based therapeutics and proteins is in fact a crucial aspect for optimizing drug design. This knowledge helps fine-tune drug formulations containing RNA and their circulation in the body, including binding to serum proteins, which significantly affects therapeutic efficacy. However, limited methodologies have hindered this understanding. Recently, academic groups and pharmaceutical companies, like Sixfold, have developed innovative analytical techniques. Sixfold's proprietary delivery system, Mergo, a chemically modified RNA, is designed for cell-specific distribution of RNA-therapeutics, exhibiting unique plasma protein binding properties. The team is actively profiling these constructs. These novel *in vitro* and *in vivo* experimental methods can also be applied to study RNA's interactions with intracellular proteins, paving the way for groundbreaking RNA-based therapeutics, such as RNA-PROTAC. RNA-PROTAC leverages known RNA binding motifs to tag RNA-binding proteins (RBP), leading to their selective degradation through the cell's proteasomal machinery. This research holds promise for advancing the field of RNA-based therapeutics and their interactions with proteins.

LECTURE ABSTRACTS

Session

**“Artificial Intelligence and Modeling in Molecular
Biology and Medicine”**

[L-15] MODELS OF CANCER INITIATION, PROGRESSION, AND METASTASIS FORMATION

Tibor Antal

Edinburgh University School of Mathematics

I'll mainly focus on a recent unpublished work on colorectal cancer initiation. Colorectal cancer typically starts with 2 APC mutations. I'll discuss some result which quantifies the relevance of the position of these mutations. I'll also review some concurrent modelling efforts on initiation. At the other end of the spectrum I'll briefly discuss some successes and more challenges of modelling metastasis formation.

[L-16] SITE FREQUENCY SPECTRA OF TUMOR GENOMES: ESTIMATING PAST DYNAMICS OF GROWTH AND MUTATION

Marek Kimmel

Rice University, Houston

We explain how models of population genetics can be used to provide quantitative inference of clonal evolution of cancer. The talk has two parts. Part 1 is devoted to the definition and mathematical properties of the Site Frequency Spectrum (SFS), one of the commonly used characteristics of cell populations undergoing growth and mutation. We explore the basic consistency of the approaches based on Wright-Fisher or Moran coalescents versus those based on birth-death processes. This provides building blocks for Part 2, which introduces the heuristic estimation equations, which employ the observable characteristics of the SFS, and allow an exact solution providing estimates of the growth and mutation rates and origin times of the clones. Examples based on simulations and available tumor data are presented. Accuracy of the estimates and their possible applications are discussed.

Contributions of Emmanuel Asante, Khanh Dinh, Roman Jaksik, Andrew Koval, Paweł Kuś, and Simon Tavaré are acknowledged.

[L-17] EXPLORING SOURCES OF VARIATION IN THE FEMALE SERUM METABOLOME IN LIGHT OF BREAST CANCER RISK FACTORS, IN HEALTHY PARTICIPANTS OF THE HUNT2 STUDY

Julia Debik

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The study analyzed pre-diagnostic serum samples from healthy women in the Norwegian Trndelag Health Study (HUNT2 study) to identify molecular biomarkers linked to breast cancer risk.

Women participating in the HUNT2 study who developed breast cancer within a 15-year follow-up period (BC cases) and age-matched women who stayed breast cancer-free were selected (n=453 case-control pairs). Using a high-resolution mass spectrometry approach 284 compounds were quantitatively analyzed, including 30 amino acids and biogenic amines, hexoses, and 253 lipids.

Age was identified as a major confounding factor responsible for large heterogeneity in the dataset, so age-defined subgroups were examined separately. The subgroup of younger women (45 years old) had the highest number of metabolites whose serum levels differentiated BC cases from controls (82 compounds). Notably, higher glyceride, phosphatidylcholine, and sphingolipid levels were linked to a lower risk of cancer in younger and middle-aged women (64 years old), while increased serum lipid levels were linked to an increased risk of breast cancer in older women (64 years old). Furthermore, while serum levels of several metabolites differed between BC cases diagnosed earlier (5 years) and later (10 years) after sample collection, these compounds were also correlated with participant age. The studys findings were consistent with those of the NMR-based metabolomics study conducted in the HUNT2 cohort, which found that higher serum levels of VLDL subfractions were associated with a lower risk of breast cancer in premenopausal women.

The study found that differences in metabolite levels indicating impaired lipid and amino acid metabolism were associated with long-term breast cancer risk in an age-dependent manner.

[L-18] RADIOMICS IN LUNG CANCER DIAGNOSIS

Joanna Polańska

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Lung cancer caused 1.76 million fatalities globally in 2018, making it the deadliest cancer. Early identification and diagnosis of lung nodules are critical in the fight against this disease.

Radiomics is a promising area for the accurate detection and treatment of lung cancer as it facilitates a more comprehensive, non-invasive and quantitative analysis of medical images. It includes extracting several quantitative features, known as radiomic features, from medical images. The aforementioned features exceed the limits of human visual detection and comprise information regarding the shape, size, and texture of lung lesions. This approach relies less on subjective interpretation, thereby producing a more objective and reproducible means of evaluating tumour characteristics. This is particularly critical in the diagnosis and treatment monitoring of lung cancer.

Radiomic features can potentially serve as biomarkers for the condition. Benign and malignant lesions can be differentiated using specific biomarkers, which may also be linked to certain lung cancer subtypes. The identification of such biomarkers can be of great assistance in early detection and prognosis.

The primary and critical stage in radiomics analysis requires the segmentation of lung nodules or lesions by outlining and delineating the region of interest in medical images. Machine learning algorithms, particularly deep learning methods, are frequently utilised to achieve precise and automated segmentation of such lesions. An unequivocal and reproducible technique for scrutinising medical imaging is vital to ensure the production of reliable and consistent radiomics outcomes. This system encompasses image preprocessing, lesion identification, feature extraction, and data analysis. The main objective is to automate this method to ensure maximal performance and minimise the possibility of human error.

We will show an exemplary pipeline developed to support low-dose-CT-based lung cancer screening programmes in clinical practice, wherein radiomics is being integrated to enhance early detection efforts.

Acknowledgements: *This work was partially financially supported by the National Science Centre, Poland, grant 2017/27/B/NZ7/01833.*

[L-19] EPIGENETIC AND 3D-GENOME CHANGES CONTRIBUTE TO THE PATHOGENESIS OF MANTLE CELL LYMPHOMA

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Mantle cell lymphoma is an aggressive B cell malignancy with poor prognosis. More than 90% of MCL cases are associated with a recurrent chromosomal translocation t(11;14)(q13;q32) that results in the overexpression of cyclin D1 (CCND1), a potent cell-cycle regulator. Nevertheless, CCND1 overexpression alone does not lead to malignancies in animal models. Thus, the development of MCL should be triggered by additional factors, which may guide the development of new therapies once discovered.

A chromosomal translocation can trigger large-scale changes in the 3D genome organization, as well as the transcriptional and epigenetic changes in the translocated loci. Here we demonstrated that the translocated CCND1 locus on derivative chromosome 14 is relocated to the nuclear center in MCL cells. This is accompanied by the appearance of a new super-enhancer (SE) inside this locus. Surprisingly, the region around the novel SE was not significantly enriched for the genes differentially expressed in MCL. Instead, most of the differentially expressed genes were located on chromosomes 19, 17 and 22 in both the MCL cell lines and the B cells from MCL patients. Among these genes, there were many related to lymphoma or other cancers. Using HiC, we detected the presence of interchromosomal contacts between chr11 and the chromosomes 19, 17 and 22. This was confirmed by FISH for chr17, and the experiments for chr19 and 22 are in progress. We hypothesize that the deregulated genes on chromosomes 19, 17 and 22 contribute to the MCL oncogenesis, and their upregulation is explained by the action of an MCL-specific SE inside the CCND1 loci. Thus, inhibiting super-enhancer activity may represent a new treatment strategy for MCL. We tested two substances with such properties, Abemaciclib and Minnelide, in MCL cell lines and the B cells from MCL patients. Both substances effectively reduced the viability of the malignant cells.

Our results provide valuable preclinical data and novel insights into the mechanisms of MCL pathogenesis.

LECTURE ABSTRACTS

Session

“Cancer Proteomics and Metabolomics”

[L-20] MASS SPECTROMETRY IMAGING OF METABOLIC PROCESSES IN 3D TISSUE MODELS

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Mass spectrometry imaging (MSI) is a powerful analytical technique that has revolutionized our understanding of biological systems by providing spatially resolved information on the distribution of molecules within a sample. 3D cell models offer a more physiologically relevant environment for studying cellular processes than 2D models, making them increasingly popular for biomedical research. Mass spectrometry imaging has emerged as a valuable tool for characterizing these models, allowing for the spatially resolved analysis of biomolecules such as lipids, proteins, and metabolites within them. We have used a multimodal imaging approach combining MALDI-MSI, DESI-MS, Imaging Mass Cytometry and MALDI-Immunohistochemistry to study drug penetration and distribution in 3D culture systems of skin and tumours. Additionally multimodal MSI has been used to study the spatial heterogeneity of tumour microenvironments and responses to treatment for small molecule and biopharmaceuticals in aggregated spheroid models and multicellular tumour spheroids. These data give an indication of the possible utilisation of these technologies in personalised medicine, and this is discussed.

[L-21] MULTIMODAL MASS SPECTROMETRY IMAGING OF OCULAR DISEASE

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Ocular disease is manifested by an array of physiological changes within ocular tissue. The metabolic, metallomic and proteomic changes that occur as a result of ocular disease can be characterised by mass spectrometry imaging (MSI).

Age-related macular degeneration- Recent studies into the innate immune system have highlighted the importance of a class of proteins known as pattern-recognition receptors (PRRs) in ocular disease. Additionally, at the turn of the century the Age-Related Eye Disease Study (AREDS) highlighted the importance of essential and non-essential trace elements within ocular tissue. Herein, MSI has been utilised to characterise WT and retinal degeneration mouse ocular tissue by MALDI-MSI and LA-ICP-MSI, in combination with Particle Induced X-ray Emission (PIXE).

Key biomarkers of ocular disease are currently under investigation through a multidisciplinary and multimodal methodology. DESI-MSI and MALDI-MSI have been employed in combination to identify key biomarkers within the murine innate immune system by characterising WT and knockout mouse ocular tissue in tandem. Lipidomic profiles alongside identification of key innate immune proteins have been observed. LA-ICP-MSI and PIXE have shown the spatial distribution of key trace elements within mouse ocular tissue. The combination of techniques allows the multi-faceted characterisation of mouse ocular tissue within the context of ocular disease, offering further insight into the pathology of ocular diseases such as age-related macular degeneration.

Uveal melanoma - Uveal melanoma (UM) is a rare adult eye cancer affecting approximately 5-6 individuals per million annually. UM arises from structures in the middle layer of the eye; the iris, choroid or ciliary body.

The existence of a metabolic switch in cancer cells leading to changes in energy metabolism compared with healthy cells is now recognised as one of the driving forces of tumour growth and progression in an otherwise inhospitable tumour microenvironment. It is essential therefore that we improve our understanding of metastatic vulnerabilities that can be targeted therapeutically in this disease. Very few studies have addressed metabolic profiles in UM. Preliminary data indicated that multi-modal MSI has the potential to provide insights into the role of trace metals and cancer metastasis. MSI data from human whole eye sections and more recently 3D spheroid models have displayed some promising findings.

[L-22] CHANGES IN CIRCULATING METABOLITES AND LIPOPROTEIN SUBFRACTIONS AFTER BREAST CANCER TREATMENT

Guro F. Giskeødegård

Norwegian University of Science and Technology, Trondheim

Breast cancer is the most common cancer disease among women, affecting one out of eleven women. Successful treatment has led to high survival rates above 90% for breast cancer patients, however several breast cancer survivors will suffer from long term late effects after treatment. Reducing overtreatment and avoiding unnecessary late effects of cancer treatment will have major impact on patient outcome and wellbeing. Molecular profiling of cancer tissue and biofluids from cancer patients at different stages of disease may contribute to increased precision medicine by better treatment stratification and monitoring of treatment response. In our research group we aim to understand the molecular signatures of breast cancer, from early cancer development, throughout diagnosis and treatment, until follow up of cancer survivors. This talk will focus on the molecular changes appearing in breast cancer patients during treatment, that may contribute to explain why some patients have poor response to certain treatments, and to understand the increased cardiovascular risk of breast cancer survivors.

[L-23] KIDNEY CANCER RECOGNITION IN MEN AND WOMEN POPULATION

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Renal cell carcinoma (RCC) is found to be one of the most common form of kidney malignancy with a 2/1 male/female incidence ratio. The serum ¹H NMR metabolic profiles matched with health control were subjected to analysis by ¹H NMR spectroscopy and chemometric methods. The common, regardless of gender, metabolites were associated with RCC namely: lower lactate, threonine, histidine, and choline levels together with increased levels of pyruvate, N-acetylated glycoproteins, beta-hydroxybutyrate, acetoacetate, and lysine. Interestingly serum lactate/pyruvate ratio was a strong predictor of RCC. During our study, we observed distinct gender-specific metabolic features among RCC patients including metabolites: glycine, pyruvate, and NAC1 for females and glucose, lysine, and betaine for males. This finding enabled us to elaborate a more specific gender-related metabolite diagnostic panel [1].

References:

[1] Deja, S.; Litarski, A.; Mielko, K.A.; Pudelko-Malik, N.; Wojtowicz, W.; Zabek, A.; Szydelko, T.; Młynarz, P. Gender-Specific Metabolomics Approach to Kidney Cancer. *Metabolites* 2021, *11*, 767.

[L-24] SERUM PEPTIDOMICS: NOVEL APPROACHES TO CANCER DIAGNOSTICS, PROGNOSIS, AND MONITORING

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Using blood (serum/plasma) for early screening and diagnosis is vital to develop an effective therapy for many diseases. Even though peptidomics has been introduced since the decade, the serum peptidome analysis has consistently been restricted for various reasons: a) enormous complexity, b) status of highly abundant proteins, c) simultaneously limit detection of low abundant peptides with diagnostic potential, d) unequal concentrations of low and high abundant proteins, e) instability of low abundant proteins or peptides, f) depletion steps, fractionation and precipitation methods for the removal of high abundant proteins are overwhelming that compromise the number, yield of diagnostic peptides identified and essential biological information can be lost in the background noise. Novel method (submitted EP application) for onestep sample preparation for qualitative and quantitative analysis of peptidome in serum based on amino acid sequencing with tandem mass spectrometry. The invention approach is simple, costeffective, fast, and comprehensive for serum peptidomics profiling. These serum peptides can be used for biomarker discovery, diagnosis, prognosis, prediction, monitoring, and differentiation of various human disease types.

[L-25] PROTEOMIC PREDICTORS OF COLORECTAL CANCER METASTASIS

Anna Wojakowska

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Colorectal cancer (CRC) patients mortality is primarily caused by aggressive metastatic disease progression, rather than the primary tumor. Despite the progress in oncological treatment, the development of metastasis is common in CRC. Regional lymph node metastasis (RLNM) is an important prognostic factor. However, there is little data on the risk factors of RLNM in CRC that can be determined before surgery. Here we used a label-free LC-MS/MS approach to search for proteomic predictors of RLNM in rectal cancer patients with different statuses of lymph node metastasis. In order to look for molecular signatures of colon cancer distant metastasis (DM) we applied proteomic profiling of FFPE primary tumors from patients with different statuses of metachronous DM.

Primary right-sided colon cancer tissue was collected during surgery from patients without synchronous distant metastasis and secured for routine histopathologic analysis as formalin-fixed paraffin-embedded (FFPE) specimens. Colon cancer patients were classified into two groups, depending on the occurrence of metachronous DM during the 5-year follow-up period. Proteomic profiling was performed using LC-MS/MS approach with an additional ion filtering stage using FAIMS interface. To look for molecular predictors of RLNM we performed a systematic assessment of proteins present both in fresh frozen tissue (tumor and proximal margins) and in circulation (plasma and exosomes).

Label-free LC-MS/MS approach allowed the identification of about 4500 proteins in FFPE specimens. An untargeted proteomic analysis revealed about 350 proteins, whose levels discriminated between patients with different statuses of DM. We proposed a panel of proteomic signatures detected in primary tumors which discriminated patients with different statuses of metachronous DM with AUC 0.90 and accuracy 80%. Moreover, we identified proteins that significantly discriminated patients with different statuses of RLNM in tumor tissue. Based on the comparative analysis we proposed DAPs whose level was upregulated in tissue of both DM+ and RLNM+ groups of patients, including ANXA2, P4HA1, CUL3, CALU, THBS1, THBS2, SCARB2, and VCAN. Interestingly significantly elevated level of VCAN were detected in RLNM+ group of exosomes. Furthermore, we detected more than 1000 proteins significantly differentiating tumors and tissue margins.

This study revealed a specific proteomic pattern of FFPE primary colon tumors, which could discriminate patients with different statuses of metachronous distant metastasis. Moreover, comprehensive protein profiling of various types of samples from patients with different statuses of RLNM allow the detection of promising candidates for signatures of regional lymph node metastasis in rectal cancer.

Funding: *This study was supported by the National Science Centre, Poland, Grant 2017/26/D/NZ2/00964.*

[L-26] PROTEOMICS UNRAVELS NOVEL MIR-363-3P TARGETS IMPLICATED IN JAK-STAT PATHWAY UPREGULATION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy arising from T-cell precursors. miRNAs are involved in post-transcriptional negative regulation of gene expression and thus are implicated in many biological processes, in health and disease. Recently we reported the upregulation of hsa-miR-363-3p in pediatric T-ALL patients and proposed it as a novel candidate oncomiR. We showed that inhibition of miR-363-3p results in increased apoptosis and decreased proliferation of T-ALL cells. Here we aimed to characterize the global effects of hsa-miR-363-3p in T-ALL *in vitro* via the combination of mRNA sequencing and quantitative proteomic analysis. We aimed to unravel the whole spectrum of direct targets of hsa-miR-363-3p as well as processes indirectly regulated by this miRNA.

DND-41 and ALL-SIL T-ALL cell lines were transduced for stable inhibition of hsa-miR-363-3p. To evaluate the influence of miRNA inhibition on growth advantage of these cells, flow cytometry GFP competition assay was performed. Total RNA from DND-41 cell line upon miR-363-3p inhibition was subjected to mRNA sequencing (150bp paired end reads, 60M reads/sample, Illumina NovaSeq 6000). These cells were also subjected to total protein Tandem Mass Tag (TMT) labeling and mass spectrometry for quantitative proteomics. Genes coding for proteins upregulated upon miRNA inhibition were analyzed to identify binding sites for miR-363-3p. Dual Luciferase Assay was performed to confirm the interaction between miR-363-3p and 3UTR of putative target genes. The upregulation of selected proteins was evaluated in DND-41 and ALL-SIL cells via Western Blot.

We identified 548 proteins upregulated upon inhibition of hsa-miR-363-3p, 138 of which were encoded by genes with at least one 3UTR putative binding site for this miRNA. One of the direct target genes was *PTPRC*, involved in negative regulation of JAK signaling. *PTPRC* acts as a tumor suppressor in JAK-STAT dependent T-ALL cases, yet the frequency of *PTPRC* inactivating mutations in T-ALL is relatively low. We also revealed *SOCS2*, another negative regulator of JAK-STAT pathway, as direct miR-363-3p target. By global analysis of RNA and protein expression we revealed overrepresentation of mRNAs and proteins (with significantly changed expression upon miR-363-3p inhibition) in JAK-STAT and downstream pathways: PI3K, mTOR, FOXO and RAS. Our results support the involvement of miR-363-3p in deregulation of these pathways in T-ALL via inhibition of *PTPRC* and *SOCS2*.

We demonstrated *in vitro* that *PTPRC* and *SOCS2* are direct targets of miR-363-3p. Our results support the involvement of miR-363-3p in deregulation of JAK-STAT and other JAK-dependent pathways in T-ALL.

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POSTER ABSTRACTS

Session I: Cancer Genetics and Genomics

[I-1] NUCLEAR FACTORS AS REGULATORY ELEMENTS IN THE FERROPTOTIC PATHWAY

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Introduction: Despite its essential role in life, excess iron is toxic due to its ability to produce reactive oxygen species (ROS) and even trigger cell death. Ferroptosis is a type of oxidative cell death that is induced by the accumulation of iron-mediated lipid peroxidation. This process is also crucially influenced by various regulators of iron in metabolism in the ferroptotic pathway. The aim of the study was to determine the role of nuclear factors involved in the ferroptotic pathway, as well as in metabolism. Assessment of the effect of death stimulators on cancer cell survival was crucial. Thioredoxin (TRX), which, as a powerful antioxidant, reduces protein disulfides to maintain redox stability in cells. Iron Regulatory Protein 2 (IRP2), which regulates cellular iron homeostasis, is important. The next nuclear factor is erythroid derivative 2 (NRF2), which regulates and controls the expression of antioxidants. Many pathological conditions are associated with an imbalance in redox homeostasis, illustrating the important role of antioxidant defence systems in preventing the pathogenic effects associated with the accumulation of reactive oxygen species (ROS). The research was conducted on two cancer cell lines, colorectal cancer HCT116 (p53+/+) and HCT116 (p53-/-).

Methods: Cells were treated with the ferroptosis inducer erastin, at doses of 5 and 10 M for 24 h. Then, the expression level of nuclear factors was evaluated by RTqPCR and cells viability was assessed by MTT assay.

Results: The studies showed an increase in IRP2 expression in all lines, an increase in thioredoxin except for the HCT116 p53-/- line, and an increase in thioredoxin reductase. However, in the case of nuclear factor NRF2, a decrease was observed in intestinal cancer lines, no changes in K562 and an increase in expression in H160 cells. Hence, the interdependence resulting from the mutual regulation of TRX and NRF2 expression, which ultimately leads to silencing of the ferroptosis pathway.

Conclusions: The main task of death inductors is its induction and, consequently, the cell death. One of the nuclear agents studied is Thioredoxin (TRX), which as a powerful antioxidant reduces protein disulfides to maintain redox stability in cells. Iron is a significant factor in the realization of ferroptosis. Consequently, many cellular processes related

to the metabolism of iron ions in cells alter the sensitivity to ferroptosis. Thus, Iron Regulatory Protein 2 (IRP2), which regulates cellular iron homeostasis, is important. The next nuclear factor is erythroid derivative 2 (NRF2), which regulates and controls the

expression of antioxidants. It was concluded that nuclear factors are crucial in the regulation of cell death, which is ferroptosis. The obtained results were used to create a decision table to assess sensitivity to ferroptosis. The results do not indicate any dependence on p53 status.

Aknowlegments: *The work was carried out thanks to the co-financing of Project-Based Education - PBL (Excellence Initiative - Research University program), in accordance with the Regulations No. 54/2020 and 55/2020 of the Rector of the Silesian University of Technology of March 13, 2020, and Silesian University of Technology grant-No 02/040/BK_23/1035 and 02/040/BKM23/1041.*

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[I-2] COMPARISON OF SEQUENCES OF SELECTED REGIONS OF APOPTOSIS GENES IN NEOPLASTIC AND NON-NEOPLASTIC SAMPLES OF LUNG TISSUE – PRELIMINARY EVALUATION

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Introduction: Lung cancer is one of the most common malignant tumors in both genders. Current knowledge in the field of lung cancer pathogenesis provides various hypotheses regarding the cascade of molecular changes related to the development of this disease. Among the molecular factors that may be related to lung cancer etiopathogenesis genes involved in the apoptosis process can be distinguished, such as: *BAX*, *BCL-2*, *CASP3*, *CASP7*.

The aim of the project was to sequence selected regions of *BAX*, *BCL2*, *CASP3*, *CASP7* genes from the apoptotic pathway in samples of non-small cell lung cancer tissue and samples of non-neoplastic lung tissue and then the comparison of results between these two types of samples.

Material and Methods: The material for the study was DNA isolated from: lung cancer tissue (4 samples), non-cancerous tissue/tumor margin (4 samples); peripheral blood samples from patients with non-cancerous lung damage (4 samples). Additionally commercially available control DNA was added into research. Based on the cBioportal and Missense3D programs, *BAX*, *BCL2*, *CASP3* and *CASP7* gene fragments in which these programs indicated the possibility of polymorphisms/mutations occurrence were selected for analysis. Next-generation sequencing was performed according to Illumina NextSeq 500 protocol. After sequencing was completed, bioinformatic analysis was performed.

Results: In analyzed subgroups no significant variants in *BAX* and *BCL 2* genes, were found compared to the reference human genome and control DNA. However, in the case of the two remaining genes of the apoptosis pathway, the presence of variants was demonstrated. In all subgroups the variants in the *CASP3* and *CASP7* genes occurred in samples. In the *CASP3* gene there occurred 3 variants c.604+56GA; c.484-34TA; c.308-4AC in introns. In the *CASP7* gene 3 missense variants were found c.765 CG (p.D255E), c.746 GA (p.R249K), c.801 CA (p.D267E). However analysis showed that variants in both genes are benign or tolerant. No differences were found between the subgroup in which cancer tissue, normal tissue and blood from patients with non-cancerous lung damage were assessed.

Conclusions: Ultimately, the sequencing results indicates that *BAX*, *BCL2*, *CASP3*, *CASP7* genes potentially do not participate in the process of carcinogenesis. However, the number of analyzed samples and results obtained in this project are too small to allow drawing final conclusions. All results obtained in the project require verification/confirmation on a larger group of patients with and without lung cancer.

Acknowledgments: *This Comparison of sequences of selected regions of apoptosis genes in neoplastic and non-neoplastic samples of lung tissue preliminary evaluation (Internal project No. 011522) is part of a project that has received funding from the European Unions Horizon 2020 research and innovation programme under grant agreement No 964997.*

[I-3] THE CIRCADIAN CLOCK DEATH-LOOP LINKS DIFFERENTIATION, EMBRYOGENESIS AND CANCER

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The circadian clock is a molecular oscillator that rhythmically coordinates the expression of around half of all protein coding genes, synchronizing it with the day-night cycle. It is closely involved in cell differentiation, with embryonic stem cells lacking core circadian clock gene expression that starts to act upon embryo implantation. Core clock genes also directly interact with cell cycle checkpoint machinery and compete with factors of embryonal stemness.

Therapy-resistant cancer cells are known to acquire highly rewired gene regulatory networks and embryonal-like stemness features through reversible polyploidization. Cell cycle checkpoint adaptation is another distinctive feature of polyploid cancer cells. This indicates that circadian deregulation could be key to cancer evolution, and that this ability of the cancer cell to stop time may promote a reversible shift from the mitotic cell cycle and Hayflick limit to the non-linear exploratory adaptation needed to reach the treatment-resistant attractor state of polyploidy and embryonal stemness.

In this study, RNA-seq data analysis has yielded evidence in favor of such a hypothesis from three sources: normal diploid and polyploid tissues from human and mouse organs, diploid and polyploid patient tumors from the TCGA database, and the MDA-MB-231 breast cancer cell line in the process of adapting to doxorubicin treatment.

[I-4] IS THERE ANY COMMON REGULATORY PATHWAY IN HO-1 DEFICIENCY?

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Heme oxygenase-1 (HO-1, HMOX1) degrades heme and protects cells against heme-induced oxidative damage. Heme stabilizes G-quadruplexes, which interfere with DNA replication. We showed that HO-1 promotes G-quadruplex unwinding and protects cells against replication stress. However, we suspect that G-quadruplex destabilization is not the only mechanism of this effect. Our aim was to indicate possible additional regulatory pathway(s).

G-quadruplexes accumulated in HMOX1-deficient cells, an effect further enhanced by increased heme levels in ALA (-aminolevulinic acid)-supplemented cells. This was accompanied by replication stress, with an increased proportion of stalled forks. At the same time, HMOX1 deficiency resulted in acceleration of ongoing forks, especially after ALA administration. The distribution of G-quadruplexes within the forks also suggests less restrictive control and continuation of DNA replication despite steric hindrance.

Replication arrest is regulated by PARP1, p53 and its transcriptional targets Cdkn1a and Plk2. Interestingly, in the absence of HO-1 we found reduced nuclear accumulation of p53 and decreased expression of p53 targets. Inhibition of the p53-dependent pathway could result from a direct regulation of p53 stability by heme or disturbed activation by PARP1. We showed that HO-1 colocalizes with PARP1 and affects its mobility after photobleaching. Moreover, HMOX1 deficiency changes the PARP1 interactome. Notably, the PARP1-p53 pathway also regulates DNA repair, nuclear envelope function, export of DNA fragments to the cytoplasm, and the resulting interferon response. All these activities are disturbed in the absence of HO-1. We postulate that PARP1-p53 dysfunction may be the common denominator of the diverse effects of HO-1 deficiency.

[I-5] IS PREMATURE CHROMOSOME CONDENSATION INDUCED BY HYDROXYUREA AND CAFFEINE IN HELA CELLS ASSOCIATED WITH ACTIVATION OF A SIGNALING PATHWAY THAT INVOLVES THE P53 PROTEIN?

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Introduction/Rationale: In HeLa cells blocked during S-phase with hydroxyurea (HU) and then induced to premature chromosome condensation (PCC) under HU and caffeine (CF) mixture, chromosomes condensed abnormally. Chromosomal aberrations were observed in part of the PCC cell population. Those aberrations that were termed G2-PCC were less extensive, while those that were termed S-PCC were very extensive. Both types of aberration were indicative of the occurrence of so-called chromosomal instability (CIN). Some cells (a relatively small proportion, but representing both subfractions: both G2-PCC and SPCC) entered the cell death (apoptosis, CD) pathway. In this study, the following hypothesis was tested: the low percentage of CD incidents in the cells studied is due to the existence of extremely efficient and efficient DNA repair mechanisms functioning in the cells studied.

Methods: In order to test the hypothesis, the method used included immunocytochemistry, which is used to detect and localise antigenic cell components using labelled antibodies.

Results/Conclusions/Novel aspect: PCC induction in HeLa cells was shown to occur via a Chk1-dependent pathway and was accompanied by DNA damage generation and micronucleus formation. An important observation was also the demonstration that the block in S-phase induced by the influence of HU occurred with the involvement of the p53 protein. The p53-dependent arrest of cells in the G1 phase of the cell cycle appeared to be an important component of the cellular stress response and had, among other things, a protective function against the massive generation of cell death incidents.

[I-6] A COMPREHENSIVE PLATFORM FOR UNRAVELING THE MOLECULAR MECHANISMS AND VULNERABILITIES OF COLORECTAL CANCER: A STEP FORWARD IN TARGET DISCOVERY

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Colorectal cancer (CRC) is a heterogeneous malignancy that originates from the colonic and rectal epithelium. It ranks among the most prevalent and deadly cancers globally, representing about 10% of cancer incidence and 9% of cancer mortality in 2018. The oncogenesis and evolution of CRC are driven by the accumulation of genetic and epigenetic alterations that dysregulate key signaling pathways, such as Wnt, RAS, TGF-, and DNA mismatch repair. However, the molecular mechanisms and vulnerabilities of CRC remain poorly understood, and many patients exhibit poor response or resistance to the current therapies. Therefore, there is an urgent need for novel and effective therapies that can target the specific features and dependencies of different CRC subtypes.

To better understand the molecular mechanisms and vulnerabilities of CRC, we established a comprehensive platform based on the CRC model cells derived from human intestinal stem cells (hISCs), patient-derived xenografts (PDXs), and clinical samples, on which we conducted integrative genomic and functional analyses. We employed CRISPR/Cas9 to introduce common CRC mutations, such as APC truncation, KRAS G12D, TP53, and SMAD4 knockouts into hISCs and generated isogenic CRC model cells with different mutational backgrounds. We also developed PDXs from CRC patients and collected biopsy samples from primary and metastatic tumors.

We characterized the transcriptomic and genomic profiles of all the samples using RNA-seq and whole-exome sequencing (WES). Then, we conducted high-throughput drug screening (drug HTS) with a collection of standard-of-care drugs and CRISPR/Cas9 dropout screening with a genome-wide library on the CRC model cells.

RNA-seq and WES analyses identified distinct molecular signatures and pathways associated with different mutational variants. We validated the presence of driver mutations in the patient-derived cultures, such as APC, KRAS, and TP53, and compared them with the model cells. Our primary cancer cell culture platform was proven suitable for high-throughput identification of new molecular targets through CRISPR/Cas9 dropout screening with a genome-wide library performed on CRC model cells. Additionally, our in-house drug HTS, utilizing a collection of standard-of-care drugs, met industrial standards of QC values such as signal-to-noise ratio, Z-factor, and robustness. The QC values, such as read depth, library complexity, and alignment rate met the established criteria and we were able to identify

essential and tumor suppressor gene sets. Moreover, by utilizing normal hIPCs, we were able to identify among the essential genes those that are critical only for the transformed cells.

This innovative platform provides a powerful tool for target discovery and validation in CRC, with potential applicability to other cancer types and personalized medicine approaches. Our findings underscore the importance of using primary material-derived cells in the process of understanding the molecular intricacies of CRC and pave the way for the development of novel, effective therapies.

[I-7] COSME CLASSIFIER AS POTENTIAL TOOL FOR RISK ASSESSMENT IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. Although cure rates in pediatric T-ALL exceed 75%, the outcome of resistant/relapsed leukemia remains poor. Novel prognostic markers are needed. Global DNA methylation profile defined by CpG Island Methylator Phenotype (CIMP) has prognostic potential in T-ALL. COSMe (CpG island and Open Sea Methylation) is an extension of CIMP based on methylation profiling using more advanced EPIC_array. There are two phenotypes: hypomethylated (COSMe-I) and hypermethylated (COSMe-II). Global methylation is related to methylation age. COSMe-I cases are epigenetically younger than COSMe-II (less methylation accumulated during cell divisions reflecting shorter leukemia onset in COMSe-I). Hence, methylation profile is currently perceived rather as a consequence of different aggressiveness

of leukemia than a reason for. Despite, we hypothesize that integrative analysis multiomic data can provide more than prognostic information and reveal biological insights into COSMe subgroups.

Aims: 1/ propose a gene expression-based classifier reflecting prognostic impact of COSMe, which could better suit diagnostic purposes than genome-wide methylation and 2/investigate biological differences underlying COSMe groups.

EPIC_array data of 109 T-ALL cases (Roels et al. Blood Cancer Discov 2020) were integrated with RNA-seq data obtained for 44/109 patients. RNA-seq reads were aligned to GRCh38 reference genome using STAR and summarized using featureCounts. Genes differentially expressed (DEGs) between COSMe groups were identified with edgeR. Using gene expression in 44 T-ALL cases labeled as COSMe_I or COSMe_II, random forest (RF) classifiers were created using caretR and evaluated by 10-fold cross-validation, with additional down-sampling to account for imbalanced dataset. Cross-validation loop included a feature selection process, set to select from 1 to max.20 features independently.

We identified 3686 DEGs between COSMe subgroups. Overrepresentation analysis for DEGs revealed processes potentially related to different aggressiveness of leukemia in COSMe groups, including: cancer cells invasiveness, resistance to cell death, and pathways frequently deregulated in cancers (PI3K-AKT, RAP1, RAS, MAPK). In line with previous reports, we found higher cumulative incidence of relapse (CIR) and lower overall survival (pOS) in the hypomethylated group (COSMe_I), which supports usefulness of COSMe in risk stratification. Using genome-wide methylation seems impossible in a routine diagnostics. Therefore, we propose a gene expression-based classifier reflecting prognostic information of COSMe, composed of 8 genes (TRIM46, BEX2, ABCA4, ZAP70, LCK, ZNF354C, NTN4) with the area under ROC curve (AUC) equal 0.985.

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[I-8] GENOMIC PROFILING OF MELANOMA TUMOR AND LIQUID BIOPSY WITH NEXT-GENERATION SEQUENCING – PRELIMINARY RESULTS

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Introduction: Genomic profiling of cancer with next-generation sequencing (NGS) is widely investigated for cancer diagnosis, prediction, monitoring, and prognosis. There are two main sources of tumour DNA for genomic analysis: standard biopsy of primary or metastatic tumour or liquid biopsy-derived circulating tumour DNA (ctDNA). Liquid biopsy is gaining interest in clinics as it is low-invasive, cheap, repeatable, and provides more representative tumour DNA in comparison to standard biopsy. Before implementing genomic profiling of cancer liquid biopsy in clinics its utility should be proved i.e. in concordance studies. Here, we present the preliminary results of genomic profiling of melanoma patient-derived DNA using both standard and liquid biopsy.

Aims of the study:

- 1) validation of the custom panel for 30 selected genes
- 2) comparison analysis of genomic profiling of melanoma DNA derived from liquid and tumour biopsy

Materials and methods: Tumour DNA (standard biopsy) and cfDNA (cell-free DNA, liquid biopsy) from 12 stage IV melanoma patients were used in the study. The cfDNA was isolated with QIAamp Circulating Nucleic Acid Kit (Qiagen). DNA libraries were prepared with the Ion AmpliSeq HD Library Kit and sequencing was performed using the Ion Torrent NGS platform (Ion GeneStudio S5 System). The custom panel of 30 genes was designed with the usage of Ion AmpliSeq Designer.

Results: A correct performance of our gene panel was confirmed using commercial, standard DNA and tumour samples with known genetic profiles. Of the 22 variants present in the reference material, only two of them were not detected by the designed panel. Subsequent genomic analysis with the validated panel identified various pathogenic mutations as well as variants of unknown significance in either tumour or cfDNA in all melanoma patients. Variants were detected in 22 out of 30 melanoma genes (73%). The most frequently mutated genes were *BRAF*, *NRAS*, *NF1*, *ARID2*, and promotor of *TERT*. In two patients known mutations associated with resistance to BRAF/MEK inhibitors were detected (genes *MAP2K1* and *RAC1*). The concordance of genomic profiles between liquid biopsy and tumour tissue was 49 % for all selected variants and 100 % for the most frequently mutated melanoma genes: *BRAF*, *NRAS*, and *ARID2*. For *NF1* and promotor of *TERT*, the concordance was 33 % (higher number of variants in liquid vs. tissue biopsy), and 80 %, respectively.

Conclusions: Our panel detects variants of selected genes and can be successfully used in the project. It enabled the identification of mutations in driver melanoma genes in all analysed patients, though some of the selected variants required further validation. High concordance of mutational profiles of main melanoma genes between tumour and liquid biopsy shows promise in the successful usage of genomic profiling of cfDNA in the clinics, though analysis of higher number of samples is required.

[I-9] EFFECT OF BETULIN DERIVATIVE - EB5 ON CELLS OF THE TOLEDO, MAVER-1 AND HH LINES

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Non-Hodgkins lymphomas (NHL) are a heterogeneous group of cancers of the lymphatic system. Currently, scientists are paying special attention to microRNA molecules. Until now, a number of miRNAs have been demonstrated to be involved in the pathogenesis of NHL. An important goal is to identify new, potential diagnostic and therapeutic markers. New drugs with lower toxicity and higher anticancer activity are still being sought. This study focused on the betulin derivative EB5.

The aim of this study was to assess the effect of betulin derivative EB5 on the expression of microRNA molecules in three cell lines representing pathogenetically and clinically diverse types of lymphomas: HH (cutaneous T-cell lymphoma - CTCL), Toledo (diffuse large B-cell lymphoma-DLBCL) and Maver-1 (mantle cell lymphoma MCL) (ATCC, Manassas, VA, USA). The assessment of changes in the expression profile of miRNA molecules in the examined NHLs treated with the EB5 compound and in untreated cells was performed using the miRNA 4.0 Array microarray method (Affymetrix, Santa Clara, CA, USA). Microarray data analysis was performed using TAC 4.0 software (Transcriptome Analysis Console). Bioinformatics analysis was performed using the GeneTrail version 3.2 tool, including the Gene Ontology and Reactom databases (<https://genetrail.bioinf.uni-sb.de/start.html>).

Analysis of the miRNA profile of the Toledo line indicated, among all the differentiating miRNAs, the highest overexpression of molecules: hsa-miR-7110-5p, hsa-miR-4530 molecules and the most decreased expression of: miR-423-5p miR-423-3p and miR-6765-5p. For the Maver-1 line, analysis showed the highest overexpression for molecules: hsa-miR-6799-5p and the most decreased expression for hsa-miR-3180-3p. The HH line showed the highest overexpression of: hsa-miR-3197, hsa-miR-3687 and the most reduced expression hsa-miR-4298.

Bioinformatics analysis showed that among all the differentiating miRNAs analyzed, there was an overrepresentation of miRNAs related to biological pathways and processes. Analysis of the Toledo line showed an overrepresentation of miRNA molecules involved in IL-37 signaling regulation. Several possible processes and signaling pathways have been demonstrated for the HH line, three enriched: Tetrahydrobiopterin (BH4) synthesis, NOTCH3 Intracellular Domain Regulates Transcription, NRAGE death signals through JNK and two depleted: GAB1 signalosome and Prostanoid ligand receptors. For the Maver-1 line, there was no overrepresentation of differentiating miRNAs in any of the analyzed biological pathways or processes.

The results of this study show for the first time the effect of the EB5 derivative on NHL cells and on the miRNA profile. The demonstrated differential miRNAs may constitute potential therapeutic and diagnostic targets.

[I-10] FEBRILE-RANGE TEMPERATURES INDUCE THE PDCD1 EXPRESSION

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Programmed cell death protein 1 (PDCD1) is typically expressed after acute T cell receptor (TCR) activation. As an immune checkpoint molecule, it suppresses immune responses to prevent autoimmune tissue damage. It is considered a molecular marker of exhausted T cells in both chronic infection and cancer and is associated with dysfunction in both cases. We found that PDCD1 expression could be increased as early as a few hours after temperature elevation. At the transcriptional level, we observed its upregulation in the human leukemia cell line HL-60 (which was associated with the binding of heat shock factor 1, HSF1, in the promoter) and in the thymus and spleen of mice. A heat shock-dependent increase in PDCD1 protein levels (especially glycosylated/active forms) was also observed in human peripheral blood mononuclear cells and the NK-92 cell line (natural killers). Interestingly, *PDCD1* transcript levels were not increased in these cells. We propose that there are different mechanisms of heat shock-induced PDCD1 upregulation: at the transcriptional and post-transcriptional levels. Protein synthesis inhibition experiments using cycloheximide showed that the PDCD1 protein is very stable (with a half-life longer than 12 hours). Our results suggest that fever (via activation of HSF1 followed by PDCD1 accumulation and/or protein modifications) may be involved in the attenuation of the immune response in various physiological states accompanied by increased temperatures (infection, heat stroke, etc.). For this reason, fever (as well as pharmacological fever reduction) can have unexpected consequences depending on the disease state. This observation may have clinical implications and therefore further research is warranted to understand the importance of fever in various disease states as well as its interaction with treatment.

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[I-11] TISSUE-SPECIFIC HSF1 ACTION: USING THE CRISPR CAPTURE METHOD TO IDENTIFY PROTEIN COMPLEXES AT A SINGLE GENOMIC LOCUS RESPONSIBLE FOR HSF1-DEPENDENT ACTIVATION OF THE TRANSCRIPTION

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The accumulation of damaged proteins caused by cellular stress (e.g., heat shock) leads to the activation of heat shock factor 1 (HSF1). In most cells, HSF1 induces the expression of genes encoding heat shock proteins (HSPs), which are chaperones that prevent cell damage and apoptosis. However, in stress-sensitive cells, HSF1 activation leads to apoptosis through the induction of pro-apoptotic *PMAIP1* (*NOXA*) gene expression. Our goal is to elucidate the tissue-specific mechanisms of *PMAIP1* activation by HSF1. Based on cell survival tests under elevated temperature conditions, we selected two human cell lines for the study: K562 (chronic myeloid leukemia; heat-resistant) and HL-60 (acute myeloid leukemia; heat-sensitive). We showed that activated HSF1 binds to the regulatory sequences of the *PMAIP1* gene in both cell lines, but induces *PMAIP1* transcription only in HL-60 cells. We assume that the activation of expression may be determined by proteins that interact with HSF1. Thus, we plan to identify HSF1 binding partners in the regulatory sequences of the *PMAIP1* (responsible for apoptosis) and *HSP* (responsible for cytoprotection) genes in both K562 and HL-60 cells.

To determine the molecular composition of locus-specific chromatin complexes in the genome, we implemented the CRISPR CAPTURE (CRISPR affinity purification in situ of regulatory elements) method. We stably introduce into cells vectors encoding biotinylated Cas9 protein devoid of nuclease activity (dCas9) and sgRNAs targeting the desired DNA sequences. After cell fixation and chromatin fragmentation, precipitation of a single chromatin fragment (bound by dCas9/sgRNA complex) and associated proteins is possible due to the strong affinity of biotin for streptavidin. Purified DNA and protein components from untreated and heat-shocked cells will be identified and analyzed quantitatively and qualitatively by qPCR, sequencing, and mass spectrometry-based proteomics.

This method will provide information on differences in the regulatory sequences of the *HSP* and *PMAIP1* genes in both heat-sensitive and heat-resistant cell lines that determine specific HSF1 activity.

[I-12] LACK OF AKT AND MAPK ACTIVATION IN SW-480 CELL LINES EXPRESSING THE RASG12V ONCOGENE

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Introduction: RAS mutants are considered to be the most important oncogenes. In fact, the GTPase activity of RAS decreases dramatically due to mutations such as RASG12V. During our study, we developed a group of compounds that restore the GTPase activity of the RASG12V protein. These compounds were tested both in vitro and in vivo.

Methods: The Promega GTPase GLO assay was used to test the GTPase activity of the wild type of RAS and its mutant form - RAS G12V. The RAS G12V, RAS WT, and GAP proteins were purified after genetic engineering from eukaryotic cells. In vitro cytotoxicity tests were performed for newly developed compounds. In vivo animal antitumor effects were analyzed. Phospho WB assays were performed to test the AKT and MAPK pathways.

Results: Molecules considered to be compounds restoring RAS G12V GTPase activity were recognized. In spite of restoring GTPase activity in vitro, in vivo analyses did not show the anticancer effects of these compounds. The biodistribution of tested molecules seems to be an important issue here. Importantly, line SW-480 was used during in vivo analyses. However, studies of the AKT and MAPK pathways in the SW-480 line showed very low activity of these pathways in spite of the RAS mutation. These results inspire to reconsider the role of RAS mutants in the late stages of cancerogenesis and the usefulness of this line for in vivo studies.

Conclusions: Recognized molecules could potentially be used in therapies based on the restoration of GTPase functionality of this mutant in tumors. The presence of a cell line showing a RASG12V mutation and a lack of AKT and MAPK pathways activation requires further explanation.

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[I-13] EXPLORING THE EFFECTS OF DGCR8 SILENCING ON MICRORNA BIOGENESIS IN HODGKIN LYMPHOMA CELLS

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MicroRNAs (miRNAs) are small, non-coding single-stranded RNAs that inhibit gene expression at the post-transcriptional level. Some miRNAs are located in intergenic regions, while others are situated in introns or, less commonly, in the exons of protein-coding or non-coding genes. MiRNAs can be encoded separately or as clusters, wherein more than one miRNA is found. The biogenesis of miRNAs comprises several steps. The initial step occurs in the cell nucleus, leading to the formation of the primary transcripts (pri-miRNAs). These transcripts are then processed by the Drosha-DGCR8 microprocessor into precursor miRNAs (pre-miRNAs), which is transported into the cytoplasm and further processed by Dicer into mature miRNAs.

In this study, we aim to determine the effects of DGCR8 knockdown on miRNA biogenesis. We used two short hairpin RNAs, shDGCR8-1 and shDGCR8-2, in lentiviral vectors to silence DGCR8 expression in L1236 Hodgkin lymphoma cells. The negative control was scrambled sequence (SCR).

Notably, DGCR8 inhibition led to apoptosis of L1236 cells. The percentage of living cells determined by flow cytometry decreased up to 3 folds for shDGCR8-1 compared to the SCR control and was only 20% at day 14 after transduction. The percentage of apoptotic cells increased up to 40 percent. L1236 cells with DGCR8 knockdown were also exposed to ionizing radiation. For a radiation dose of 4 Gy, the percentage of cells in the G2/M phase increased by approximately 20% in the SCR control. However, this effect was not observed in DGCR8-inhibited cells, where the percentage of cells in the G0/G1 phase remained at around 70% regardless of the radiation dose.

To analyze pri-miRNA levels, total RNA sequencing was performed for L1236 cells transduced with lentiviral vectors containing shDGCR8-1 or SCR. Pri-miRNA was defined as a pre-miRNA with a 150 bp sequence upstream and downstream of the pre-miRNA. We found that the levels of only 15 (of 767) pri-miRNAs increased by a minimum of 2 folds in DGCR8-silenced L1236 cells compared to the SCR control. Selected pri-miRNAs and miRNAs were validated using qRT-PCR. Levels of mature miR-155, miR-103, miR-146a, miR-17, and miR-24 decreased by up to 5 folds, while levels of pri-miR-155, pri-miR-17~92 (pri-miR-17), and pri-miR-146a increased by up to 60 folds after shDGCR8 transduction compared to SCR in L1236 cells.

In conclusion, DGCR8 inhibition induced apoptosis. Interestingly, not all miRNAs were equally affected by DGCR8 knockdown, as the levels of most pri-miRNAs were not altered compared to SCR. DGCR8 silencing prevented biogenesis of several miRNAs, including exonic miR-155, miR-146a. Further studies that involve analysis of additional miRNAs and pri-miRNAs using PCR are ongoing.

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[I-14] UNRAVELING THE WNT/B-CATENIN PATHWAY IN CLEAR CELL RENAL CELL CARCINOMA: MOLECULAR COMPLEXITIES, DYSREGULATION, AND THERAPEUTIC PROSPECTS

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Introduction: Clear cell renal cell carcinoma (ccRCC) is the most common renal neoplasm among adults, and its incidence is steadily increasing. Its occurrence is characterized by a complex, heterogeneous genomic landscape and tendency to metastasize through the bloodstream, leading to limited treatment options and poor overall survival rates. While prognostic models for ccRCC primarily rely on clinical parameters and pathological characteristics, the need for molecular markers capturing the disease's biological behavior is evident. Among the many pathways implied in ccRCC, the Wnt/-catenin pathway has emerged as a key contributor. To overcome these challenges, acquiring a comprehensive understanding of the underlying biology of ccRCC is crucial.

Our group has already shown that during the development of ccRCC, the level of Monocyte Chemoattractant Protein-1 Induced Protein (MCPIP1) decreased together with the alteration of the WNT pathway. The loss of MCPIP1 RNase activity led to the upregulation of miRNA-519a-3p, -519b-3p, and -520c-3p, which inhibited the expression of Wnt pathway inhibitors. Moreover, we report that MCPIP1 protein levels are decreased during renal cancer progression. Our results illuminate how MCPIP1 serves as a key nodal point in coordinating tumor growth, angiogenesis, and metastatic spread in ccRCC. Recent results have shown that MCPIP1 may act as a tumor suppressor that prevents EMT by stabilizing Wnt inhibitors and decreasing the levels of active -catenin and EMT inducers. However, there are many unknown pathways altered during progression.

Method: The level and distribution of genes and proteins were studied by realtime PCR, western blot, immunofluorescence staining. Microarray analysis was conducted on samples from patients suffering from ccRCC. We used 6-week-old female NOD-SCID mice injected subcutaneously with Caki-1 cells with stable overexpression and mutant form of the MCPIP1. Tumor growth was monitored for 6 weeks then resection of tumors and analysis were performed.

Results: Our research showed that the RNase activity of the MCPIP1 protein significantly affects the regulation of the level of post-translational modifications of -catenin. The level of inactive, degradation-promoted, phosphorylated -catenin (Ser45) decreases dramatically in advanced stages of ccRCC compared to early clinical stages. However, the level of active, phosphorylated -catenin (Tyr654) increases significantly with the progression of the disease. Moreover, the RNase activity of the MCPIP1 protein regulates the level of transcriptionally active -catenin (Tyr654) and its inactive forms promoted for proteasomal

degradation, i.e. phosphorylated -catenin (Ser45) and (Ser33/37/Thr41). Our studies suggest that MCPIP1 plays an important role in the progression of clear cell renal cell carcinoma by regulating the level of post-translational modifications of -catenin.

Novel aspect: Our current results indicate the importance of MCPIP1 as a suppressor of tumor development and progression. We show that MCPIP1 is crucial in the activation of Wnt/-catenin pathway. In our research we plan to identify ccRCC-specific molecular targets that regulate tumor progression and developed resistance to therapy.

[I-15] RADIOGENOMIC PROFILING OF NON-SMALL CELL LUNG CANCER

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Rationale: Prognostic models in cancer are constructed based on various types of data. In lung cancer, potential biomarkers have been identified, for example, among genetic variants [1] or in radiomics [2]. In recognition of the complexity of the disease, there is a growing interest in multi-omics, e.g. radiogenomics, aimed at integrating different layers of available information [3]. Based on a unique dataset, we explored the associations between clinical, genetic, and imaging data, and investigated their potential in predicting clinical outcomes.

Methods: The study group comprised 61 patients treated with chemoradiotherapy for non-small cell lung cancer. The median age was 61 years and the majority of patients were males (69%). Out of all patients, 66% individuals had squamous cell carcinoma. Most patients had advanced disease, but only those without distant metastases detectable at diagnosis were included, since metastasis-free survival (MFS) was one of the studied endpoints. The genetic variants with known functional significance in solid cancers were selected (n = 33), including 31 single-nucleotide polymorphisms (SNPs) and 2 indels. Genotyping was performed on DNA from frozen peripheral blood, using the polymerase chain reaction-restriction fragment length polymorphism method or TaqMan genotyping assays. PET images acquired for radiotherapy planning, standardized using body weight and interpolated to CT resolution, were used to extract 100 radiomic features from the primary tumor region. Relationships between the genetic and radiomic features were assessed using the point-biserial correlation coefficient. Features related to overall survival (OS) and MFS were identified with the regularized Cox regression model.

Results: Strong correlations were observed between several shape features and the rs7667298 polymorphism in *KDR* gene encoding VEGF receptor 2, a key angiogenesis-related factor. For the considered endpoints, both radiomic and genomic features were selected for the regularized model, with the largest coefficients corresponding to ShortRunEmphasis, RunLengthNonUniformityNormalized, and *XPD* gene for MFS, as well as SmallAreaLowGrayLevelEmphasis and *EGFR* gene for OS.

Conclusion: Genetic variants and radiomic features extracted from medical imaging can be treated as complementary information allowing for better characterization of the complex landscape of lung cancer.

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[I-16] INDEL MICROHOMOLOGY IN OVARIAN CANCER GENOMES

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Ovarian cancer is a significant challenge to gynecologic oncology, despite substantial efforts to achieve early detection and to develop effective treatments. Approximately half of all ovarian tumors are characterized by homologous recombination deficiency (HRD). These tumors stimulate the activity of DNA polymerase theta (POLQ), increasing their dependence on the microhomology-mediated end joining (MMEJ) DNA repair process. The MMEJ repair pathway is inherently mutagenic, as it employs homologous nucleotides from both broken DNA ends to initiate repair, resulting in multi-nucleotide deletions in the DNA sequence. This mechanism allows the use of specific treatment methods, based on PARP inhibitors, which are very effective due to a concept known as synthetic lethality. PARP inhibitors exploit the vulnerability of cells with the HRD phenotype by further hindering an alternative DNA repair pathway - the base excision repair, mediated by poly (ADP-ribose) polymerase (PARP). For this reason accurate detection of the HRD phenotype, induced by various DNA damages that are difficult to identify, can significantly enhance the chances of successful therapy for ovarian cancer patients.

The aim of this study was to develop an algorithm, capable of identifying the increased dependence of cells on MMEJ, through the detection of MMEJ-resulting deletions. This is achieved through the nucleotide sequence analysis of both the deleted sequence and its genomic context, in search for microhomologies required by the MMEJ repair pathway. The algorithm was tested using ovarian cancer genomic data, from The Cancer Genome Atlas, obtained through whole-genome sequencing (WGS) and exome sequencing (WES). WGS and WES data enable the identification of somatic mutations, including deletions, to various extents, especially in intronic and intergenic regions.

The results obtained allowed to determine the influence of the mutation detection algorithms and the type of sequencing (WES/WGS) on the statistics of microhomology-associated deletions. We were also able to show that not only mutations within BRCA1/2 genes can lead to an increased dependence on MMEJ but also within the RB1 gene. Furthermore survival analysis of patients revealed that a higher number of deletions with microhomologies is associated with an increased patient survival, even in the absence of PARP inhibitor treatment. Our findings provide new insights into the mechanism behind microhomology-associated deletions, which, in the long term, could serve as predictors of a tumors susceptibility to PARP inhibitor therapy.

Session II: Proteomics and Metabolomics

[II-1] EFFECT OF NATURAL NF-KB INHIBITORS ON NORMAL MUSCLE CELLS AND TUMOR CELLS OF MUSCLE ORIGIN – IN VITRO

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NF- is a protein complex that controls DNA transcription and cytokine production. NF- occurs in all types of animal cells and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, heavy metals. NF- plays a key role in regulating the immune response to infection. Abnormal regulation of NF- has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection and abnormal immune development [1]. The cytotoxic effects of five selected cytotoxic substances of natural origin were analyzed - i.e. cucurbitacin E (CurE) [2], biochanin A [3], caffeic acid phenethyl ester (CAPE) [4], berberine [5] and curcumin [6]. The substances effects are tested *in vitro* (on the L6 control line - isolated from rat skeletal muscle and WEHI-164 - isolated from mice with fibrosarcoma).

The MTT test determined the viability of cells after treatment (24 and 48 hrs) with the tested cytotoxic substances. Comet Assay - was performed to analyze the type of cell death after treatment with the tested substances. Additionally, molecular docking simulation of cucurbitacin E (CurE) and caffeic acid phenethyl ester (CAPE) to cytoskeleton proteins was performed by Swissdock software and the results were evaluated in Chimera. The obtained results indicated that the anticancer activity revealed CAPE and CurE.

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[II-2] STUDIES ON N-GLYCOSYLATION OF C-REACTIVE PROTEIN IN THE COURSE OF GRAVES' DISEASE

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Introduction: Graves disease (GD) is becoming an increasingly important health problem. In addition to elevated levels of thyrotropic hormone receptor antibodies (anti-TSHR) and hyperthyroidism, GD is characterized by an inflammatory response leading to the destruction of thyroid gland tissue. One of the non-specific responses to disrupted homeostasis is an acute phase reaction involving C-reactive protein (CRP), which is synthesized mainly by the liver in the presence of IL-6. Physiologically, CRP is secreted into the blood at low concentrations and does not undergo *N*-glycosylation, unlike in inflammation, when *N*-glycans were identified in CRP. Moreover, CRP level has been shown to increase in hyperthyroidism and normalize in euthyroidism. Therefore, the objectives of this study were to optimize the isolation of CRP protein from human sera using affinity chromatography and to analyze the *N*-glycosylation of CRP by lectinoblotting and MALDI-Tof mass spectrometry.

Methods: Sera were collected from patients before methimazole treatment (GD) and after TSH normalization (GD/T), and from healthy subjects (CTR). Glycosylation analysis was performed for CRP present in whole serum as well as isolated by affinity chromatography with p-aminophenylphosphorylcholine as the ligand in the deposit. CRP purification efficiency was assessed by immunoblotting, while the content and composition of CRP *N*-glycans were assessed by enzymatic de-*N*-glycosylation, lectin precipitation, and MALDI-Tof mass spectrometry.

Results: The presence of CRP was confirmed in the study groups. Using lectinoblotting, CRP was shown to be glycosylated in GD, with sugar structures enriched in sialic acid and fucose. In addition, immunosuppressive treatment was found to impair fucosylation and increase CRP sialylation. MALDI-Tof MS analysis revealed 24 structures in the *N*-glycan pool of CRP. These were mainly diantennary complex-type structures, some of them sialylated and fucosylated. Oligomannose structures were present in the minority.

Conclusions: The obtained results provided the accurate characteristics of CRP *N*-glycan structures in GD serum samples. To our knowledge, this is the first analysis of CRP glycosylation in GD and one of the few done for this protein to date. Given the growing number of reports on the importance of glycosylation for normal protein structure and function, and the changes that glycosylation undergoes during disease, it may be crucial to better understand the regulation of CRP protein function during inflammatory reactions.

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[II-3] MELANOMA CELLS GLYCOSYLATION AFFECTS PROTEIN SORTING TO EXTRACELLULAR VESICLES

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Introduction: The glycosylation profile of tumor cells intensively changes during carcinogenesis. Tumor-associated carbohydrate antigens (TACAs) arise as a result of alternations to the already existing glycoepitopes, the formation of completely new glycoepitopes, or the reoccurrence of fetal glycoepitopes. Tumor-derived extracellular vesicles (EVs) are well-known carriers of proteins involved in metastasis, invasion, drug and immunological resistance, and angiogenesis. The aim of the study was to examine how changes in the glycosylation profile of melanoma cells influence the qualitative and quantitative composition EV cargo.

Methodology: EVs (ectosomes and exosomes, separately) were isolated by differential centrifugation combined with low-vacuum filtration. The purity and quality of isolated EVs were verified by transmission electron microscopy (TEM), nanoparticle tracking analyses (NTA), and Western blot for EV markers. In order to identify the proteins present in the cargo of EVs released by WM266-4 melanoma cells treated with tunicamycin (TM) for 72 hours, and control cells, tandem mass spectrometry coupled with high-performance liquid chromatography (LC-MS/MS) was performed. During the bioinformatics analysis, gene ontology analysis and label-free quantitative analysis were performed. The analysis of WM266-4 cell glycosylation was performed using flow cytometry with a panel of six lectins.

Results: During the LC-MS/MS analysis, 1159 proteins were identified in ectosomes derived by control cells (not treated with TM) and 1125 proteins in ectosomes from cells treated with TM. In exosomes derived from control cells 578 proteins were identified, while in exosomes derived from TM-treated cells 622 cells were identified. Quantitative proteomic analyses showed that 57 ectosomal and 95 exosomal proteins were upregulated in EVs derived from TM-treated cells compared to the control. Moreover, 53 ectosomal and 112 exosomal proteins were downregulated in EVs derived from TM-treated cells compared to the control. Additionally, a variety of identified proteins were involved in cancer-related processes. The use of TM changed the surface expression of glycoepitopes (sugar structures) recognized by used lectins. For most stainings, a decrease in RFI was observed after 72 h of inhibitor exposure.

Conclusions: The presented research showed that protein N-glycosylation plays an important role in protein sorting into EVs. Alternations to melanoma cell glycosylation induced by TM-treatment were partially reflected in EVs, and resulted in qualitative and quantitative differences in their protein cargo. Such extensive changes in glycoprotein content of EVs that occur during carcinogenesis may determine their pro- or anti-cancerous potential.

[II-4] SHOTGUN PROTEOMICS OF THYROID CARCINOMA EXOSOMES – INSIGHT INTO THE ROLE OF EXOSOMAL PROTEINS IN CARCINOGENESIS AND THYROID HOMEOSTASIS

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Transport of molecules via exosomes is one of the factors involved in cancer development, and transported molecules may serve as specific tumor biomarkers. The aim of this study was to perform the LC-MS/MS proteomic analysis of exosomes released by FTC and 8305C thyroid cancer cells, and reference Nthy-ori 3-1 normal thyroid follicular cells. A total of 1769 unique proteins were identified in the exosome samples. For exosomes derived from Nthy-ori 3-1 cells, the highest number of 1504 proteins was identified, while exosomes from thyroid carcinomas FTC and 8305C cells had 730 and 1304 identified proteins, respectively. For the identified proteins, gene ontology analysis was performed in terms of their cellular location, molecular function and involvement in biological processes. For proteins that only appeared in tumor derived FTC- and 8305C-derived exosomes, enriched categories were related to cancer progression and included cell adhesion and positive regulation of cell migration. Among these unique proteins were also proteins related to protein N-linked glycosylation, drug resistance, and cell response to NK and T cell cytotoxicity. Finally, label-free quantification (LFQ) was performed for identified differentially expressed proteins between all possible group pairings. For exosomes derived from thyroid cancer cells, the most differentially expressed proteins included collagen alpha-2(I) chain, tenascin, matrix metalloproteinase 1, interstitial collagenase and C-type lectin domain family 11 member A. The obtained results expanded the knowledge concerning the role of exosomal proteins in thyroid cancer and indicated potential biomarkers for further evaluation in the clinical settings.

[II-5] AGE-DEPENDENT DIFFERENCES IN SERUM METABOLITES LINKED TO BREAST CANCER RISK: A HIGH-RESOLUTION MASS SPECTROMETRY STUDY OF PRE-DIAGNOSTIC SERUM SAMPLES FROM THE NORWEGIAN TRØNDELAG HEALTH STUDY

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The study analyzed pre-diagnostic serum samples from healthy women in the Norwegian Trndelag Health Study (HUNT2 study) to identify molecular biomarkers linked to breast cancer risk.

Women participating in the HUNT2 study who developed breast cancer within a 15-year follow-up period (BC cases) and age-matched women who stayed breast cancer-free were selected (n=453 case-control pairs). Using a high-resolution mass spectrometry approach 284 compounds were quantitatively analyzed, including 30 amino acids and biogenic amines, hexoses, and 253 lipids.

Age was identified as a major confounding factor responsible for large heterogeneity in the dataset, so age-defined subgroups were examined separately. The subgroup of younger women (45 years old) had the highest number of metabolites whose serum levels differentiated BC cases from controls (82 compounds). Notably, higher glyceride, phosphatidylcholine, and sphingolipid levels were linked to a lower risk of cancer in younger and middle-aged women (64 years old), while increased serum lipid levels were linked to an increased risk of breast cancer in older women (64 years old). Furthermore, while serum levels of several metabolites differed between BC cases diagnosed earlier (5 years) and later (10 years) after sample collection, these compounds were also correlated with participant age. The study findings were consistent with those of the NMR-based metabolomics study conducted in the HUNT2 cohort, which found that higher serum levels of VLDL subfractions were associated with a lower risk of breast cancer in premenopausal women.

The study found that differences in metabolite levels indicating impaired lipid and amino acid metabolism were associated with long-term breast cancer risk in an age-dependent manner.

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[II-6] COMPARISON OF SERUM METABOLOME PROFILES OF FOUR TYPES OF SOLID CANCERS BY MS AND NMR APPROACHES

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Metabolic analysis of cancer-specific profiles is a powerful method for understanding complex molecular changes characteristic to the promotion and progression of different types of tumors. In our study, we implemented two analytical approaches: high-resolution mass metabolomic features of four types of solid cancer. We analyzed metabolites presented in sera collected from women patients with breast (n = 35), head and neck (n = 32), lung (n = 35), and colorectal (n = 30) cancers to reveal patterns characteristic for each group of patients. Metabolic signatures of all four malignancies were compared based on both types of analytical approaches. The metabolic profile of colon cancer patients was the most distinct while the profiles of lung cancer and head and neck cancer were the most similar to each other. Colon cancer was characterized by the lowest levels of lipids (lysophosphatidylcholines, cholesteryl esters, and triglycerides in particular), lipoproteins, and amino acids. On the other hand, breast cancer patients were characterized by relatively high concentrations of lipids (cholesteryl esters and sphingomyelins in particular) and low concentrations of glycans. Noteworthy, only a minor correlation between cancer stages and metabolic patterns was observed, which indicated that cancer-type-specific features might be more important than cancer-stage-specific features when metabolic patterns are observed at the systemic level in patient's serum. We concluded that the analysis of serum metabolome provides new information about molecular differences between different types of solid cancers.

This work has been supported by the Norwegian Financial Mechanism 2014-2021, Project 2019/34/H/NZ7/00503.

[II-7] PROTEOMIC SIGNATURES OF THE RESPONSE TO RADIOTHERAPY IDENTIFIED IN TUMOR AND SMALL EXTRACELLULAR VESICLES OF RECTAL CANCER PATIENTS

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Introduction: Identification of biomarkers that could be used for the prediction of the response to neoadjuvant radiotherapy (neo-RT) in locally advanced rectal cancer (LARC) still remains a challenge. Small extracellular vesicles (sEV) and other types of vesicles circulating in patients blood represent a novel type of liquid biopsy and a source of potential cancer biomarkers. Hence, the search for molecular signatures for assessing the radio-responsiveness of rectal cancer represents a relevant issue. Here we applied an MS-based proteomic approach to reveal molecular components characteristic for tumor tissue in sEV isolated from serum of rectal cancer patients with different responses to radiotherapy.

Methods: Tissue and serum samples were collected from 20 LARC patients diagnosed with adenocarcinoma treated by neo-RT in a total dose of 39 - 54Gy. Blood samples were collected directly before surgery and tissue specimens were obtained during a standard surgical treatment. Collected samples were classified into two groups depending on the response to the treatment and the presence of tumor cells in studied material: good responders (GR) - 10 patients with RT-sensitive tumors (TRG 0-1), and poor responders (PR) - 10 patients with RT-resistant tumors (TRG 2-3). sEV were isolated from serum using SEC and characterized according to MISEV2018 criteria. Tissue samples after lysis with SDC and trypsin digestion were subjected to LC-MS/MS-based proteomic profiling.

Results: Label-free LC-MS/MS approach allowed the identification of around 2700 proteins in tissue samples and around 260 in sEV. Among all identified proteins 160 overlapped between specimens. These co-identified proteins were associated with pathways relevant for the response of rectal cancer patients to neo-RT, including response to stress, immune and inflammatory response, leukocyte mediated immunity, neutrophil degranulation, complement activation, hemostasis, platelet activation, ECM-receptor interaction, post-translational protein phosphorylation and plasma lipoprotein particle assembly. Moreover, there were 1854 proteins in tissue samples (FDR0.05) and 29 in sEV (p0.05) that showed significantly different abundance between good and poor responders. Among all differentially accumulated proteins (DAPs) 9 overlapped between specimens. Based on comparative analysis we proposed DAPs common for serum-derived exosomes and rectal tumor tissue that included LUM and SERPINF2, which will be further validated with targeted MS techniques, immunohistochemistry and by fluorescence microscopy.

Conclusions: This study revealed specific proteomic signatures of the response to radiotherapy identified in both tumor and vesicles of rectal cancer patients. Proposed proteins characteristic for tumor tissue and present in serum-derived sEV could be promising candidates for non-invasive biomarkers of rectal cancer radioresponsiveness. Hence, further study is necessary to validate the proposed molecular signatures.

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[II-8] LEWIS C ANTIGEN AND A2-6-LINKED SIALIC ACID RESIDUE IN SPECIFIC PROTEINS CAN POTENTIATE THE DIAGNOSIS OF CUTANEOUS MELANOMA

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The unique glycosylation pattern of proteins in cancer cells can be a valuable marker to identify them, determine the stage of the disease, and be a target of anticancer therapy. The objectives of our research were (i) to identify, unique to melanoma cells, *N*-glycans containing Lewis C antigen (Gal13GlcNAc epitope) and 2-6-linked sialic acid, and (ii) to evaluate the molecular weights of proteins that carry these glycomarkers.

The study was carried out using three human cell lines HEMa-LP (melanocytes), WM793 (primary skin melanoma), and WM266-4 (metastatic skin melanoma). *N*-glycans, released from proteins by digestion with endoglycosidase F, underwent linkage-specific derivatization of sialic acids or digestion of oligosaccharides with a mixture of *Arthrobacter ureafaciens* sialidase and *Streptococcus pneumoniae* galactosidase, were analyzed using MALDI-MS spectrometry. Furthermore, Western blot immunodetection of Lewis C-bearing proteins was performed using mAb-A4 antibodies, as well as sialic acid-bearing proteins were detected using 2,3- and 2,6-specific lectins, respectively, from *Maackia amurensis* (MAA) and *Sambucus nigra* (SNA) using the lectin blot method.

Mass spectrometry analysis has shown that melanoma cells, in particular the metastatic melanoma cell line WM266-4, have more *N*-glycans with 2-6-linked sialic acid compared to melanocytes. For example, core fucosylated and nonfucosylated triantennary structures containing one residue of 2-6-linked sialic acid were present only in melanoma cells. Similarly, melanoma cells have a higher amount of *N*-oligosaccharides, especially triantennary structures, with Gal1-3GlcNAc unit compared to melanocytes. Immunodetection and lectin binding suggested that the largest number of protein bands containing the Lewis C antigen and 2-6-linked sialic acid were found in metastatic melanoma cells. Five protein bands bearing Lewis C epitope, among them the most intense 34 kDa-band, were not present in the protein fractions of melanocytes. Multiple protein bands with molecular weights ranging from 250 to 140 kDa as well as below 30 kDa, and reacted with SNA lectin specific for 2,6-linked sialic acid, were present only in melanoma cell lines.

Our results suggest that these proteins that during tumorigenesis underwent specific glycomarkers, such as Lewis C antigen and/or 2-6-linked sialic acid, could be a highly-specific diagnostic markers of melanoma. In a future, we plan to identify these glycoproteins.

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[II-9] EFFECT OF INHIBITORS TARGETING HEAT SHOCK FACTOR 1 ON THE GROWTH OF BREAST CANCER CELLS

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Breast cancer is the most common type of malignancy in women worldwide. Approximately 70% of all cases are ER-positive (ER+; expressing estrogen receptors) and estrogen-dependent. They are first treated with hormonal therapy, yet about 20-40% of ER+ breast cancer patients develop metastases, which are the major cause of cancer-related mortality. Our recent studies have demonstrated the potential role of Heat Shock Factor 1 (HSF1) in the growth of ER+ breast cancer and its resistance to hormonal therapy. HSF1 is a well-known transcription factor, playing a key role in the cell response to stress. Moreover, it is activated by estrogen via ER/MAPK signaling in ER+ breast cancer cells. Here, we studied the effect of several chemical compounds targeting HSF1 (CCT251236, KRIBB11, and DTHIB) on the growth of ER+ breast cancer cells (MCF7, T47D, and BT-474). Treatment with HSF1 inhibitors resulted in the repression of heat shock-induced HSPA1 (molecular target of HSF1) expression in all tested cell lines. Unlike CCT251236 and KRIBB11, DTHIB (Directed Targeted HSF1 Inhibitor) caused a decrease in the level of HSF1, ER, and HSP90 proteins in MCF7 cells. All tested compounds inhibited the proliferation of tested ER-positive breast cancer cells with accompanying changes in the cell cycle. Our data suggests that pharmacological inhibition of HSF1 may affect the growth of ER+positive breast cancer cells.

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[II-10] CHANGES IN PROTEOME OF PROTEASOME INHIBITOR RESISTANT MULTIPLE MYELOMA CELL LINES

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Multiple myeloma (MM) is a monoclonal gammopathy caused by clonal proliferation of abnormal plasma cells. Despite the huge progress in the treatment of MM, the disease remains incurable, due to relapse occurring in most cases. This fact is closely related to acquired resistance to the treatment. Proteasome inhibitors (PIs) are drugs commonly used in MM therapy. By blocking specific proteasome subunits, PIs lead to accumulation of the misfolded proteins in the cell and in consequence enhanced apoptosis or cell cycle inhibition. However, this approach, although effective, also inevitably leads to drug resistance and relapse.

The aim of presented study was proteomic analysis of multiple myeloma cells, to determine proteins and pathways involved in the acquired resistance to proteasome inhibitors. We used two MM cell lines: U266B1, RPMI8266, primary sensitive to PIs, to establish their daughter cell lines - resistant to one of two approved drugs: bortezomib (BTZ) or carfilzomib (CFZ).

Long-term cell culture with increasing concentration of the drug, allowed us to obtain MM cell lines resistant to wide range of both PIs concentrations. After protein isolation, samples were prepared for analysis by nanoLC-MS/MS using a Dionex UltiMate 3000 RSLCnano System coupled with a Q-Exactive Orbitrap mass spectrometer. The raw files were analyzed by Proteome Discoverer. Next, the comparative proteome analysis between the sensitive and resistant cells was performed, including identification of differentially expressed proteins (q-value 0.05; fold change 2; 2 unique peptides), enrichment analysis using Ingenuity Pathway Analysis (IPA) software and identification of the top canonical pathways, biological functions, and upstream regulators associated with the observed differences in protein profile.

We observed high enrichment in unfolded protein response and ubiquitination pathways in all analyzed cell lines. We observed negative correlation of PIs concentration and protein abundance in pathways including deregulation in cellular respiration: TCA cycle/ glycolysis, oxidative phosphorylation and mitochondrial dysfunction. We also identified enrichment of proteins involved in pathways, not previously connected with response of MM cells for PIs treatment: EIF2 signaling pathway and sirtuin signaling pathway. In U266B1 PIs resistant cell lines, the abundance of proteins involved in both pathways was negatively correlated with all PIs concentrations. In case of RPMI8266 PI resistant cell lines, we observed negative correlation of protein abundance with both PIs for sirtuin signaling pathway while for EIF2 signaling protein abundance was more clearly correlated with BTZ concentrations.

In conclusion, in our study we analyzed the changes of proteome of MM cell lines resistant to proteasome inhibitors. Two new pathways deregulated in MM resistant cell lines as compared to drug sensitive cell lines were pointed: EIF2 signaling pathway and sirtuin signaling pathway.

[II-11] TOWARDS EFFECTIVENESS OF LIQUID BIOPSY IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Head and neck squamous cell carcinoma (HNSCC) constitute the entity of tumors located in the upper aerodigestive tract. They develop from epithelial cells covering the larynx, pharynx, oral cavity, and sinonasal tract. Liquid biopsy is a minimally invasive, diagnostic procedure, that utilizes body fluids analysis to detect and characterize cancer fingerprints. It is of great potential in oncology, however there are challenges related to the appropriate handling of liquid biopsy samples that need to be addressed to implement such analysis in patients care. Therefore, the main aim of our study was optimization of pre-analytical conditions and thorough characterization of cfDNA fraction (concentration, length, integrity score) in HNSCC patients (n= 152) and healthy volunteers (n=56).

We observed significantly higher cfDNA concentration in cancer patients compared to healthy controls. Moreover, we pointed up the need to consider time necessary for epithelial regeneration of at least 100 days to avoid elevated levels of cfDNA that are not a consequence of active cancer. Our results revealed also a significant increase of cfDNA concentration with age in both, healthy volunteers as well as HNSCC patients, indicating age-related accumulation of nucleic acids together with inefficient elimination of cfDNA. Another meaningful finding of our study, regarding the multitude of HNSCC locations, is the lack of difference in concentration of cfDNA depending on the anatomical location of the tumor. Furthermore, we proved a trend toward higher cfDNA length (range 35-10 380 and 500-10 380 bp) in the group of patients with recurrence in follow-up, suggesting predominance of tumor necrosis as a prognosticator of recurrence.

In conclusion, our findings provide a broad characterization of cfDNA fractions in HNSCC patients and healthy controls and point to several aspects necessary to consider when implementing liquid biopsy in clinical practice.

Session III: Immunity and Cancer

[III-1] NO DETECTABLE 3-NITRO-L-TYROSINE (3-NT) LEVELS IN NSCLC PATIENTS WITH AND WITHOUT ADJUVANT CHEMOTHERAPY AFTER TUMOUR RESECTION

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Currently, 3-nitrotyrosine (3-NT) has raised great interest concerning its potential as a biological tool for the therapeutic monitoring of various diseases involved in nitrosative stress. A significant increase in the extent of this process may result in elevated 3-NT levels in biological samples and has been associated with a wide range of diseases.

The research objective was to evaluate the assumption that 3-nitro-L-tyrosine concentrations, and hence the level of nitrosative stress, is different in NSCLC patients with and without adjuvant chemotherapy after tumour resection.

The study comprised 43 patients: 28 patients diagnosed with NSCLC (study group) and 15 age and sex-matched blood donors (control group). The mean age of study group was 66.4 years, 21 men and 7 women were included in the study. The NSCLC subgroup comprised 16 adenocarcinomas and 12 squamous cell carcinomas, varied in terms of TNM staging and grading. Out of 28 patients, 10 was active smokers. Plasma samples were collected in a short postoperative or post chemotherapy course follow-up (~24H). In total, 43 serum samples were subjected into analysis, 15 taken from patients with NSCLC after tumour resection, 13 from NSCLC patients, who were provided with adjuvant chemotherapy and 15 from healthy controls. An LC-MS/MS assay for quantitative determination of 3-NT in human blood plasma was developed. The specificity in respect to target analyte and human blood plasma components were achieved, with the lowest limit of quantification (LLOQ): 0.1ng/mL (based on calibration curve). Working concentration ranged from 0.1ng/mL to 5.0ng/mL (repeatability at LLOQ: 15%; accuracy at LLOQ (RE): 15%). Mass spectrometry parameters were obtained experimentally by direct infusion of 3-nitro-L-tyrosine and 3-nitro-L-tyrosine-[d3] standard solutions. Calibration plot consisting of six concentration levels was constructed; each concentration was tested in triplicate. Response was linear over the tested range 0.1ng/mL 5ng/mL of 3-NT in human plasma (Correlation coefficient: $r = 0.9995$, $r^2 = 0.999$).

The 3-NT was undetectable in all selected cases. As the constructed calibration curve was correct, quality check samples were performed repeatedly through analysis and all tested validation parameters met the assumed criteria - it confirmed that results were truly negative.

The preliminary results indicate that 3-NT is not a suitable marker for nitrosative stress indicator among NSCLC patients after tumour resection with and without adjuvant chemotherapy, because no detectable concentrations were found among investigated groups. To the best of our knowledge this is the first such type of analysis that is why no final

conclusion can be drawn. The experiment should be repeated on a larger study group, possibly taking into account the dynamics of changes in time after surgery.

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[III-2] HSF1 STIMULATES MOTILITY OF ESTROGEN RECEPTOR POSITIVE MCF7 BREAST CANCER CELLS TO PROMIGRATORY CYTOKINES

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Introduction: Breast cancer is the most common type of malignancy in women worldwide. Approximately 70% of all cases are estrogen-dependent. Estrogen receptor-positive (ER+) breast cancer patients develop metastases, mainly to bone, lung, liver and brain, which are the major cause of cancer-related mortality. The process of metastasis can be directed by extracellular signaling proteins (mainly cytokines). The paracrine action of these molecules is primarily to stimulate cell motility. In addition, some of these proteins act as chemoattractants for cancer cells. Our recent studies have demonstrated the potential role of Heat Shock Factor 1 (HSF1) and 17-estradiol (E2) in the growth and motility of ER+ breast cancer cell lines (MCF7 and T47D). HSF1 is a transcription factor, playing a key role in the cell response to stress, especially to proteotoxic stress such as heat shock. It activates the production of Heat Shock Proteins, which allow cells to survive during unfavorable conditions. We found that E2 leads to the activation of HSF1 via ER and MAPK in MCF7 cells. Moreover, activated HSF1 directly cooperates with ER and supports its action participating in the transcription stimulation.

Methods: In this work, we studied the effects of HSF1 and E2 on the response of MCF7 cells to extracellular signaling proteins involved in metastasis formation. MCF7 cells with different HSF1 status (HSF1-proficient versus HSF1-deficient cells), were pretreated or not with E2 for 14 days. The cells were then tested for chemotaxis (directed migration in the Boyden chambers) and chemokinesis (undirected migration analyzed by tracking the movement of single cells using live imaging microscopy) under the influence of selected signaling molecules.

Results: We found that MCF7 cells are attracted to IGF1, EGF1 and calcium ions (Ca²⁺) but not to TGF, HGF, TNF, bFGF and SDF1. The stimulation of chemotaxis by IGF1 in Boyden chamber assay is less efficient for HSF1-deficient cells, also after 14 days E2 pretreatment. IGF1 strongly enhances HSF1-proficient and HSF1-deficient cells motility/chemokinesis. Also, EGF1 strongly attracts HSF1-proficient, but does not attract HSF1-deficient cells, as well as MCF7 cells treated with 17-estradiol, regardless HSF1 status. EGF1 weakly stimulates MCF7 motility. TGF stimulates MCF7 cells chemokinesis, but chemotaxis assessed by Boyden chamber assay was not observed. Ca²⁺ ions are chemotactic and motogenic factor for MCF7 cells. Nevertheless, HSF1-deficient cells treated with E2 and EGF1 or TGF or Ca²⁺ ions move less efficiently in comparison to control cells.

Conclusion: Our results suggest that IGF1 (insulin-like growth factor 1), EGF1 (epidermal growth factor 1) and calcium ions have the greatest effect on directed migration of MCF7 cells, which is supported by HSF1. They may be most important in the metastasis of ER+ breast cancer.

[III-3] CDK8/19 AND MEK INHIBITION FOR HORMONE-NEGATIVE BREAST CANCER

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Introduction: Breast cancer (BC) encompasses diverse subtypes characterized by progesterone and estrogen receptor expression, categorizing them as hormone-negative (HR-neg) - lacking both receptors - or hormone-positive (expressing at least one receptor). Hormone-negative BC, including triple-negative BC (TNBC), presents challenges due to the absence of precise targeted therapies. CDK8 and CDK19, components of the mediator complex, have recently emerged as potential therapeutic targets. Elevated CDK8 expression aligns with unfavorable prognosis across various BC subtypes. This study assessed BC cell lines response to RVU120, a potent CDK8/19 inhibitor in clinical trials for diverse malignancies. Moreover, given previous CDK8-MEK inhibitor synergy in neuroblastoma, MEK inhibitors (MEKi) were explored as potential partners in HR-neg BC treatment.

Methods: To evaluate CDK8/CDK19 and MEK inhibition effects, BC cell lines were treated with RVU120 alone or in combination with selumetinib, trametinib, binimetinib, or avotemetinib in mammospheres and spheroids assays. Cell viability was assessed using CellTiter-Glo 3D reagent. Synergistic interactions between RVU120 and MEKi were analyzed by Combenefit and Compusyn software employing the Loewe additivity model. Transcriptional profiling was performed using RNA expression data from the Cancer Cell Line Encyclopedia (CCLE) repository and Gene Set Enrichment Analysis. The Ras activation score, predicting responses to PI3K and Ras pathway inhibitors, was calculated using RNA expression data from CCLE according to *Loboda A. et al, BMC Med Genomics 3, 26, 2010*.

Results: Investigation of various BC subtypes in cell lines demonstrated high sensitivity of TNBC and HR-neg HER2+ cells to RVU120. Transcriptional profiling of cell lines revealed that sensitivity to CDK8/CDK19 inhibition correlates with heightened STAT3 phosphorylation and amplification of TNF/NFKB and STAT target genes. Interestingly, TNBC sensitivity to RVU120 was notably influenced by EGF presence in the culture. Considering the role of EGFR in activating downstream signaling pathways and previous observations of synergy between CDK8 and MEKi in neuroblastoma, MEKi were investigated as potential combination partners in HR-neg BC. 5 out of 15 tested BC cell lines exhibiting synergy between CDK8/CDK19 and MEK inhibitors, 5 showing antagonism, and 5 displaying no significant effect. Responsive cell lines to this combination therapy exhibited EGFR amplification and a positive Ras score, consistent across different inhibitors.

Conclusions: The study emphasizes the potential of combining RVU120 with MEK inhibitors, particularly in TNBC with specific genetic characteristics. Successful development relies on validating partner drugs targeting the RAS pathway and identifying suitable biomarkers like EGFRamp or Ras score. The findings propose the combination of CDK8/19 inhibitor RVU120 and MEKi as a potential targeted therapy strategy for HR-neg BC.

[III-4] EFFECT OF HORMONES ON MIRNA EXPRESSION

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MiRNAs are non-coding RNA molecules that are commonly involved in regulating the expression levels of genes. Translational silencing is achieved when a specific miRNA interacts with the 3-UTR of its target gene and prevents protein synthesis from the transcript. On the other hand, miRNA levels are influenced by external and internal factors. In the present project, based on literature studies, the miRNA regulated by sex hormones was chosen. To examine miRNA-target interactions, we used predicted and experimentally validated databases (miRTar Base, TargetScanHuman). Next, using the KEGG database, the main pathway regulated by hormone-dependent miRNA was identified.

One family of proteins that can be regulated by hormone-dependent miRNA are aquaporins (membrane proteins that transport water and other small molecules). However, the mechanism inducing the change in aquaporin and miRNA expression is not yet fully understood. Since endocrine disruption is common in civilization diseases, in the present study, we tested the potential relationships between the expression levels of selected miRNAs (miR-221; miR-378) and the presence of different levels of hormones.

The study was carried out on the HCT116 cell line. Cells were treated for 24 hours with water (control) or various concentrations of estradiol, progesterone, or insulin. After this time, RNA was isolated from the cells and expression levels of miRNA-221, miRNA-378 were determined by RT-qPCR. The Ct method was used to determine the relative expression levels of the genes studied, the calculation referring to the expression level of the reference gene SNORD44 for miRNA. The results showed that the miRNA expression levels of the studied genes depend on the concentration of hormones. Until now, miRNA-221 and miRNA-378 have not been experimentally verified for their interaction with the AQP genes; however, the binding site miRNA to the sequence of the AQP3 and AQP8 gene.

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[III-5] DOES P53 SENSITIZE CANCER CELLS TO THE ATTACK FROM NATURAL KILLER CELLS?

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The p53 protein is best known as the guardian of the genome. Our earlier study has shown that p53 protein regulated some genes involved in immunity. In this project we are focusing on how SLAMF7, KLRG2 and NCR3LG1 are regulated by p53. All of these genes are engaged the innate immune response, but the details of their functions are poorly known. Of the three genes, SLAMF7 is best studied. This protein is present on natural killer (NK) cells and also on cancer cells. The mutual interaction between SLAMF7 molecules on tumor cells and on NK cells is a part of the activating signal promoting killing of cancer cells. SLAMF7 protein is used as a target for immunotherapy of multiple myeloma with Elotuzumab because this protein is highly expressed in these cancer cells.

We found that activation of *SLAMF7*, *KLRG2* and *NCR3LG1* genes is mediated by p53 in cells exposed to the innovative combination of drugs actinomycin D and nutlin-3a (A+N). We observed the weakening of expression of all these genes in p53-deficient cells. In addition, we observed that one of the forms of SLAMF7 protein (50 kDa) is secreted into the extracellular space. The reporter tests demonstrated that cloned regulatory regions of these genes are activated by p53, both ectopically expressed and endogenous. Strong activation of SLAMF7 by A+N opens a perspective for treatment of cancers other than multiple myeloma with Elotuzumab.

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[III-6] THE IMPACT OF STRONG P53 ACTIVATION ON INNATE IMMUNITY OF CANCER CELLS

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The innate immunity is the first line of defense against infections and cancer development, and the p53 protein is the key tumor suppressor. The role of p53 in regulating the cell cycle and apoptosis is well studied, but much less is known about the effect of the protein on the immune system. We have discovered that two substances actinomycin D (A) and nutlin3a (N) act synergistically in the activation of p53 and stimulation of a subset of p53-target genes. Actinomycin D stimulates kinases involved in the activation of p53, while nutlin-3a prevents the interaction of p53 with its inhibitor, the MDM2 protein. A+N activates the expression of many genes not stimulated by the compounds acting alone. Some of these genes code immunity proteins, e.g., CASP1, STING, and proteins affecting anti-virus mechanisms, e.g., IFIT1, IFIT2. A+N synergize in p53 activation in various cell lines, however, there is a subset of genes activated by A+N in p53-independent fashion. Moreover, we have transcriptomic data concerning the activation of genes by A+N and we wanted to combine them with the proteomic data to find out which genes actually produce the proteins and which proteins are upregulated in transcription-independent fashion, e.g. by protein stabilization.

To this end we performed proteomic analysis using mass spectrometry on the following set of samples derived from A549 lung cancer cell line: wild-type A549 cells, p53-deficient A549 cells engineered using CRISPR/Cas9 technique, controls for CRISPR/Cas9 technique modification. The cells grew in control conditions or were exposed to A+N for 48h. We extracted the proteins from cell lysates and in conditioned medium to analyze the secretome of cells. In this fashion we found which proteins upregulated by A+N in cells and in secretome are controlled by p53. Many proteins secreted to extracellular space can influence the neighboring immune cells, hence we got data about how p53 can impact on the relationship between cancer cells and immune system.

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[III-7] RADIATION-INDUCED ALTERATIONS IN MIRNA BIOGENESIS IN B-CELL LYMPHOMA CELLS

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MicroRNA (miRNA) are short non-coding single-stranded RNAs that play a role in the regulation of gene expression at the post-transcriptional level. The biogenesis of miRNA involves several steps, with the first occurring in the cell nucleus, resulting in the production of primary miRNA (pri-miRNA), which is further processed by the Microprocessor to generate precursor miRNA (pre-miRNA). Subsequently, pre-miRNA is transported to the cytoplasm, where it is processed by Dicer to form mature miRNA.

In this study we aim to determine the effect of irradiation on miRNA biogenesis in B-cell lymphoma cells. We used three Hodgkin lymphoma cell lines of: KMH2, L428, and L1236 and three Burkitt lymphoma cell lines: CA46, DG75, and ST486. All cells were irradiated with a dose of 4Gy and after 1, 4, 8, 12, 24, 48 and 72 hours (h) the percentage of apoptotic cells and cell cycle distribution was measured using flow cytometry.

We observed that apoptosis was induced in all cell lines 24 hours after irradiation, although the percentage of apoptotic cells varied between cell lines. Furthermore, we found that irradiation with 4Gy resulted in cell cycle arrest in the G2 phase in all cell lines.

Next, we performed RNA sequencing to analyze the levels of pri-miRNA and miRNA. We defined pri-miRNA as pre-miRNA with the 150 nt sequence upstream and downstream of pre-miRNA. We did not observe significant and consistent changes in the levels of pri-miRNA and miRNA in Hodgkin lymphoma cells following irradiation. We observed that ionizing radiation impacted the biogenesis of a small number of miRNAs in Burkitt lymphoma cells, with the specific changes depending on the cell line. For instance, irradiation of ST486 cells caused a 2-fold decrease in miR-155 at the first and fourth hours and at least a 1.5-fold decrease in miRNA-146a and miR-21a at 4 and 12 h. In DG75 cells, we observed a 1.5-fold increase in miR-155 and miR-146a, with a statistically significant increase in pri-miRNA-155 at 4 h. In the CA46 cell line, there was at least a 2-fold increase in miR-146a and miR-449a/b at 4h and 12h. Additionally, we observed a 1.5-fold increase in miR-29b at 4h. Next, we selected several miRNAs and confirmed the altered miRNA and pri-miRNA levels upon irradiation using qRT-PCR.

In conclusion, we showed that irradiation of B-cell lymphoma cells resulted in G2 cell cycle arrest and apoptosis after 24 hours. Furthermore, we determined that irradiation affected the biogenesis of a small number of miRNAs, including exonic miRNAs such as miR-155 and miR-146a, along with their pri-miRNAs. The altered expression occurred at different time points upon irradiation depending on the cell line.

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[III-8] THE IN VITRO AND IN VIVO ASSESSMENT OF THE SUNITINIB-BASED ANTITUMOR THERAPY AGAINST MELANOMA

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Introduction: Tumors require a blood supply to grow and metastasize and by blocking the formation of new blood vessels, anti-angiogenic drugs can inhibit tumor progression. Sunitinib (SUN) is a tyrosine kinase inhibitor that targets multiple receptors involved in angiogenesis and cell proliferation. Those processes have a significant impact on the tumor microenvironment (TME). The TME refers to the cellular and non-cellular components surrounding tumor cells, including blood vessels, immune cells, fibroblasts and extracellular matrix. These components can play a critical role in tumor growth, progression and response to treatment. Understanding the complex interactions between SUN and the TME is essential for optimizing its use in therapy. In this study, we evaluated the impact of SUN on selected cell lines *in vitro*. We also checked how various routes of SUN administration and types of vehicles affect the antitumor response in the murine melanoma model.

Methods: Cell viability and mechanisms of cell death after SUN treatment have been tested. The experiments were conducted on several murine cell lines: B16F10 RedF-luc melanoma cells, HECa10 endothelial cells, H5V heart endothelioma cells, NIH3T3 fibroblasts, J744A.1 and RAW264.7 macrophages. Cytotoxicity of SUN over a wide range of concentrations (1-100M) was performed using the MTS assay. In vivo study, we checked the therapeutic effect of different routes (oral, intraperitoneal, and intratumoral) of SUN administration in B16F10 RedF-luc melanoma tumors. We also compared two types of vehicles for SUN: DMSO with PBS and carboxymethylcellulose (CMC) buffer.

Results: Our results show that SUN reduces cell viability in a dose-dependent manner in all tested cell lines. Mouse macrophage lines turned out to be the most sensitive to the drug's action even at the lowest tested concentrations, while the HECa10 cell line turned out to be the least sensitive. However, concentrations above 25M caused complete death in every cell line. Other analyses confirm the pro-apoptotic effect of SUN. Additionally, we observed that SUN in a single dose of 50mg/kg, injected directly into tumors, inhibits the growth of tumors more efficiently, compared to the four times oral or intraperitoneal administration of SUN. The antitumor therapeutic effect was the most effective when SUN was dissolved in the CMC buffer.

Conclusions: Sunitinib causes the death of tumor and other cells in a dose-dependent manner *in vitro*. The results indicate that SUN may be used as a tumor microenvironment modulating cell death-inducing agent in anticancer therapy. Moreover, only one dose of 50mg/kg of SUN dissolved in the CMC buffer is needed to effectively inhibit melanoma tumor growth. The obtained data are preliminary and further research may lead to the optimization of the sunitinib-based antitumor therapy.

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Session IV: Bioinformatics and Mathematical Modeling

[IV-1] CLOUD-BASED SOLUTION: AN ATTRACTIVE APPROACH FOR CHIP-SEQ DATA ANALYSIS

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NGS (*Next Generation Sequencing*) computational analyses are time-consuming and computationally demanding. One of NGS methods - ChIP-Seq (*Chromatin Immunoprecipitation followed by sequencing*) is a method used to identify and characterize protein-DNA interactions. In this study, a solution enabling ChIP-Seq data analysis in the cloud and on-premises environment was proposed. The implemented solution consists of commonly used ChIP-Seq analysis steps: read quality analysis (FastQC) [1], adapter removal (TrimGalore) [2], read mapping (BWA) [3], SAM file conversion to BAM (Samtools) [4], duplicate removal (picard-tools MarkDuplicates) [5], BigWig file generation (DeepTools BamCoverage) [6], peak calling (MACS3) [7] and differential analysis (DiffBind) [8]. The computations were performed using Microsoft Azure infrastructure. Azure Batch [9] was used as the main service. Nextflow [10] was used as the task manager, enabling parallelization of the stages. The cloud-based solution using Standard_D2s_v3 virtual machines was compared with an average specification personal computer (Intel Core i7-1165G7 Processor, 4 cores, 8 threads, 3.0GHz, Level 3 Cache 12MB, Hyper-Threading, 16 GB RAM, M.2 PCIe SSD). Over 10% acceleration was achieved compared to the personal computer for the entire analysis. Approximately 22.4% acceleration was achieved for one of the most computationally demanding stages, i.e., read mapping, compared to the personal computer. The use of a greater number of virtual machines reduced the computation time. The cost of conducting a single cloud analysis for input data (.fastq.gz) totaling over 192 million reads did not exceed 2 EUR. Considering the excellent cloud computation time and low analysis costs, the use of Microsoft Azure Batch seems to be a very attractive solution for conducting bioinformatic analyses.

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[IV-2] COMPARATIVE ANALYSIS AND SEGMENTATION OF MOUSE BRAIN MRI

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Introduction: The aim of this study was to investigate the comparative analysis of different fluorescence imaging processing parameters of mouse brains. Different groups of mice were included in the study to assess the effect of the BMP8b protein on the structure of the brain vascular network.

The image data contains the complete structure of vessels of mouse brains, which requires significant computational power for analysing differences among the experimental mice. Because of that, a comparative analysis was conducted in order to reduce the computational cost and increase the segmentation quality while retaining the robustness of the data. In addition, various parameters of the segmentation and identification of the vascular networks were tested to obtain the best results.

Materials and methods: The raw data was initially processed with Imaris, followed by adjustments made using ImageJ (Fiji).

The influence of different imaging processing parameters in Imaris was investigated, and the analysis was performed on data at different resolutions to estimate its effect on the vascular network parameters. Additionally, key sections of the scan were identified and exported as separate files to optimize computational efficiency.

The processed data in different resolutions was then reintegrated into Imaris for the final analysis, allowing for a comprehensive comparison of results across various resolutions. Different workflows for network tracing were tested, in particular the computation of tracing segments between pairs of seed points together.

Results: The results were obtained depending on the size of the network and the resolution of the images. The selection of appropriate parameters allowed us to obtain satisfactory results. The statistics obtained confirm the validity of our analysis for applications of the blood vessel network.

Conclusions: Due to the substantial computational resources required for the analysis of the entire scan, there is a clear justification for implementing compression techniques. It is worth noting that only minor disparities were detected between images at full, half, and quarter resolutions.

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[IV-3] VOLUMETRIC ANALYSIS OF PATIENTS WITH PARKINSON'S DISEASE BASED ON MRI STUDIES OF BRAIN STRUCTURES

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Introduction: Parkinsons disease (PD) is the second most common neurodegenerative disorder after Alzheimers disease, affecting more than ten million individuals worldwide. The aim is to analyze volumetric changes in the brain resulting from developmental processes and neurodegenerative pathologies of the central nervous system, with Parkinsons disease serving as an illustrative example.

Materials and methods: The data were obtained as part of a collaboration with the Maria Skłodowska-Curie National Research Institute of Oncology, in which ten patients with Parkinsons disease underwent structural magnetic resonance imaging (MRI). The analysis was performed using the FMRIB Software Library (FSL). First, the MRI image was de-noised using the SUSAN (Smallest Univalued Segment Assimilating Nucleus) module. Unwanted tissue was then removed from the image until only the brain remained using FSL BET (Brain Extraction Tool). The next step was to use the FSL FAST (FMRIBs Automated Segmentation Tool) modules and FSL FIRST (FMRIBs Integrated Registration and Segmentation Tool) modules to obtain a subdivision of the structures into grey matter, white matter, and cerebrospinal fluid and 14 subcortical structures. Finally, the joint volume and the volume of each structure separately were calculated using MATLAB software and the Medical Image Processing Toolbox library.

Results: The analysis was conducted for 10 patients with Parkinsons disease. The three main brain structures - grey matter, white matter, and cerebrospinal fluid and 14 subcortical structures for the left and right cerebral hemispheres were segmented. Their volumes were then calculated using MATLAB and FSL software to ensure minimal measurement error. The received volumetric values from segmented structures of patients with Parkinsons disease were compared to the volumetric values from segmented structures of healthy volunteers.

Conclusions: Volumetric analysis will allow the construction of a model to distinguish Parkinsons disease patients from healthy volunteers. Understanding observable structural brain changes in Parkinsons disease in people at increased risk of developing dementia may provide an integrated biomarker to identify and treat people who will develop Parkinsons dementia.

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[IV-4] ILLUMINATING INSIGHTS: DECIPHERING THE GENOMIC NETWORK - ADVANCED ANALYSIS AND DYNAMIC VISUALIZATION IN CYTOSCAPE

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This study explores genomic network analysis and visualization in Cytoscape, shedding a profound light on its intricate structural properties and sophisticated clustering characteristics. Our in-depth analysis not only reveals the networks underlying architecture but also deciphers complex connectivity patterns. We systematically investigate node distribution and arrangement, exposing local clusters characterized by exceptional clustering coefficients while identifying influential nodes with unparalleled centrality, primarily governed by incoming connections.

In addition to conventional analyses, we introduce an innovative mathematical model that integrates two pivotal network metrics: betweenness centrality (BC) and clustering coefficient (CC). This model is further augmented by two essential parameters, α and β , offering a unique perspective on network dynamics. The α parameter captures the profound influence of network density on clustering tendencies, with higher values emphasizing stronger clustering within densely interconnected regions. Meanwhile, the β parameter extends beyond CC, providing a nuanced understanding of node centrality, thus enhancing the accuracy and depth of our network analysis. This approach paints a comprehensive picture of network properties, illuminating the subtleties of information flow dynamics. Our gene network analysis, a culmination of precision and rigor, is conducted on 43 nodes intricately connected by 53 edges. Notably, it uncovers a striking average of 2.465 neighbors per node, revealing the intricate web of connections within the network. The networks diameter, an astounding 14, denotes the farthest distance between any two nodes, while the clustering coefficient, a remarkable 0.020, signifies the networks innate tendency to form localized clusters. Furthermore, the networks singular connected component ensures that every node is readily accessible. Remarkably, the runtime analysis highlights our methodologys efficiency, with results delivered in a mere 0.055 seconds. To further enrich our insights, we employ a comprehensive statistical analysis, meticulously scrutinizing node degree distribution and edge count. This insightful analysis unravels hidden patterns within the network, providing invaluable revelations about its structural properties and intricate clustering tendencies. Our pioneering mathematical model revolutionizes network analysis by dynamically adapting node BC values based on CC, network density, and node centrality. Although immensely promising, the real-world application of this model necessitates empirical validation due to the inherent complexities of biological networks. In conclusion, this groundbreaking study not only deepens our comprehension of gene network structure and 1 behavior but also introduces an innovative mathematical model, brimming with potential applications in network optimization, precise node identification, and predictive behavior modeling.

[IV-5] THE USE OF DIVERSITY PROFILES TO DESCRIBE DIFFERENTIALLY SKEWED TCR REPERTOIRES

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Introduction: Adaptive immunity relies on diverse B and T cells to mount specific responses against pathogens. T cell receptors (TCRs) ensure specificity by binding to antigens presented by infected or aberrant cells. Complementarity determining region (CDR3) is the most diverse region of TCR exon, providing the specificity of responses. Assessing T cell diversity involves analyzing CDR3 sequences and evaluating distinct clones abundance and richness. Here we intend to evaluate the use of diversity profiles to describe the heterogeneity of TCR repertoires.

Methods: We used two datasets of human TCR repertoires obtained from 587 healthy donors (HD, Dean et al <https://doi.org/10.1186/s13073-015-0238-z>) and 21 melanoma patients (MP, Huuhtanen et al - <https://doi.org/10.1038/s41467-022-33720-z>). TCR clonal diversity was calculated based on CDR3 clones and was defined as renyi entropy of order alpha ranging from 0 to 10 with step 1. Small alpha values put more emphasis on rare clones, whereas as alpha gets bigger, more focus is put on abundant TCRs. Skewness of TCR repertoires was estimated using power law distribution. The dispersion of diversity for a given alpha was estimated using coefficients of variations.

Results: HD repertoires were more skewed than the MP repertoires based on power law distribution. The diversity was higher for small alpha values when compared to large alpha values for both analyzed datasets. For small alpha values, the mean diversity was lower for less skewed repertoires with regard to more skewed repertoire (for alpha=0 mean95%CI for MP: 10.340.20 vs HD: 12.160.04). For large alpha, there were no significant changes between diversity values and the profiles remain relatively stable for both more (HD) and less (MP) skewed repertoires. The dispersion of diversity is bigger for higher alpha values (for alpha=10, CV = 24.68 and 30.56 for HD and MP respectively) when compared to smaller alpha values (for alpha=0, CV = 3.62 and 4.27 for HD and MP respectively).

Conclusions: Diversity profiles provide a valuable tool for observing the trajectory of changes that cannot be captured by individual diversity indices. When examining small values of alpha, the diversity is higher than for large alpha values, meaning that there are more TCR clones with low abundance, than the frequent ones. The diversity profile is rather stable for large alpha values. Dispersion of diversity values at different levels of alpha allows for comparative studies focusing on either low- or high-abundant clones.

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[IV-6] GENETIC AND CLINICAL DATA INTEGRATION FOR PREDICTION OF THE RISK OF DISTANT METASTASES IN LUNG CANCER

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Non-small cell lung cancer is characterized by high metastatic potential, with approximately 60% of patients developing distant metastases. The presence of distant metastases is a turning point in cancer treatment as the survival rates of patients with cancer that disseminated to distant organs are significantly lower than those with local tumors. Thus, assessing the risk of metastatic spread is important from a clinical point of view. However, this assessment is very difficult and requires sophisticated statistical and machine-learning models.

This project used genetic data (31 single-nucleotide polymorphisms, SNPs, and 2 indels) and clinical data to predict the risk of distant metastases in 335 non-small cell lung cancer patients. In our case, the outcome is metastasis-free survival (MFS) which measures elapsed time from diagnosis to metastasis and whether distant metastases occurred. Thus, the data are in the form of survival data. We tested three machine learning models (regularized, boosting, and random survival forest) that automatically select the most important features. The performance of the models was assessed using the concordance index (c-index).

We found that the panel of selected SNPs could predict the risk of distant metastases with good accuracy (c-index=0.7). The inclusion of clinical patient characteristics did not improve the models performance. Therefore, it is possible to predict the risk of distant metastases solely on the basis of a panel of selected SNPs identified in DNA from peripheral blood. As clinical data are subjective and their accuracy is lower than SNP identification in cancer patients, the selected panel of genetic variants could potentially be used in the patients treatment planning.

[IV-7] A CONVOLUTIONAL NEURAL NETWORK FOR THE CLASSIFICATION OF COVID-19 PATIENTS USING CHEST X-RAY IMAGES

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Introduction: The most recent global pandemic, COVID-19, caused by the SARS-CoV-2 virus, has underscored the importance of chest X-ray images (CXR). The use of X-ray images for detecting and diagnosing COVID-19 has become a vital tool in the battle against the worldwide crisis. These images offer crucial information about how the disease affects the lungs. This work aimed to build a neural network for classifying COVID-19 patients based on their lung changes that could support the diagnosis and treatment.

Materials and methods: In the study, the BIMCV COVID-19+ from the Valencian Region Medical ImageBank dataset was used containing chest X-ray images of the infected patients with the virus and their radiological findings and locations. A convolutional neural network was proposed to classify COVID-19 patients based on lung change characteristics into subcategories: Negative for pneumonia and Typical appearance. To prepare the images, segmentation of lungs was performed, and high-resolution dataset images were downsampled to a resolution of 256x256 pixels. In addition, data augmentation techniques were applied to the training dataset to enhance dataset diversity and improve model evaluation. The dataset was split into a training set (70%) and a testing set (30%) with a consistent ratio of categories. The trained model was evaluated using several metrics, including sensitivity, specificity, and balanced accuracy.

Results: The constructed CNN model comprises three convolution layers, each followed by ReLU activation function and a max pooling layer. The balanced accuracy of 79% demonstrates that the network accurately predicted the category of most COVID-19 patients. However, the sensitivity of 50% indicates that the network might face challenges in identifying patients diagnosed with typical appearance of COVID-19. On the other hand, the specificity of 93% suggests that the network better identified cases of patients diagnosed as negative for pneumonia.

Conclusion: In conclusion, the Convolutional Neural Network for the classification of COVID-19 patients represents a significant and valuable contribution to the field of medical imaging and disease diagnosis. The results of this study underscore the potential and effectiveness of CNNs in assisting medical professionals with the critical task of patient categorization. This study has demonstrated that CNNs are indeed valuable tools for classification of lung changes on CXR. However, further research is needed to fully unlock their potential.

[IV-8] PROTEOMIC ANALYSIS OF NON-SMALL CELL LUNG CANCER INCLUDING SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA

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Introduction: Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are two major subtypes of lung cancer and constitute about 70% of all lung cancer cases. Since many biological functions are mainly executed by proteins, proteome plays a key role in identifying biomarkers that can facilitate diagnosis and further treatment of patients. Protein levels and functions are not precisely referenced in the genomic and transcriptomic analysis of patient tumours. Therefore, direct examination of the functional proteome can provide information that complements and extends the genomic, epigenomic, and transcriptomic analysis. This study aims to compare LUAD and LUSC in terms of their proteomic profiles.

Methods: To identify potential differentiating biomarkers of non-small cell lung cancer subtypes (NSCLC), protein level measurements available with The Cancer Genome Atlas (TCGA) project and generated by Reverse Phase Protein Arrays (RPPA) were collected in a normalized form. The proteomic dataset of the two major lung cancer subtypes consisted of 682 samples (LUSC n=322, LUAD n=360) and the records for 216 proteins. As the assumption of normality of distribution was not met for a substantial proportion of the proteins considered, the non-parametric Mann-Whitney U test and Glass rank biserial correlation effect size (ES) were used to compare LUAD and LUSC cases. P-values were corrected for multiple testing with the Benjamini-Hochberg (BH) method.

Results: The levels of nine proteins showed significant differences between the NSCLC subtypes (4EBP1, BECLIN, CD49B, DUSP4, FASN, LCK, SMAD4, STAT3_pY705, TFRC). However, no proteins were selected as differentiating ones following the BH correction for multiple testing. The ES was at least small ($|ES| \geq 0.1$) for BECLIN, LCK, and TFRC, suggesting these proteins may be relevant in the context of subtype identification. The identified proteins participate in crucial biological processes, such as translation initiation, cell growth, autophagy, facilitating iron transport and metabolism, selection and maturation of developing T-cells.

Conclusion: The obtained results indicate that protein expression is an important factor modulating the behavior of lung cancer cells depending on the subtype. Potential changes in expression and, consequently, in regulating cellular processes provide a chance to achieve a therapeutic goal in the treatment of lung cancer and better understand its background. Further studies are needed to better characterize the proteomic profiles of NSCLCs. Moreover, the acquired proteomic data should be integrated with genomic and transcriptomic analyses to identify the broader portrait of NSCLC.

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Session V: Basic Research and Medical Biotechnology

[V-1] STYRYLQUINAZOLINONE-BASED MOLECULE FOR GLIOBLASTOMA: MULTIMECHANISTIC EFFECTS PROVEN ON 2D AND 3D MODELS

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The cancer treatment landscape is predominantly evolving around domains like immunotherapy, precision-based approaches, targeted interventions, and multifaced novel drugs. Within these key areas, there is a growing demand for innovative macromolecules and minor molecular entities. However, aggressive cancers, notably glioblastoma, continue to challenge researchers with a dearth of definitive treatment solutions. Our teams longstanding commitment is anchored in uncovering therapeutic agents derived from the quinazoline and quinazolinone structures. A standout among these is IS11, a styrylquinazolinone-centered molecule with a broad range of therapeutic potentials. Our cytotoxic evaluations on 3D cell cultures underscore IS11s potent inhibitory actions on multiple glioblastoma strains, with IC50 values spanning from 7.61 M in T98G cells to 22.38 M in LN-18 cells. In contrast, 2D culture analyses spotlighted IS11s discerning nature, remaining inert against non-malignant astrocyte cells (IC50 25 M). Delving deeper into its modus operandi, IS11 was observed to significantly hinder the G2/M phase cell cycle progression in U-251 cells and exhibited enhanced tubulin polymerization than the renowned Paclitaxel. Furthermore, IS11 disrupts metabolic balance and oxidative equilibrium within glioblastoma cells. Our data accentuate its prowess in amplifying ROS concentrations, leading to a cascading effect on cellular antioxidant mechanisms through GSH depletion and ATP reduction, culminating in oxidative stress-mediated cell termination via apoptosis or autophagy. This cascade might be indirectly triggered by IS11s interference with tyrosine kinases, disrupting integral cellular pathways involved in growth and cellular dynamics. Recent assessments on 3D glioblastoma spheroids further attest to IS11s unyielding anticancer attributes. Further assessment has shown some problems with crossing of Blood-Brain-Barrier on TransWell@ models of IS11. To overcome these problems, we developed liposomes and cross-linked albumin nanocarriers. In essence, IS11s quinazoline makeup emerges as a beacon of hope in both 2D and 3D glioblastoma scenarios, underlined by its intricate action spectrum. However, a more exhaustive exploration is paramount to decode its full range and potential drawbacks, motivating our upcoming *in vivo* experiments.

This research was financed by National Science Centre grant 2019/35/B/NZ5/04208 (KM).

[V-2] IMPROVEMENT OF THE PHTHALOCYANINE PHOTODYNAMIC THERAPY WITH POLYMERIC NANOPARTICLES

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As one of the most promising methods of cancer treatment, photodynamic therapy (PDT) is of interest to many researchers around the world. The main element of this strategy consists of a chemical compound capable of absorbing a specific wavelength, which further leads to emission of energy generating cytotoxic effect in cancer cells. Because of the required activation via radiation, PDT causes minor side effects and allows for high precision of treatment. Many groups of compounds exhibit properties necessary in PDT, of which phthalocyanines are considered especially potent due to their high activity. Unfortunately, the main limiting factor of phthalocyanines in treatment is their low water solubility, leading to numerous issues with drug delivery. The solution to this problem proposed by our group was to encapsulate a compound with polymeric nanoparticles, increasing their solubility and allowing for easier permeability through membranes.

Efficiency of PDT with two phthalocyanines used as photosensitizers was examined. Results of therapy with free (Pc4, TT1) and encapsulated (Pc4-NPs, TT1-NPs) molecules were tested on the human breast cancer cell line SK-BR-3. Performed cytotoxicity studies with and without excitation showed an improvement of the therapeutic effect due to the lower IC50 values for compounds closed in nanoparticles. The next stage of research focused on understanding the mechanism of action, with initial hypothesis of increasing the levels of oxidation stress. The annexin V binding assay confirmed the occurrence of apoptosis in treated cells with stronger effect caused by Pc4-NPs and TT1-NPs. Further analysis showed the presence of cell cycle arrest at the G0/G1 phase. Photosensitizers induce cytotoxic effect through generation of singlet oxygen, as confirmed by quantum efficiency measurements. Predicted molecular mechanisms of phthalocyanines were checked with protein expression analysis. Western Blot results showed the decrease of BID protein suggesting the initiation of apoptotic processes and changes in heme oxygenase expression are a result of higher concentration of reactive oxygen species. All of the aforementioned results were stronger and more distinct in cells treated with encapsulated photosensitizers.

Data obtained during the experiments prove that used polymer nanoparticles are an effective method of drug delivery for PDT and can help overcome difficulties encountered during treatment.

The work is the result of research projects No. 2020/39/O/NZ5/02342 (AMW, PZ) and 2019/35/B/NZ5/04208 (KM) funded by the National Science Center.

[V-3] NOVEL 2,3,4-TRIHYDROXYBENZALDEHYDE DERIVATIVES AS A HK2 INHIBITORS IN ANTICANCER THERAPY

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Hexokinase 2 (HK2), as a glycolytic enzyme, converting glucose into glucose-6-phosphate, plays a key role in glucose metabolism in cancer cells [1]. It is well known that HK2 is overexpressed in cancer cells, especially in rapidly growing tumors, making it an interesting molecular target for new cancer treatments [2]. According to known HK2 inhibitors reported so far in literature, no efficient inhibitor was identified as yet [3]. In our study, we designed and synthesized 2,3,4-trihydroxybenzaldehyde derivatives which belong to the class of hydrazine and hydrazone derivatives (figure 1) as potent HK2 inhibitors.

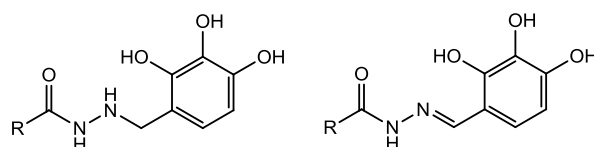


Figure 1.

The spectroscopic methods (NMR and MS) confirm the structure of final compounds. Commercially available test for evaluation of inhibitory activity against HK2 was used. In the primary investigation we identified a group of compounds with potent inhibition activity against HK2.

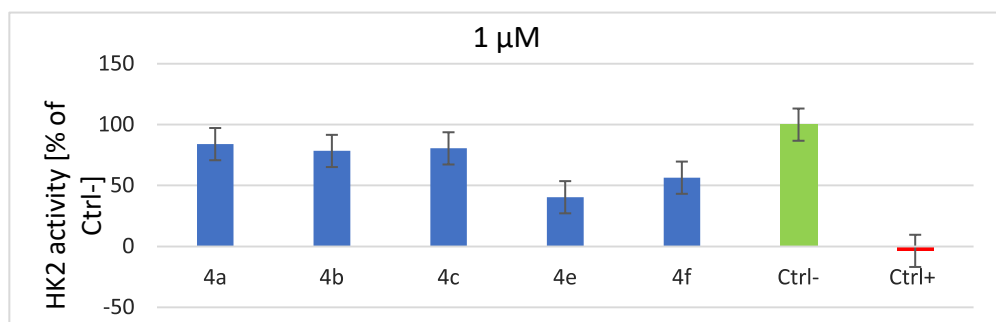


Figure 2.

Some of obtained compounds reduce activity of HK2 to 40-60% at concentration of 1 μ M (figure 2). This research is a good start point to the design of more effective hexokinase 2 inhibitors that can be used in combined anticancer therapy.

Keywords: hexokinase 2, enzyme inhibition, drug discovery.

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[V-4] IRON CIRCULATORY PATHWAY UNDER THE STRESS CONDITION

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Introduction: Ferroptosis is a type of programmed cell death resulting from the excessive accumulation of iron in the body. Iron is involved in chemical reactions that lead to damage to cell membranes and loss of structural integrity. The excessive concentration of iron contributes generation of an excess of reactive oxygen species (ROS) in cells, which can damage DNA and protein molecules. Iron is involved in ferroptosis, a type of programmed cell death resulting from the excessive accumulation of oxidation products. Iron is important in redox reactions, influences various oxidation states of cofactors, anti-oxidative etc. [1,2,3,4].

Materials and methods: Keratinocyte wild type (WT), HaCaT and GPX4-knockout (KO) obtained by CRISPR/Cas9 method were used for the study. Cells were incubated for 24h with a ferroptosis inducer - erastin, in two doses of 5 and 10 M. The relative marker genes expression level, TFRC, DMT1, IRP2 and SLC40A1 was evaluated by RT-qPCR, while ROS was measured through flow cytometry. The iron level was determined by the iron-specific Prussian blue.

Results: The level of expression of the TFRC gene in WT cells decreases with the dose of erastin. GPX4 KO cells no expression was observed at a lower dose whereas an increase was observed at a higher dose what resulted in the accumulation of iron in cell. The expression of the SLC40A1 gene in HaCaT wild type cells increases after erastin addition, while in GPX4 KO it does not change. DMT1 gene expression in WT cells decreases at both doses while in GPX4 KO decreases slightly at both doses. CRISPR modification resulted in changes in marker gene expression level, but no impacted cells viability. IRP2 is responsible for genes SLC40A1 down regulation and TFRC upregulation. An increased of IRP2 expression was observed in wild-type cells, which may be a feedback-like response to the low concentration of the iron ions in cells. In the KO mutant cells, IRP2 expression was silenced, due to excess iron accumulation.

Financing: The work was carried out thanks to the co-financing of Project-Based Education - PBL (Excellence Initiative - Research University program), in accordance with the Regulations No. 54/2020 and 55/2020 of the Rector of the Silesian University of Technology of March 13, 2020, and grants No. 02/040/BK_23/1035 (M.S.); 02/040/BKM23/1041 (M.A.O.) and Silesian University of Technology in Gliwice, Poland, grant No 02/040/BKM23/1042 (A.D.).

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[V-5] COMBINATION OF MESALAZINE WITH LACTOFERRIN - A PROMISING RESULT OF DRUG REPOSITIONING?

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Introduction: Despite medical advances, colorectal cancer is still one of the most common causes of malignant tumor incidence, with a high mortality rate. Mesalazine and lactoferrin are compounds selected based on the idea of repositioning drugs that are currently recommended for colorectal cancer chemoprevention. They exhibit antitumor effects against colon cancer cells, but through different mechanisms. We hypothesize that if each compound administered individually inhibits tumor progression, then simultaneous administration of mesalazine with lactoferrin will result in lower tumor cell viability.

Methods: Cytotoxicity evaluation was carried out using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay and Apo-Tox Glo-Triplex Assay. The tests were conducted on 3 colorectal cancer cell lines: HCT-116, DLD-1, HT-29 and on normal intestinal epithelial cells CCD 841 CoN. Mesalazine was used at concentrations of 10, 20, 30, 40, 50 mM, while lactoferrin was used at concentrations of 100, 200, 400, 800 g/ml.

Results: The combination of mesalazine and lactoferrin resulted in a lower viability of tumor cells than either substance given separately. In addition, a significant increase in the viability of normal intestinal epithelial cells was observed after the combination of compounds.

Conclusion: The combination of mesalazine and lactoferrin holds promise in the battle against colon cancer. Following the administration of this compound combination, cancer cell viability decreased, while that of normal intestinal epithelial cells increased. This suggests

a potential future application of the tested compound combination in the treatment of patients post-tumor resection and chemotherapy, with the aim of eradicating residual cancer cells and promoting the regeneration of normal intestinal epithelial cells. The preliminary test results are promising; however, further studies are necessary to comprehensively assess the potential of this combination.

[V-6] ANTICANCER DRUG DELIVERY VIA ELECTROACTIVE POLYMER MICROSPHERES FOR GLIOBLASTOMA MULTIFORME TREATMENT

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Introduction: Glioblastoma multiforme is a type of cancer that attacks glial tissue of the brain. Its treatment is challenging due to its location limiting surgical intervention, and the presence of blood-brain barrier limiting the efficiency of chemotherapy. Due to the unreliability of traditional therapies, a drug delivery system has been proposed whereby an anti-cancer drug can be delivered directly to a target site. The system is based on 3,4-ethylenedioxythiophene (PEDOT) microspheres loaded with a model drug, curcumin, which is known from its anticancer, anti-inflammatory, and antioxidant properties. Despite its many advantages, its use in traditional therapy is problematic due to its low bioavailability.

Methods: Polymer microspheres were prepared by polymerizing EDOT-OH on the surface of polystyrene (PS) beads of different sizes. After polymer deposition, PS beads were dissolved with toluene. PEDOT microspheres were characterized by scanning electron microscopy (SEM) and infrared spectroscopy (IR). Curcumin was immobilized inside microspheres and its release into phosphate buffer saline solution was studied by UV-Vis spectroscopy. Spontaneous release and electro-stimulated release modes were compared.

Results: SEM images proved the formation of microspheres of different sizes. The presence of curcumin in the microspheres after the immobilisation process was detected by IR. On the basis of UV-Vis spectra analyses, the concentration of released drug in time was determined. It was noted that the drug is released gradually at low doses, in the case of both spontaneous and electro-stimulated release modes.

Conclusions: Polymer microspheres were found to have the ability to immobilize and release curcumin with appropriate kinetics in both spontaneous and electro-stimulated release modes. The next step will be to investigate the biocompatibility of the obtained carriers and to test their performance with other model drugs. Studies indicate that the developed polymeric microspheres show high potential and application value as a novel anti-cancer therapy, particularly in the treatment of glioblastoma multiforme.

Funding: Students received funding as a part of the 10th education financing competition project-oriented - PBL (Excellence Initiative Program Research University) in accordance with Regulation No. 55/2020 of the Rector of the Silesian University of Technology.

[V-7] THE IMPACT OF ELECTROPORATION-INDUCED EXTRACELLULAR VESICLES ON MELANOMA CELL BEHAVIOR

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Introduction: Electroporation (EP) is a well-established technique primarily employed in oncology to modulate the permeability of the cell membrane of cancer cells. The precise adjustment of EP parameters enables the achievement of specific clinical goals, such as enhancing drug uptake or inducing apoptosis of cancer cells. This exposure of cells to electrical stress triggers the release of various transmitters, including extracellular vesicles (EVs). These EVs are a diverse group of small, membrane-bound structures that are released by various types of cells into the extracellular environment. They are crucial in cell-to-cell communication, protecting and transporting bioactive cargo over long distances.

Objectives: Our aim was to investigate how EVs derived from cells previously exposed to an electrical field impact other cells, particularly their proliferation, migration, and invasiveness.

Materials and Methods: The study involved two human melanoma cell lines (A375 and Me45) and immortal keratinocytes (HaCaT). We assessed the effect of EVs on the target cells using viability assay, real-time cell analysis based on impedance and wound healing assay.

Results: The application of electrical pulses (8001600 V/cm; 0.1-ms duration; 1 Hz) led to the increased release of EVs. Isolated EVs added to the culture medium of cancer cells slowed down their growth and reduced their ability to migrate. The effect depended on the electroporation parameter used and the cell line. No similar effect was observed for cells not treated with EVs.

Conclusions: The inhibition of the proliferation and migration of melanoma cells after transferring to their culture medium EVs isolated from electroporated cells was visible in all conducted tests. The experiments have shown a bystander effect where cells exposed to electrical pulses may transmit a signal in the form of EVs to non-electroporated cells reducing their viability.

[V-8] LYSYL OXIDASE PROPEPTIDE (LOX-PP) INHIBITS OVARIAN CANCER CELLS PROLIFERATION AND SENSITIZES CELLS TO CHEMOTHERAPEUTIC AGENTS

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Introduction: Lysyl oxidase (LOX) is an enzyme engaged in the cross-linking of the extracellular matrix proteins. LOX proenzyme is secreted to extracellular space, then proteolytically cleaved resulting in the LOX enzyme and LOX propeptide (LOX-PP). Various studies showed contradictory results, indicating that LOX may act either as an oncogene or a tumor suppressor. More detailed analyses suggest that LOX enzyme may be responsible for oncogenic effects, while LOX-PP may act as tumor suppressor. Our aim was to investigate LOX-PP role in ovarian cancer cells.

Materials and methods: Ovarian cancer cell lines (SKOV3, A2780, ES2) with stable LOX-PP overexpression were established using lentiviral transfer system. Cell lines were validated by semi-quantitative RT-PCR and immunoblotting. Cells proliferation were assessed by MTS assay. In vitro cytotoxicity assay was performed with two major drugs used in ovarian cancer treatment: cisplatin (0,1-20M range) and paclitaxel (1,56 nM - 30M range).

Results: RT-PCR showed that the LOX-PP-containing cells had higher LOX-PP expression compared to control cells with an empty vector. Immunodetection was successful both in cells lysates and in culture medium suggesting that the protein is efficiently secreted. Cell proliferation assay showed lower proliferation rate of A2780_LOX-PP as compared to controls. No differences in proliferation rate was observed among cell lines SKOV3 and ES2. In vitro cytotoxicity assay demonstrated that LOX-PP overexpressing SKOV3 and A2780 cells were more sensitive to both cisplatin and paclitaxel. The A2780_LOX-PP cells showed a 8,5% and 16% higher sensitivity to paclitaxel and cisplatin, respectively, while SKOV3_LOX-PP cells showed 15% and 24% higher sensitivity to those drugs.

Conclusion: LOX-PP seems to exert an anti-cancer function in ovarian cancer, either by decreasing cells proliferation rate and/or by sensitizing of cells to chemotherapeutic agents.

[V-9] THE INFLUENCE OF THE TYPE OF PROTECTING GROUPS IN SELECTED URIDINE GLYCOCONJUGATES ON THEIR CYTOTOXICITY AND SELECTIVITY

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Over the past few decades, the introduction of numerous antibiotics and vaccines significantly reduced mortality from viral and bacterial infections. Consequently, cancer diseases become one of a leading global cause of death. Many therapeutic strategies used to treat cancer often prove insufficiently effective. In addition, the high systemic toxicity of the drugs used limits the possibility of achieving successful results of anticancer therapies. Therefore, it is necessary to find new, effective chemotherapeutics with low systemic toxicity and a high selectivity profile.

Disorders of the glycosylation process generate incorrect exchange of information between cells, which leads to pathogenesis [1]. The enzymes responsible for post-translational glycosylation, are glycosyltransferases (GTs) [2]. For this reason, methods to control the activity of these enzymes are being sought. Selective GTs inhibitors can provide the control of glycosylation associated with cancerogenesis and may lead to the development of new therapeutic agents. The designing of GTs inhibitors is generally based on similarity to their natural substrates: NDP-sugars (donors of a sugar unit) or sugar residue acceptors. In the case of designing sugar donor analogues, one of three structural parts: the carbohydrate part, the diphosphate linkage or the nucleoside moiety may be modified. The analogues containing the modified pyrophosphate moiety should be able to interact with a bivalent metal cation present in an enzyme active site (should have an anionic character or have lone pairs of electrons). An anionic character of such compounds prevents their entry into cells through the phospholipid bilayer. That's why analogues with a linker containing lone electrons pairs which would interact with metal cation are being designed more and more often. It seems advantageous to introduce into the linker structure a heteroaromatic system, e.g. the 1,2,3-triazole ring. Application of the copper-catalyzed azide-alkyne cycloaddition reaction can be used to synthesize this type of compounds. A series of uridine derivatives were synthesized in which a diphosphate bridge was replaced with a linker containing 1,2,3-triazole. In order to check the compounds lipophilicity effect on their biological activity and selectivity, both: unprotected glycoconjugates, as well as glycoconjugates with different types of protecting groups were obtained. The influence of the presence and type of protecting groups on the activity and selectivity of the obtained glycoconjugates was tested on cancer cell lines for which GTs overexpression was confirmed. The results of these assays will be presented.

Acknowledgement: Research studies part-financed by the project of student science clubs Excellence Initiative Program Research University (31/010/SDU20/0006-10).

Keywords: uridine glycoconjugates, anticancer activity, glycosyltransferase inhibitors

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[V-10] NO SPARING EFFECT OF FRACTIONATION WHEN LOW RADIATION DOSES (0,5 GY) ARE COMBINED WITH CHEMOTHERAPEUTICS

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National Research Institute of Oncology in Gliwice, as the first in Poland, has started a phase II clinical trial using low-dose fractionated radiation (LDFR, 0.5 Gy fractions) to potentiate the effect of induction chemotherapy (carboplatin and paclitaxel) in patients with locally advanced squamous cell carcinoma of head and neck (SCCHN). It is generally believed that the effectiveness of such treatment results from the phenomenon of low-dose hyper-radiosensitivity (HRS). However, the incidence of the HRS effect has never been studied in SCCHN patients treated with low-dose fractionated radiation. Therefore, the aim of the study is to determine whether the chemopotentiating effects of LDFR depend on the HRS status and/or on other molecular and clinical factors (e.g. type of cytostatic, HPV status etc.).

Until now, primary normal skin fibroblasts from 25 patients with SCCHN (13 HPV-positive and 12 HPV-negative) enrolled in the clinical trial were studied. To investigate the presence of HRS effect, fibroblasts were irradiated with doses ranging from 0.1 to 4 Gy (6-MV X-ray beam) and cell radiosensitivity was estimated by flow cytometry-based clonogenic survival assay. The effects of LDFR 4 x 0.5 Gy versus a single dose of 2 Gy on carboplatin and paclitaxel were compared using clonogenic, pATM and gH2AX foci assays.

To date, the HRS response (confirmed by the induced-repair model) was demonstrated for normal fibroblasts of two of the 18 SCCHN patients. However, regardless of HRS status, in cells of all 18 patients, potentiating effects of LDFR 4 x 0.5 Gy on carboplatin or paclitaxel were greater (reduction of cell survival) or at least the same as those of a single 2 Gy dose. The fact suggests no sparing effect of fractionation when low radiation doses (0.5 Gy) are combined with chemotherapeutic agents. Therefore, the results demonstrate that chemopotentiating effects of low-dose fractionated radiation in normal cells of patients with head and neck cancer are independent of the HRS status.

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[V-11] MITOREGULIN PROMOTE CELL SURVIVAL AND IS UPREGULATED IN HODGKIN LYMPHOMA CELLS

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Mitoregulin (MTLN) is a 56 amino acid micropeptide localized in the inner mitochondrial membrane. Mitoregulin was first identified as long non-coding RNA, LINC00116. LINC00116 was shown to be upregulated in B-cell lymphoma compared to normal B cells, yet the function of LINC00116/MTLN in B-cell lymphoma remains unknown.

In this study, we focused on the role of MTLN in B-cell lymphoma. We have shown that Mitoregulin is expressed in Hodgkins lymphoma (HL) and diffuse large B-cell lymphoma (DLBCL) and that cells MTLN protein levels were significantly higher in Hodgkin lymphoma (HL) cells compared to normal B-cells. Next, we silenced MTLN with two hairpin RNAs (shRNAs) in lentiviral vectors and used two negative control vectors (NT and SCR). Reduced levels of both LINC00116 and MTLN were confirmed upon both shRNAs compared to negative controls. The vectors contained GFP, thus we examined the number of GFP+ transduced HL cells compared to wild-type (WT) cells. Within 22 days, the percentage of HL cells with inhibited MTLN decreased significantly, whereas the percentage of HL cells transduced with negative control vectors did not change compared to WT. Furthermore, we demonstrated that the decrease in the number of L540 cells with MTLN knockdown was not caused by apoptosis, since no significant changes in the apoptosis rate was observed using flow cytometry with Annexin V-APC staining for L540 HL cells with inhibited MTLN compared to negative controls. Additionally, using the miRNA prediction algorithm DIANA-microT 2023, we showed that MTLN is a potential target of miRNAs from the miR-17 and miR-430 families.

In conclusion, we demonstrated that MTLN expression was higher in HL cells compared to normal B-cells, and MTLN silencing significantly inhibited HL cell growth, but not through induction of apoptosis. In further studies, we aim to demonstrate possible differences in the functions of LINC00116 and MTLN in B-cell lymphoma.

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[V-12] IN-VITRO MODELS REVIEW – NEUROVASCULAR UNIT

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Neurological disorders are the leading cause of disability and the second leading cause of death worldwide [1]. The report from 2019 of the Neurological Alliance estimates that at least 1 in 6 people living with one or more neurological conditions [2]. Therapies against neurological disorders have the lowest approval rates with prolonged approval periods [3]. Given the gruesome data, research on biology and prevention is done extensively in the hope of expanding knowledge on the topic. Years of scientific research ensured development of tools that help in the neurological processes exploratory such as in-vitro models. Here, we focus on the review of the in-vitro models, from transwells through organoids and 3D cultures to microfluidic devices that incorporate cells of a neurovascular unit.

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[V-13] RIANS MODEL AND LOW-DOSE HYPER-RADIOSENSITIVITY PHENOMENON

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The RIANS model (Radiation-Induced ATM NucleoShuttling) describes the activation and transition of the ATM kinase from the cytoplasm to the nucleus after cell exposure to ionizing radiation (IR). In the nucleus, phosphorylated ATM monomers initiate activation of the DNA damage checkpoint, including phosphorylation of the H2AX histone. The RIANS model suggests that delayed ATM transport from the cytoplasm to the nucleus is associated with increased radiosensitivity of cells and may be involved in the phenomenon of low-dose hyper-radiosensitivity (HRS). The occurrence of the HRS effect results in increased cell death after the exposure to low radiation doses (usually below 0.5 Gy) when compared to that predicted by the linear-quadratic model. Moreover, data from the literature suggest that low-dose fractionated radiation (LDFR 4 x 0.5 Gy) potentiates the cytotoxic effects of chemotherapeutic drugs more effectively than a single 2 Gy dose. This study aims to investigate the kinetics of γ H2AX, pATM and MRE11 nuclear foci formation and disappearance and to correlate them with the chemopotentiating effects of LDFR 4 x 0.5 Gy in HRS-positive and HRS-negative head and neck cancer cell (HNCC) lines. The potentiating effects of LDFR 4 x 0.5 Gy versus a single dose of 2 Gy on carboplatin and paclitaxel are compared using clonogenic survival, pATM, γ H2AX, MRE11 foci assays. For combined experiments, the cells were treated with cytostatic drugs 1 hour before irradiation with 6MV X-ray beam. To date, we assessed HRS status (using FACS-based clonogenic survival assay) for six HNCC lines. For the experiments we chose one HRS-positive FaDu cell line (with extremely pronounced HRS effect) and two HRS-negative SCC-25 and SCC-152 cell lines. Preliminary results showed that LDFR 4 x 0.5 Gy enhanced the effects of carboplatin and paclitaxel at the same level as a single dose of 2 Gy in HRS-positive as well as HRS-negative HNCC cells. This fact suggests, that the chemopotentiating effects of LDFR (4 x 0.5 Gy) on carboplatin and paclitaxel are independent of the HRS status. Research is ongoing and the results on the role of RIANS model in the mechanism of the chemopotentiating effects of LDFR will be presented.

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Session VI: Biotechnology

[VI-1] INFLUENCE OF ARTIFICIAL DIETS ON THE BIOLOGICAL ACTIVITY OF THE SECRETION OF LUCILIA SERICATA MAGGOTS

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Introduction: Maggot therapy has been in use for a long time in the successful treatment of wounds that are difficult to heal. Extracting compound promoting the wounds healing has become a promising medical aid in respect to antibiotic crisis and bacterial resistance. *Lucilia sericata* larvae are involved in the wound healing process by secreting peptides and low molecular weight compounds that help to eliminate infecting bacteria and promote tissue regeneration. Furthermore, to enhance the efficacy of maggot therapy and facilitate research, a number of studies have been carried out to develop an artificial diet for growing larvae to improve the medical standards. The use of such a diet allows the larvae to be reared in an environment that is free of zoonotic pathogens and ensures that breeding conditions are reproducible. At our lab, an artificial diet recipe was developed and used for the production of larvae secretion.

Method: Oviposited eggs of *Lucilia sericata* were surface sterilized and incubated with the artificial diet or beef liver (control) at 32 C in the dark. The larvae were growing for 3 days on artificial food or on liver as a control. 2/3rd instar larvae were collected, washed to remove any food or debris, followed by incubation in sterile water in the dark for further washing.

The cleansed larvae were again incubated with fresh and sterile water for 2-3 hours until they were active. The liquid secretion was collected, spun, filter sterilized and then separated into 10 kDa smaller fractions using amicon columns. Antimicrobial assays were performed. The fractions were tested against normalized reference bacteria. Additionally, active fractions were then separated into smaller fractions using Size EXclusion chromatography. Collected samples were sent to NIO-PIB in Gliwice for the testing of their influence in endothelial cells development and growth.

Results: Compared to the control liver diet, fractions from the artificial diet showed biological activity. The fractions tested were active against the reference strain of *Pseudomonas aeruginosa*. The influence of the sample varied from inhibiting pigment production to the formation of zones of inhibition against the lawn growth of *P. aeruginosa* during the agar well diffusion method.

Conclusion: The samples collected were found to be active against the reference strains. The results indicate promising applications for the development of artificial diets as a food source for larvae to obtain biologically active secretions.

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[VI-2] EXPLORING NEURO-OSTEOGENIC NETWORKS: BIOACTIVE BETULIN AND PEG LOADED POLY(VINYL ALCOHOL) NANOFIBERS FOR ENHANCED BONE IMPLANTS IN TISSUE ENGINEERING

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Recent literature findings have highlighted the interconnection of the nervous and skeletal systems, forming a neuro-osteogenic network crucial for proper skeletal development and fracture healing. In this study, poly (vinyl alcohol) (PVA) nanofibers containing biodegradable polyanhydrides based on betulin disuccinate (DBB) and dicarboxylic derivatives of poly (ethylene glycol) (PEG) were synthesized and evaluated for their potential in coating bone implants. The morphology of the nanofibers and surface properties were analyzed using scanning electron microscopy (SEM) and water contact angle evaluations.

The *in vitro* bioactivity of the samples was examined by immersing them in the simulated body fluid (SBF) solution with a pH of 7.40. Formation of the Hydroxyapatite (HA) was confirmed in all the samples. Moreover the cellular adhesion evaluation indicated that this coating improve the cell adhesion. Considering the favorable biological and surface properties of the developed coating, it can be considered a promising candidate for future application, particularly for the modification of metallic scaffolds used in bone tissue engineering.

[VI-3] THE IMPACT OF AERATION METHODS ON THE ENZYME ACTIVITY DURING THE ELIMINATION PROCESS OF ANTICANCER DRUGS BY FUNGI

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White-rot fungi, are increasingly used in biotechnological and environmental applications. Those organisms can degrade a wide variety of recalcitrant compounds, due to secretion substrate non-specific ligninolytic enzymes. Fungi have the potential even to eliminate anticancer drugs that are resistant to wastewater treatment.

One of the critical factors influencing enzymes production by fungi organisms is aeration. Therefore, the aim of the study was to determine the influence of different types of aeration: continuous, periodic, and agitation-induced, on the laccase activity during cytostatic drugs removal by chosen white-rot fungi. The selected organisms were: *Trametes versicolor* (strain CB8) and *Hypholoma fasciculare* (strain CB15). Tests with drugs: bleomycin and vincristine at an initial concentration of 10 mg/L were conducted for 10 and 3 days, respectively. The enzymatic activity was measured by monitoring the oxidation of 2,6-dimethoxyphenol in sodium malonate buffer (pH 4.5) at = 468 nm.

The results showed that in the biodegradation process of cytostatic drugs mediated by *H. fasciculare* (CB15), any type of aeration did not have a significant positive effect on laccase activity. On the other hand, for *T. versicolor* (CB8), agitation at the beginning of the process resulted in good enzyme activity, while continuous and periodic aeration proved to be the most effective for longer period. This pattern was observed for both drugs, indicating that laccase activity, in relation to the specific kind of aeration, was not dependent on the type of added cytostatic. Such experiment results provide important information about influence of culture conditions employed during biodegradation process and inspire further research into the application of fungi and their enzymes in the elimination of pharmaceuticals.

The study was financed by the National Science Centre, Poland, project: Research on white-rot fungi ability to remove cytostatic drugs on an example of: bleomycin and vincristine (UMO-2020/37/N/ST8/01077).

[VI-4] BIODEGRADABLE VASCULAR SCAFFOLD: PLGA/PISEB COMPOSITE FOR REGENERATIVE MEDICINE

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Introduction/Rationale: Advancements in regenerative medicine demand the development of biodegradable vascular scaffolds, overcoming the limitations of non-degradable materials like PTFE and PET. This study explores the potential of a composite material merging poly(L-lactide-co-glycolide) (PLGA) and poly(isosorbide sebacate) (PISEB), aiming to design a highly efficient and biocompatible vascular scaffold.

Methods: Utilizing the electrospinning technique, PLGA/PISEB composite scaffolds were fabricated. Structural integrity, morphology, and mechanical properties of the composite were examined through microscopic and spectroscopic techniques. A 12-week hydrolytic degradation study was used to evaluate the scaffolds biodegradability.

Results: The PLGA/PISEB composite scaffolds exhibited uniform fibers and excellent mechanical strength, making them suitable for withstanding physiological blood flow pressures. The 12-week degradation study demonstrated gradual biodegradation, indicating its potential for in vivo applications.

Conclusions/Novel Aspect: This study presents a promising biodegradable vascular scaffold composed of PLGA and PISEB. The scaffold exhibited structural stability, biodegradability, and supported endothelial cell growth, essential for vascular regeneration. The combination of PLGA and PISEB introduces a novel aspect in the field of regenerative medicine, offering a potential solution for vascular tissue engineering.

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Keywords: vascular scaffold, biodegradable materials, PLGA/PISEB composite, regenerative medicine, endothelial cell growth

[VI-5] THE EFFECT OF ASYMMETRICAL PULSED ELECTRIC FIELDS CONSISTS OF MICRO- AND NANOSECOND-RANGE PULSES AND INTERPHASE DELAYS ON BIPOLAR CANCELLATION PHENOMENON

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Introduction: The application of electrical pulses featuring a negative (–) polarity following positive (+) polarity pulses may potentially induce a phenomenon known as bipolar cancellation (BPC). This physiological response is primarily associated with nanosecond electroporation (nsEP). The currently available scientific literature has not thoroughly investigated the outcomes of bipolar electroporation (BP EP) using protocols that involve varying pulse durations, spanning from nanoseconds to microseconds. Additionally, the impact of time intervals between pulses with reversed polarities should be taken into account.

In this study, we opted to assess how the combination of asymmetric pulses and calcium ions (Ca²⁺) affects the efficacy of the BPC phenomenon. Moreover, the effect of interpulse delays on BPC was also investigated. The primary objective of this investigation was to evaluate the efficiency of non-uniform electroporation procedures in terms of their impact on the survival of human ovarian carcinoma cells. Additionally, we examined the effects of alterations in the time intervals between sequential electrical pulses and the presence of calcium ions (Ca²⁺) on the BPC phenomenon.

Methods: The analysis used the human ovarian clear cancer cell line (OvBH-1) model. Cells were exposed to pulses delivered in bursts but as uni- or bipolar, symmetrical, or asymmetrical sequences with a duration of 500 ns or 50 s and electric field strength equal to 4.0 or 14 kV/cm, respectively. The MTT assay was used for the cells viability examination. Cell membrane permeabilization changes were observed using Yo-Pro-1 uptake, and fluorescent staining of plasma membranes and α -tubulin was also performed.

Results: The application of a second pulse with reverse polarity resulted in a decrease in cellular membrane permeability and a consequential increase in cellular viability. Furthermore, the study demonstrated the influence of temporal intervals during interphases (ranging from 1 to 10 s) on bipolar cancellation (BPC).

Conclusions: The findings observed in the study suggest that it is possible to control the bipolar cancellation (BPC) phenomenon by altering the pulse asymmetry or by introducing a time gap between the positive and negative polarities of the pulse.

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[VI-6] DEVELOPMENT OF AN ANALYTICAL PROCEDURE FOR THE DETERMINATION OF STEROID HORMONES IN WATER

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In the past few years, research has focused on the problem of endocrine-disrupting compounds (ECDs) in the environment. They specifically influence living organisms by disrupting the natural hormonal balance, which results in different diseases and mutations.

Steroid hormones such as progesterone (P4) or estrogens (-oestradiol (E2), ethinylestradiol (EE2)) belong to ECDs. Their presence in the environment, especially in waters, is a result of medicines containing P4 and E2 such as preparations for menopausal disorders or birth-control pills[1][2]. Therefore, many studies focus on developed sensitive and selective methods for determination of ECDs in environmental samples.

This study focused on selecting liquid chromatography with tandem mass spectrometer (LC-MS/MS) conditions for the determination of E2, EE2, and P4. Hormones were detected using electrospray ionization (ESI) in the negative mode for E2 and EE2, or the positive mode for P4. Kinetex F5 column was used as the stationary phase. The mobile phase was composed of acetonitrile and 0.02% ammonia solution in water in a ratio of 70:30. Elution was carried out in an isocratic system and the mobile phase flow rate was 0.5 mL min⁻¹.

Further research will focus on choosing the extraction parameters of selected ECDs from liquid environmental samples. The main goal will be monitoring the spread of P4, E2 and EE2 in the environment.

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[VI-7] MICELLAR DRUG-LOADED SYSTEMS VIA ENCAPSULATION OF P-AMINOSALICYLATE IN AMPHIPHILIC LINEAR COPOLYMER MATRIXES

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Introduction: Polymeric micelles are common nanostructures formed by amphiphilic macromolecules in aqueous environments [1]. Their self-assembling behavior allows for the formation of various types of polymeric micelles, depending on the composition of copolymers, the nature of interchain interactions, and the micellization method. Polymeric micelles have emerged as the controlled release which enable accurate precise targeting of the place in the body where medication should be administered, thereby improving the efficacy of the drug while reducing potential side effects.

Methods: This study has developed the utilization of amphiphilic linear choline-based copolymers as a matrix for drug encapsulation, with the goal of creating micellar drug-loaded systems. First, these copolymers have been synthesized by employing the monomeric ionic liquid, specifically [2-(methacryloyloxy)ethyl]trimethylammonium chloride (ChMACl) [2]. In the next step, these copolymers were employed to encapsulate *p*-aminosalicylate acid (PASA) or *p*-aminosalicylate sodium (PASNa) [3], a drug with demonstrated efficacy against *Mycobacterium tuberculosis*.

Results: The self-assembly capacity of the copolymers was assessed using goniometry, which determined the critical micelle concentration (CMC) within the range of 0.04-0.13 mg/mL. The hydrophilicity level quantifying the surface wettability by the water contact angle (34-53° for the copolymer matrix, 43-49° PASA loaded, and 48-60° PASNa loaded). The drug content in the copolymers was ranged in 43-96% (PASNa) vs. 73-100% (PASA). Furthermore, this study evaluated the micellar performance of drug delivery systems through *in vitro* drug release experiments under physiological conditions (PBS, pH 7.4 at 37°C). In the case of systems with the encapsulated PASA, the drug was released within half an hour in a percentage of 88- 100%. For encapsulated PASNa, an initial burst release was observed within the first hour, followed by a slower release over a period of up to 12 h with a release efficiency of 50-100%.

Conclusions: The strategy of micellar systems have demonstrated a great ability to encapsulate drug in the copolymer containing chloride anions. This highlights the potential of PAS-loaded polymer micelles for drug delivery at a satisfactory level, offering promising prospects for therapeutic effectiveness.

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[VI-8] MICROSYSTEM FOR ENCAPSULATING BIO-SAMPLES BASED ON MICROFLUIDICS

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Introduction: Cell immobilization is a set of techniques designed to limit, in whole or in part, the free movement of cells. There are three methods of immobilization: immobilization without the carrier, immobilization on the surface of the carrier and immobilization within the carrier. The last method is to physically close the cells in the matrix. An example is encapsulation, which involves coating or sealing within a system or material (coating/carrier/shell) another material or mixture of materials (core). The core may be solid, liquid, or gaseous and it represents between 10% to even 90% of the total weight of the capsule. The function of the capsule is to prevent cells from escaping without disrupting the flow of small molecule products and transformation products. Encapsulation technology is used, among others, in the pharmaceutical and printing industries. One can encapsulate oils, oleoresins, enzymes and so on.

Methods: Microfluidic systems are systems that allow control of liquids on a microscale, including mixing them with other liquids and substances in precise proportions. Active systems microfluidic operate using pumps that set the liquid in motion. Design of the proposed system assumes the use of a syringe pump as the main way of moving the medium, which will be transported to the laboratory cuvette in which tiny capsules with bio-samples will form. Then, photos will be taken using a digital camera and sent to be analyzed by a connected computer utilizing computer vision algorithms. Based on this analysis, the appropriate flow and pressure will be selected in real time. A peristaltic pump will be installed behind the cuvette to help maintain the set pressure.

Results: The resulting microsystem is able to reliably create encapsulated and immobilized biosamples in a controlled environment. Moreover, the design allows seamless switching between automated control based on cameras and computer vision, constant and permanent flow and fully manual precision control of the microfluidic system. Additionally, the resulting system can be used to obtain capsules with a very precise geometry - homogeneous spheres, that have high quality and resiliency.

Conclusion: The solution we propose has a number of potential applications in various branches of biotechnology and bioengineering. Thanks to multiple modes of control as well as modularity of parts allows easy ways of production and prototyping encapsulated biosamples.

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[VI-9] ENZYMATIC ATRP: A GREEN CHEMISTRY IN THE SYNTHESIS OF STAR POLYMERS FOR BIOMEDICAL APPLICATIONS

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Star-shaped polymers are a unique class of macromolecules characterized by a singular core, from which arms extend radially. They exhibit distinctive physicochemical properties, which can be finely tuned by altering parameters, such as the number of arms, the length of arms, and the composition of the core and arms. Due to their branched structure, star polymers possess enhanced stability and functionality in comparison to their linear counterparts. Their versatile nature has led to a myriad of applications, including drug delivery systems, nanotechnology, and the creation of advanced materials. Through judicious design, star polymers can be engineered to meet specific requirements, thus broadening the scope of polymer science.

The aim of the research was to obtain star polymers via enzymatically assisted atom transfer radical polymerization (ATRP), also known as breathing ATRP from oligo(ethylene glycol) methyl ether methacrylate (OEOMA500 and OEOMA300) using a (2-hydroxypropyl)-cyclodextrin based initiator. The polymerization reaction was carried out in a PBS buffer solution with a pH of 7.4 in the presence of glucose oxidase, which was responsible for removing oxygen from the reaction mixture. The obtained polymers were characterized by ¹H NMR, SEC/MALLS and AFM. Their cloud point temperature (TCP) was also determined using UV-Vis.

The conversion rates in the polymerization reactions stayed in range between 65-95%, with these values contingent on the ratio of monomer to initiator and the method of initiator introduction. The molecular weight distributions of the obtained polymers remained in the range of 1.23-1.75, indicating a controlled synthesis process. The TCP values of the polymer solutions decreased with increasing polymer concentration and number of DP per arm. The various lengths of arms and cores based on cyclodextrin can be useful for future applications in controlled drug release systems, biomedicine, cosmetics, the food industry, as a polymeric absorbent of metals in wastewater treatment and in agriculture.

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[VI-10] AFFECTING CELL ADHESION BY SURFACE MODIFICATION WITH DIAZONIUM SALTS AND POLYLYSINE

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Introduction: Cell adhesion, i.e. the ability of a cell to adhere to a surface, has become crucial in the design of biomedical implants. Depending on the purpose of the implant, it is necessary to use modifications that either increase or decrease cell adhesion. For example, tissue regeneration scaffolds and neural implants should promote adhesion, while intravenous implants, such as stents, should decrease adhesion to prevent clot formation. One of the common methods to increase surface adhesion is to use adhesion promoters, such as positively charged polylysine that interacts electrostatically with negatively charged cell membrane. However, polylysine-coated surfaces are not durable because of the lack of covalent bonds between the surface and the polymer. For this reason, a technique was developed to permanently attach polylysine to the platinum surface using diazonium salts acting as molecular glues.

Methods: 4-nitrobenzodiazonium salt was grafted to the electrode surface and then nitro group was reduced to an amino group, which was able to interact with the lysine molecule to form an amide bond. Subsequently, polylysine was obtained via electropolymerization. The presence of polylysine was proven by infrared spectroscopy. The adhesion of SH-SY5Y [K1] cells was investigated on the surface modified with diazonium salt and diazonium salt coated with polylysine.

Results: Slightly more efficient cell adhesion was found for surfaces modified with 4-nitrobenzenediazonium salt. The presence of amino groups on the surface led to a significant enhancement of cells adhesion. Further modification with polylysine did not improve cell adhesion, providing a similar number of cells as in the case of surface coated with an amino moiety.

Conclusions: A procedure for surface-initiated growth of polylysine by the electropolymerization of lysine was developed. Although as-formed layers were found to improve cell adhesion when compared with bare electrode surface, the effect was similar as in the case of surface modified with 4-nitrobenzenediazonium salt with subsequent reduction of nitro group to amino moiety.

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[VI-11] POLYANILINE-POLY(VINYL PYRROLIDONE)@GRAPHENE COMPOSITE AS AN EFFICIENT ELECTROCHEMICAL SENSOR FOR CIPROFLOXACIN IN THE HUMAN URINE SAMPLE

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Ciprofloxacin (Cif) is a fluoroquinolone-containing second-generation antibiotic which typically prescribed for patients infected by gram-positive/negative aerobic pathogens. Owing to the medical and food applications target-specific determination of Cif is a major concern.

To resolve this issue, the present work aimed to develop a graphene (G)-polyaniline (PA)-polyvinylpyrrolidone (PVPy) composite electrode material by polymerization method. The prepared GPA and GPA-PVPy composites were investigated by scanning electron microscope and X-ray photoelectron spectroscopy. The GPA and GPA-PVPy composite were coated on a glassy carbon (GC) electrode and investigated by cyclic voltammetry in 0.1 M H₂SO₄. The surface coverage (Γ) of GPA/GC and GPA-PVPy/GC electrodes were measured as $1.8310 \cdot 10^{-9}$ and $1.0010 \cdot 10^{-9}$ mol cm⁻², respectively. The GPA-PVPy/GC revealed excellent oxidation peak current of Cif and UA, also the operating potential shifted to the negative direction as compared with the G/GC, GPA/GC electrodes. The GPA-PVPy/GC electrode demonstrates simultaneous UA and Cif sensor (10–250 mM). The excellent sensitivity and limit of detection for UA and Cif were yielded. The designed electrode displays excellent selectivity of Cif determination even in the presence of a 10-fold excess of UA medium. In addition,

GPA-PVPy/GC electrodes exist well separate xanthine oxidation peak potential in the real human urine sample. Therefore, the modified electrode, GPA-PVPy/GC will be a potential candidate for the simultaneous determination of Cif in the presence of UA in human urine.

[VI-12] COMPARISON OF HOMOGENEOUS AND IMMOBILIZED CULTURE

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Introduction: *Rhodospirillum rubrum* is a Gramme-negative, spiral-shaped, facultative anaerobic bacterium in the Alphaproteobacteria class therefore it is able to grow under aerobic conditions. It thrives in aquatic environments and is known for its capacity to produce molecular hydrogen through photo-hydrogen production.

The process of hydrogen production is divided into three main parts: photosynthesis, hydrogen production involving additional enzymes (nitrogenase) and hydrogen release- in which molecular hydrogen can be used as a source of energy or as a raw material for various industrial purposes.

Research into *Rhodospirillum rubrum*'s hydrogen production is promising for clean energy, but optimizing the process involves challenges such as controlling environmental conditions for higher efficiency and production of biohydrogen.

Methods: The project used a bioreactor equipped with a temperature probe and a pH probe to measure and regulate key parameters. To optimize the process, the bioreactor was also equipped with an external light source for controlled lighting conditions. It is worth noting that data acquisition takes place continuously, which facilitates real-time monitoring and collection of information on reactor parameters which allow to continue improving of the cultivation process and the collection of data necessary to create a precise mathematical model. Such data analysis is a key step towards optimizing hydrogen production in the bioreactor culture.

To optimize medium exchange and assess the utility of bacteria in hydrogen production, we conducted an immobilized culture using a trapping method described in previous presentations. The microspheres were then resuspended in a standard culture medium and placed in a microreactor with a proton membrane, monitoring hydrogen production, temperature, and pH. In particular, the immobilized culture was left unstirred to prevent mechanical disruption of the microspheres.

Results: Both cultures use the same nutrient type and environmental conditions (anaerobic, pH 6-7, 30C, well-illuminated). The primary distinction lies in the vessel size: the first culture employs a one-litre reactor with dispersed cells due to continuous mixing, while the second culture uses a 50-milliliter reactor where cells occupy 70% of the volume without mixing. The first culture yields a rapid but one-time hydrogen production process, which can be addressed by immobilizing bacteria for subsequent or continuous cultures.

Conclusions: Immobilized culture seems to be promising in the production of biohydrogen in a more sustainable way and on a larger scale than floating bacteria.

Funding: Project financed from: PBL project 31/010/SDU20/0006-10.

[VI-13] WHAT CAN NON-LINEAR CHARACTERISTICS OF THE PATCH-CLAMP RECORDINGS TELL US ABOUT THE CONFORMATIONAL DYNAMICS OF THE ESTRADIOL- AND PROGESTERONE-STIMULATED BK CHANNELS IN GLIOBLASTOMA CELLS?

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Sex steroids can significantly affect the biology of the cells, both the ones that possess the appropriate hormone receptors but also the ones that are not typically hormone-dependent, through some more intricate mechanisms. Our previous studies showed that the big-conductance voltage- and Ca²⁺ channels (BK) in glioblastoma U-87 MG cells are inhibited by estradiol (E2) and progesterone (P) in a dose-dependent manner. Moreover, the hormone stimulation of these cells results in a decrease in their viability. In this work, we focus on the effects of E2 and P on the conformational dynamics of the BK channel protein to (at least partly) uncover the underlying mechanism of the hormone-BK interactions. We analyze the single-channel recordings obtained by the patch-clamp method at two different concentrations of hormones (i.e., physiological-like [E2] = 0,0018 g/ml and pharmaceutical [E2] = 1,8 g/ml; [P] = 0,025 g/ml and [P] = 25 g/ml) and the appropriate controls. In our investigations, we utilize the popular methods used in time series analysis, including the cross-correlation function, autocorrelation, Hurst (R/S), and information entropy. These characteristics are then interpreted in terms of the changes in the number of available states of the channel (stable macroconformations) and their mutual connectivity. The obtained results suggest that the sex hormones may directly bind to the channel protein (since it has multiple potentially active ligand-sensing sites) and destabilize some of its conformations.

Acknowledgments: *This study was funded by the Silesian University of Technology under project 31/010/SDU20/0006-10 (in the 10th edition of grants supporting the Project-Based Learning projects).*

[VI-14] PREPARATION AND CHARACTERIZATION OF MODIFIED DENTAL COPOLYMERS UTILIZING NOVEL MONOMERS POSSESSING QUATERNARY AMMONIUM GROUPS

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Aim of research: Preparation and characterization of copolymers based on dental dimethacrylate monomers and novel urethane-dimethacrylate monomers possessing quaternary ammonium groups.

Methodology: Two novel monomers, QA10+TMXDI and QA12+TMXDI were synthesized in three-step process, where final step was addition of 2-(methacryloyloxy)ethyl-2-alkylhydroxyethylmethylammonium bromide, having -C10H21 and -C12H25 N-alkyl substituent, respectively, to 1,3-bis(1-isocyanato-1-methylethyl)benzene (TMXDI).

Copolymers were obtained by photopolymerization of liquid monomer compositions with 0,4 wt. % CQ and 1 wt. % DMAEMA as initiating system. Compositions consisted of 40 wt. % Bis-GMA, 20 wt. % UDMA, 20 wt. % TEGDMA and 20 wt. % QA10+TMXDI or 20 wt. % QA12+TMXDI. Resulting copolymers were named as 20(QA10+TMXDI) and 20(QA12+TMXDI), respectively. The reference copolymer of 40 wt. % Bis-GMA, 40 wt. % UDMA and 20 wt. % TEGDMA - 40(UDMA) was also prepared.

Results: The density of researched copolymers (ρ_p) was similar. The $\rho_p=1.2 \text{ g/cm}^3$. Glass transition temperature (T_g) of 20(QA10+TMXDI) and 20(QA12+TMXDI) were lower than that of 40(UDMA). Respectively, $T_g=53.46, 60.30, 62.97^\circ\text{C}$. Degree of conversion (DC) was the lowest for reference copolymer. Its DC=53.60%, whereas 20(QA10+TMXDI) had DC=57.33% and 20(QA12+TMXDI) DC=69.06%. Polymerization shrinkage (S) was 7.42, 7.04, 7.72% respectively for 40(UDMA); 20(QA10+TMXDI); 20(QA12+TMXDI). The water contact angle (WCA) of the copolymers surface was measured with a goniometer. Surfaces of 40(UDMA) and 20(QA10+TMXDI) were hydrophilic WCA=74.07, 87.03 respectively. 20(QA12+TMXDI) showed a hydrophobic surface with WCA=91.30. Water sorption (WS) and water solubility (SL) were also determined. 40(UDMA) showed their lowest values, WS=5.31 g/mm³ and SL=0.17 g/mm³. 20(QA10+TMXDI) had WS=10.43 g/mm³ and SL=2.18 g/mm³ and 20(QA12+TMXDI) had WS=10.35 g/mm³ and SL=2.46 g/mm³.

Novel copolymers showed satisfying mechanical properties. The reference copolymer had hardness (HB) of 225.9 MPa, modulus (E) 3597.7 MPa and flexural strength (FS) 125.4 MPa. 20(QA10+TMXDI) had HB=234.8 MPa, E=3244.9 MPa and FS=100.6 MPa. 20(QA12+TMXDI) had HB=194.6 MPa, E=3020.6 MPa and FS=90.3 MPa.

Microbiological activity was tested against *S. aureus* and *E. coli*. The number of *S. aureus* at the surface of 40(UDMA) was 6.21 log(CFU/ml) and for *E.coli* 6.41 log(CFU/ml). The minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) were over 50 mg/ml for both bacteria. 20(QA10+TMXDI) showed the number of *S. aureus* and *E. coli* 6.41 log(CFU/ml) and 5.57 log(CFU/ml) respectively, MBC=12.5 mg/ml and MIC=6.25 mg/ml for both bacteria. The number of bacteria at the surface of 20(QA12+TMXDI) was 6.39 log(CFU/ml) for *S. aureus* and 6.21 log(CFU/ml) for *E. coli*, MBC, and MIC for *S. aureus* were over 50 mg/ml, whereas for *E. coli* were 25 mg/ml and 12.5 mg/ml respectively. For all copolymers and both bacteria inhibition zone were not observed.

[VI-15] IONIC GRAFT CONJUGATES FOR DELIVERING PHARMACEUTICAL ANIONS IN TUBERCULOSIS TREATMENTS

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Advancements in drug-delivery methods are crucial due to the complexities of diseases and potential side effects of medications. These methods focus on enhanced therapeutic outcomes and controlled drug release. Polymeric drug delivery systems, owing to their versatility, are crucial for improving drug efficacy and controlled release [1]. This study aimed to design carriers for antituberculosis drug using a biomodified polymerizable ionic liquid (IL) derived from choline methacrylate.

The commercially available IL, [2-(methacryloyloxy)ethyl]trimethylammonium chloride (TMAMA/Cl) was biofunctionalized through an ion exchange of chloride anion with anion of *para*-aminosalicylate (PAS) [2] known as a standard tuberculosis antibiotic. Then the resulting biocompatible IL monomer (TMAMA/PAS) was copolymerized with methyl methacrylate producing ionic graft polymer conjugates [3]. The degree of grafting varied between 18 % and 48 %, controlled by multifunctional macroinitiators. Functionalized monomer and copolymers were analysed using ¹H NMR spectroscopy. The synthesized drug carriers were further examined using *in vitro* tests via dialysis method, including the release of pharmaceutical anions by exchange with phosphate anions in a PBS medium (37°C, pH = 7.4).

The ion exchange efficiency for PAS monomer was almost complete (92 %). Side-chain lengths of copolymers varied from 16 to 89 repeat units. Ionic graft copolymers had 40-73 mol% ionic units, with 35-49 % of conjugated PAS. *In vitro* tests showed a PAS release of 76-100 % (8.4-9.8 g/mL).

The controlled polymerization of TMAMA/PAS produced the well-defined graft polymer conjugates with significant drug contents. The satisfactory quantities of PAS were released from carriers. These PAS-conjugates show promise as drug delivery systems for tuberculosis treatment.

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[VI-16] STRUCTURAL ANALOGUES OF PHENOTHIAZINES AS A NEW GROUP OF SUBSTANCES WITH ANTI-CANCER POTENTIAL THAT ACTIVATE P53-DEPENDENT CELL SIGNALING PATHWAYS

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Phenothiazines are organic, three-ring, heterocyclic chemical compounds that contain nitrogen and sulfur heteroatoms in the central ring. Their aliphatic derivatives have antibacterial, antiviral and antiprotozoal properties. Their biological activity against cancer cells was examined depending on the modification of the aromatic system and the possibility of using them as substances inhibiting proliferation was assessed. Four structural analogues were selected for experiments in which the benzene rings were replaced by azine systems - pyridines (BM1: 10H-1,9-diazaphenothiazine and BM2: 10-propargyl-1,9-diazaphenothiazine) or quinolines (MJ1: 6H-9-fluoroquinobenzothiazine and MJ2: 6-propargyl-9-fluoroquinobenzothiazine). The research was conducted on two cell lines: HCT116 and K562, which express the p53 protein. Additionally, the HCT116 line was selected with a mutated variant of this protein. Cancer cells are characterized by numerous mutations in the TP53 gene. Under pathological conditions, MDM2, MSM4 and MDMX regulators are also produced, which promote cancer progression.

Based on the MTT tests, IC50 values were calculated for the tested chemical compounds. The best IC50 values in all cell lines were achieved by MJ2 (for the K562 line IC50 = 16.37 M, for the HCT116 p53 line with wild-type p53 protein status and with mutated p53 protein, respectively IC50 = 36.37 M and IC50 = 52.82 M). In the clonogenic test, the most promising results were obtained for pyridine derivatives. Compound concentrations equal to IC50 values after 14-day incubation effectively reduced the number of cell fractions surviving HCT116, especially those with mutated p53 protein.

Real-time polymerase chain reaction was also performed. These experiments were aimed at determining the expression level of selected genes in HCT116 cell lines containing two different variants of the p53 protein - the mutant and wild type. These genes were an indicator of the mechanism of action of the tested phenothiazines. The research was carried out for the reference gene RPL41 and the tested genes: AIFM2, BCL2, MDM2, which play an important role as a factor regulating apoptosis in the p53 protein-dependent signaling pathway. For the HCT116 line with wild-type p53 protein status, an increased level of expression of selected genes was observed after incubation with phenothiazines compared to the control. For the HCT116 line with mutated p53 protein, a reduced or silenced expression level was observed compared to the control. The results demonstrate the influence of the p53 protein status on the level of gene expression after the use of phenothiazines.

The anticancer and antiproliferative properties of the tested phenothiazine compounds and their properties modulating p53 protein-dependent signaling pathways were confirmed.

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Session VII: Varia

[VII-1] POSSIBILITIES OF NITROGEN AND PHOSPHORUS RECOVERY BY PRECIPITATION OF STRUVITE AND VIVIANITE

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Currently, there is a shift in the approach to wastewater and waste management. The future of both municipal and industrial wastewater management lies in embracing new biological processes that go beyond the traditional focus on disposal. Nitrogen and phosphorus recovery is now a widely studied emerging trend. The potential for their recovery is primarily linked to wastewater treatment plants. This aligns with the concept of a circular economy and aids in mitigating detrimental environmental consequences, such as eutrophication and the greenhouse effect. A particular advantage of recovery is also the obtaining of valuable products that can be used as a cost effective alternative to industrially produced fertilizers.

The scientific aim of the project is to test several selected methods for recovering phosphorus and nitrogen from wastewater and sewage sludge to reclaim valuable nutrients. On one hand, technologies primarily focused on the recovery of phosphorus in the form of vivianite and struvite using iron and magnesium are being tested. Additionally, the goal is to develop a technology for nitrogen recovery in the form of ammonium nitrate using a two-stage biological-chemical process.

In the first stage of the research, the focus was on testing physicochemical methods for phosphorus recovery from reject water and sewage sludge in the form of struvite and vivianite. Initially, the emphasis was on determining the optimal process conditions, with particular attention to the appropriate pH range and the dosing of magnesium salts (to optimize struvite precipitation) and iron salts (to optimize vivianite precipitation). During the conducted studies, it was also observed that it is crucial to determine whether vivianite precipitation should occur under anaerobic conditions at an elevated temperature, or if maintaining such conditions is unnecessary. Besides the technological aspect, this determination also holds significance for the economic efficiency of the studied processes. Subsequently, there are plans to verify the obtained results on real wastewater and sludge.

In the second stage, the aim is to investigate the possibility of nitrogen recovery in the form of ammonium nitrate, which is the preferred form of nitrogen in fertilizers. This stage of research will require a combination of biological and chemical methods, where, in the first step of an innovative shortened nitrification process, a mixture of NH_4^+ and NO_2^- will be produced. In the second chemical step, under acidic conditions, the conversion of nitrite to nitrate will occur. Given the presence of ammonium ions in the mixture, such a reaction will facilitate the formation of ammonium nitrate (NH_4NO_3).

Our results suggest that recovering nitrogen and phosphorus as vivianite and struvite minerals is feasible, offering a promising avenue for reducing resource consumption costs. Subsequent research will investigate their potential use as fertilizers.

[VII-2] A HELPFUL APPLICATION FOR SCIENTIFIC LITERATURE ANALYSIS

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Introduction: Scientific databases are often the main source for searching scientific literature in various fields. The ever-increasing number of scientific documents makes finding information of interest more time-consuming. Metadata provided in database queries allows for accurate information retrieval. In this work, we present a tool that allows searching scientific publications in the IEEE Xplore database based on input metadata and additional analysis of obtained records.

Methods: The proposed application allows to perform complex search queries for all scientific documents in the IEEE Xplore using the API of the available database. The established records are serialized to a file and contain information such as title, unique identifier, link to the document in the database, number of citations, list of authors, keywords, abstract, type of publication, name of the journal/conference and link to the GitHub repository (if provided).

Results: An example analysis of the results obtained through the proposed application was prepared for the following query: deep learning techniques in histopathology imaging. We received 444 records containing all the words we defined in the metadata. From that, 365 records were conference papers, while 79 were published in scientific journals. Ninety-two papers were published in the year 2023, from which only 4 papers provided a GitHub repository address.

Conclusions: The prepared tool can support the process of literature review performed during research work. Based on the results obtained, quantitative analysis can be carried out for the selected query. The application can be used in any field, for example, medical imaging, text detection, or face recognition. Currently, searching is limited to the results found in the IEEE Xplore database, but it is planned to extend the functionality.

Code is available here: <https://github.com/ZAEDPolSI/PyXplore>.

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[VII-3] CAN ESTROGEN CONCENTRATIONS IN ENVIRONMENTAL WATERS AFFECT THE VIABILITY OF HUMAN CELLS?

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Micropollutants found in environmental waters are often biologically active substances, which despite their low concentrations (on the order of ng/l), can adversely affect both the aquatic ecosystem and human health. The widespread production and use of pharmaceutical products results in an increase in the concentration of endocrinically active substances in the aquatic environment. Although even very low concentrations of hormone compounds can lead to endocrine disorders and the development of diseases (including diseases of civilisation), there is currently no obligation to monitor the level of concentrations of hormones and their derivatives in environmental waters. Additionally, the literature on the effects of hormones and their derivatives dissolved in environmental waters on the functioning of human cells at the molecular level is very scarce. Therefore, the present study focused on the effect of environmental waters (water samples were collected from reservoirs located in the Silesian Voivodeship between April and June 2023) on the viability of human cells (HCT116) determined by the CCK-8 assay.

To test whether the substance/substances present in the environmental water samples affect the viability of HCT116 cells in proportion to the concentration of the environmental water samples, medium solutions containing successive dilutions of the environmental water were concentrated and then prepared. The results confirmed that the viability of HCT116 cells, especially after 24 h of incubation, is inversely proportional to the concentration of the environmental water samples.

On the basis of studies in the literature, it was concluded that both natural and synthetic hormones can be present in environmental waters. Therefore, the effects of estradiol and progesterone on HCT116 cell viability were investigated. The results indicate that both standard hormone solutions and substances in environmental waters affect the viability of HCT116 cells. The response of HCT116 cells to pure hormones is dependent on hormone concentration and incubation time with hormones. The nature of the changes in viability of HCT116 cells treated with the environmental waters tested suggests that the compounds responsible for the observed change in HCT116 cell viability may be estrogens.

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[VII-4] PRO-HEALTH ACTIVITIES OF SPENT HOPS (HUMULUS LUPULUS L.) EXTRACT AGAINST CROHN'S DISEASE

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Introduction: Crohns disease (CD), one of two major subtypes of inflammatory bowel diseases, is chronic, relapsing disorder which affects the gastrointestinal tract, including small intestine, in both children and adults. The inflammatory response and oxidative stress, as well as associated with them the production of various mediators, e.g. free radicals or matrix metalloproteinases (MMP), are responsible for progression of the disease and occurrence of complications, such intestinal fistula or stenosis. Currently available therapeutic options are not fully effective, and their use results in side effects, high cost and little improvement in quality of life. The spent hops, prepared by the hops (*Humulus lupulus* L.) extraction, are the source of polyphenols with high biological activity, including anti-inflammatory and anti-oxidative. The aim of the present study was to obtain spent hops extract (SHE), characterize its polyphenolic composition and anti-oxidant activity, as well as to determine the impact of the extract on the expression and activity of type IV collagenases, matrix mettaloproteinase-2 (MMP-2) and -9 (MMP-9).

Methods: The SHE was extracted by solid liquid extraction with subsequent green method extraction using ultrasounds. Total polyphenol content was determine using Folin-Cioclteu method. Anti-oxidant capacity of SHE was carried out using FRAP method. The epithelial cell line from small intestine (HIEC-6) was cultured at 37C in an atmosphere of 5% CO₂ plus air, and the inflammatory condition was stimulated by tumor necrosis factor alpha (TNF-). The influence of SHE on cell viability was tested using the MTT test. In turn, quantitative real time polymerase chain reaction (Q-PCR) and zymography were performed to evaluate mRNA expression and activity of MMPs, respectively. Budesonide, one of corticosteroids, was used as a positive control.

Results: The total polyphenolic content of SHE was 49.34 mg GAE/g of dry weight of the extract. In turn, FRAP assay indicated high anti-oxidant capacity of the extract. SHE also significantly inhibited MMP-2 and MMP-9 expression and activity in *in vitro* model of Crohns disease.

Conclusions: Our data shows that SHE has anti-oxidant properties, as well as it may inhibit the expression and activity of gelatinases in Crohns disease. The tested extract may be an effective agent for the inhibition of progression of Crohns disease.

[VII-5] THE INFLUENCE OF POLY(METHYL METHACRYLATE) NANOPARTICLES ON SELECTED HUMAN CELL LINES

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Introduction: The aim of the study was to obtain nanoparticles (NPs) from poly(methyl methacrylate) (PMMA) characterized by different topology and examine their potential influence on selected cell lines. The tested polymers had following topology: linear (PMMA-L); star-shaped (PMMA-S) comb-shaped (PMMA-C) and star-comb-shaped (PMMA-SC).

Methods: PMMA NPs were prepared by nanoprecipitation using a KD Scientific LEGATO 180 syringe pump and designed by us 3D printed microfluidic reactor. Determination of mitochondrial activity and oxidative stress of colon cancer cells (HCT-116); normal human dermal fibroblasts (NHDF) and human bronchial epithelial cells (BEAS-2B) treated with polymeric nanoparticles for 24h, 48h and 72h, was performed using the Alamar Blue assay and dihydrofluorescein diamide dye respectively. For both tests 96-well plates were read on a Thermo Scientific Varioskan LUX spectrometer.

Results: PMMA nanoparticles did not affect cell proliferation in the studied range of concentration (100- 1,5625 ug/ml). Results concerning reactive oxygen species (ROS) showed that HCT-116 cell line treated with PMMA nanoparticles for 24h exhibited up to 60% stronger response in comparison to control for PMMA-L, up to 75% stronger for PMMA-S and PMMA-SC, twice as strong for PMMA-C for the highest concentration. However, the same cell line subjected to PMMA nanoparticles for 48 and 72h demonstrated reduced ROS levels. NHDF cell line reacted similarly to all PMMA NPs and ROS levels slightly increased with each day of the test. Whereas BEAS-2B cells reaction was as follows: for PMMA-S ROS level was similar to the control, and for other NPs the ROS levels were increased.

Conclusions: Comparing this study results to literature PMMA NPs effect on different human cell lines is very similar NPs are non-toxic also to A549 and THP-1 [1], IMR-90 [2] and K562 [3]. ROS levels were different to ones found in literature. For IMR-90 ROS levels were lower (only 30% stronger after 24 h for the same concentration of NPS) in comparison to our result [2]. To extend this study more cell lines could be tested, not only human ones because PMMA NPs can be toxic to animal cells like BHK-21 where cell viability was decreased to 68% after 24h of incubation in the highest concentration [4].

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[VII-6] THE ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF ARONIA LEAF EXTRACTS IN PREVENTING COLORECTAL CANCER

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The incidence of colon cancer (CRC) is on the rise globally, largely due to lifestyle factors, particularly in highly developed countries. Therefore, new compounds capable of supporting alternative treatment options for CRC are urgently needed. Aronia leaves, which are rich in polyphenols, appear to be a valuable waste material that can be used for this purpose.

In this study, we evaluated the antioxidant capacity (FRAP assay), total polyphenol content (Folin-Ciocalteu and LC/MS/MS methods), and cytotoxic potential (MTT test) of four aronia leaf extracts. Chokeberry leaves were subjected to extraction with water or 70% ethanol supported by 400W and 600W ultrasonic waves.

The results showed that the ethanol extract incubated at 25oC with an ultrasonic wave power of 400W (referred to as ALE400) had the highest antioxidant potential (316,272,01 mM TE/g), followed by the ethanol extract incubated at the same temperature with an ultrasonic wave power of 600W (297,011,18 mM TE/g), referred to as ALE600. Meanwhile, the water extract, referred to as ALW25, had an antioxidant potential of 245,411,23 mM TE/g when incubated at 25oC with an ultrasonic wave power of 400W, and the next water extract, referred to as ALW50, exhibited an antioxidant ability of 211,331,16 mM TE/g when incubated at 50oC with an ultrasonic wave power of 400W. Additionally, the ALW50 water extract contained the highest amount of total polyphenols at 111.450.5 mg GAE/g, only slightly more than the ethanol extract with the highest antioxidant capacity, ALE400 (106.920.49 mg GAE/g). Two other extracts had values of 98.480.90 mg GAE/g for ALE600 and 84.050.75 mg GAE/g for ALW25.

All tested extracts were found to be more effective in inhibiting the growth of colon cancer SW-480 cells than normal intestinal CCD-841CoN cells. After 48 h of incubation, ethanol extracts revealed the strongest cytotoxic potential, achieving 50% inhibition of SW-480 cell growth (IC₅₀) at a concentration of 374 g/mL of ALE400 and 501 g/mL of ALE600. Water extracts had an IC₅₀ of 1455 g/mL for ALW50 after 48 h and 500 g/mL for ALW25 but only after 72 h of incubation. Moreover, the viability of colon cancer cells was significantly reduced in a concentration- and time-dependent manner. None of the tested extracts showed IC₅₀ values for CCD-841CoN after 24, 48 or even 72 h of incubation.

The obtained results provide essential information that can help support the chemoprevention of CRC, but more efforts are needed to understand their health-promoting

properties. Therefore, we have decided to conduct further research on the ethanol extract (ALE400).

[VII-7] SINGLE AND CO-DELIVERY DRUG SYSTEMS BASED ON PHARMACEUTICALLY MODIFIED GRAFTED PIL-BASED COPOLYMERS

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Drug delivery systems (DDS) offer many application possibilities due to their versatility. Beneficial for the use of polymer carriers is possibility of regulating the release kinetics profiles and physicochemical properties through the polymer structure [1]. One of the main problems in pharmacotherapy is the drug resistance overcoming and therapy improvement. The possibility of the co-delivery systems in a combination therapy that includes various pharmaceuticals with synergistic action, can exclude this issue.

The studies were focused on the well-defined graft copolymers containing choline-based IL units in the side chains [2]. Due to the ionic structure the anion exchange with the use of fusidate (FUS) sodium salt was possible, and the polymer conjugates were received as single drug systems. The amphiphilic properties of polymer matrix allowed for the encapsulation of rifampicin (RIF). Therefore, the micelles were obtained as single and dual drug systems. The potential of these types of DDSs was verified by determination of drug contents and loading efficiencies, *in vitro* drug release in phosphate buffered saline (PBS, pH=7.4, 37°C), and cytotoxicity by MTT assay on BEAS-2B cell line.

The amphiphilic properties were confirmed by the critical micelle concentration (CMC), which exhibited a significant increase after ion exchange (from 0.011-0.020 mg/mL to 0.012-0.025 mg/mL). The drug loading content of RIF reached 40.5-66.9 mol.% in single drug systems, while in dual ones acquired 48.9-66.1 mol.%. *In vitro* drug delivery studies designated that the release of FUS reached 45-81% (3.8-8.2 g/mL) and 31-55% (4.3-5.6 g/mL) from single and dual drug systems, respectively. The release of encapsulated RIF attained 20-37% (3.4-4.0 g/mL) from the single drug systems and 19-31% (3.3-4.0 g/mL) from the dual ones. The basic *in vitro* cytotoxicity studies showed negligible effect of polymer matrices on cell viability at low concentrations on human bronchial epithelial cells (BEAS-2B).

These studies proved that the systems based on choline-PIL grafted copolymers seems to be a good candidate for single and dual-drug delivery systems [3]. The rapid release with a relatively high drug concentration can be beneficial in the antibacterial treatment.

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[VII-8] MODULATION OF ANGIOGENESIS PROCESSES ON BIODEGRADABLE POLYMERIC MATERIALS INTENDED FOR CHRONIC WOUND TREATMENT

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Introduction: Widespread cases of congenital defects, cardiovascular conditions, and traumatic external hemorrhages underscore the urgency of developing treatment for external wounds. In situations where the health of skin and tissues is compromised, the introduction of biodegradable and biocompatible support becomes essential to aid in wound healing. The projects objective is to develop a degradable and biocompatible structure to assist in wound healing, utilizing biocompatible polymers, followed by an extensive analysis of the tissue healing process.

Methodology: Electrospun scaffolds were fabricated using a blend of poly(L-lactide-co-glycolide) (PLGA) and poly(isosorbide sebacate) (PISEB). PLGA was synthesized through a ring-opening polymerization of L-lactide and glycolide, while PISEB was produced via a polyesterification process. In vitro biological analysis employed human umbilical vein endothelial cells (HUVEC). The MTT test determined the cell viability on the fabricated materials. Angiogenesis process scrutiny entailed the examination of HUVEC cell morphology using confocal microscopy and assessment of marker gene expression for angiogenesis (MMP2, MMP9, TIMP1, TIMP2, VEGF), alongside selected inflammation-associated biomarkers (IL6, IL8).

Results: Cell analysis after 7 and 14 days of incubation on the surface of PLGA/PISEB scaffolds revealed a potentially pro-angiogenic expression profile and anti-inflammatory effects of this material.

Financing: The work was carried out thanks to the co-financing of Project-Based Education - PBL (Excellence Initiative - Research University program), in accordance with the Regulations No. 54/2020 and 55/2020 of the Rector of the Silesian University of Technology of March 13, 2020.

[VII-9] SYNTHESIS AND CHARACTERIZATION OF TREHALOSE-CONTAINING NANOCARRIERS FOR POTENTIAL CANCER TREATMENTS

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Introduction: Trehalose is a naturally occurring disaccharide that can stimulate autophagy. Autophagy-based therapy is considered an effective treatment in early cancer development, mainly due to its role for degradation of ferritin-induced cancer ferroptosis (iron-dependent cell death). The use of nanocarriers to transport the autophagy inducer trehalose for cancer treatments offers multiple benefits, including improved targeting and ingestion by cancer cells, stabilization of trehalose against enzyme-caused hydrolysis, decreased doses, and improved efficacy. One of the potential nanocarriers is nanogel, a nano-sized hydrogel particle.

Aims: This research aims to synthesize and characterize nanogels containing releasable and non-releasable trehalose for potential cancer treatments.

Methods: A series of trehalose-releasing and non-releasing nanogels were synthesized *via* a photoinitiated free radical polymerization with a miniemulsion technique. Trehalose monoacrylate and acrylamide were employed as the primary monomers and *N,N*-methylenebisacrylamide as the primary crosslinker to create trehalose-releasing nanogels. On the other hand, trehalose monoacrylate and *N,N*-dimethylacrylamide were used as the primary monomers to create trehalose-non-releasing nanogels as a control with three distinct crosslinkers (ethylene glycol diacrylate, triglycerol diacrylate, and poly(ethylene glycol) diacrylate) being evaluated.

Results: The nanogels were characterized their hydrodynamic diameter (dH) and zeta potential (ζ), trehalose content, and trehalose release profile.

Conclusions: Trehalose-containing nanogels are characterized by appropriate physicochemical properties to continue the studies and further evaluate their potential for cancer treatments.

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[VII-10] RESEARCH ON THE SOLUBILITY OF CASEIN IN VARIOUS SUBSTANCES. METHOD SELECTION FOR PROCESS OPTIMIZATION

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Introduction: Casein is an essential component of milk, existing in the form of micelles. It belongs to phosphoproteins and glycoproteins. Casein is characterized by its ability to form a gel-like structure in the presence of acids, which is crucial in the milk coagulation process during cheese production. Casein has a wide range of applications in the food and pharmaceutical industries, as well as in the production of biomedical materials.

Aim: The aim of this research was to assess the solubility of casein, a protein naturally occurring in mammalian milk, in various chemical compounds to find the best solvent.

Methodology: The research was conducted under laboratory conditions, monitoring the process of dissolving casein from cows milk in samples at different concentrations and conditions.

Results: Casein did not dissolve in water, forming an heterogeneous mixture (additionally, swelling of casein was observed), even under elevated temperatures. The dissolution of casein occurred effectively in an alkaline environment, forming a salt, caseinate. Therefore, the focus of the research was on substances with an alkaline reaction, primarily hydroxides.

Conclusions: These studies suggest that the solubility of casein is closely related to its molecular structure and environmental conditions. Understanding the behavior of casein in various solutions and finding optimal solvents has significant implications for both the food industry and scientific fields related to proteins. This will facilitate further research involving casein.

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[VII-11] A SPACE ODYSSEY - CELL CULTURE VERSION. A PROPOSAL FOR A SYSTEM THAT ALLOWS THE STUDY OF THE IMPACT OF COSMIC RADIATION ON MODEL CELL LINES

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Since the 1950s, after the creation of the first cell line in the world, HeLa, *in vitro* research has been conducted on a massive scale, examining cellular responses to various factors, applied in chemo- and radiotherapy. One of the most frequently investigated physical factors affecting cellular responses is the influence of electromagnetic and particle radiation. Various types of electromagnetic radiation, with different wavelengths, were employed within these studies, ranging from visible light to UV and ionizing radiation. Ionizing radiation, characterized by the highest penetrability, leads to the most significant damage in cells, such as the formation of double-strand DNA breaks. Numerous publications have elucidated cellular responses after exposure to radiation.

Consequently, the question arises: What happens when cells are exposed to cosmic radiation, which is more complex and dangerous than X-ray radiation? Will the cellular responses resemble those to X-ray radiation? Will cell survival be similar? Will there be an increase in the expression of cancer markers in healthy model cell lines? Our study aims to address these questions by developing a system to support the maintenance of cell cultures. This system will enable live cells to be sent to the stratosphere, where they will be exposed to cosmic radiation. We present systems and technological proposals designed to address issues related to maintaining suitable conditions within the mission capsule.

This study was funded by the Silesian University of Technology under the project in the 5th edition of grants supporting Project-Based Learning projects.

[VII-12] TRANSFER OF CF₃-COUMARIN DERIVATIVES WITH POTENTIAL ANTIBACTERIAL AND ANTICANCER ACTIVITY FROM BLOOD TO THE CEREBROSPINAL FLUID IN SHEEP

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The aim of this study was to evaluate the bactericidal activity of eight newly synthesized pharmacophores of coumarin and α -aminodimethylphosphonate moieties against selected strains of gram-negative bacteria *E.coli* K12 ATCC 25404, R2 ATCC 39544, R3 ATCC 11775, and R4 ATCC 39543 containing LPS and gram-positive bacteria *Staphylococcus aureus* strain ATCC 23235, *Acinetobacter baumannii* ATCC 17979, *Pseudomonas aeruginosa* ATCC 15442, *Enterobacter* ATCC 49141, *Porphyromonas gingivalis* ATCC 33277, *Treponema denticola* ATCC 35405 strains. The next goal was to investigate the transfer of these coumarin derivatives across the blood-brain/blood-cerebrospinal barrier (BBB/BCSFB) in sheep. The tested compounds have a trifluoromethyl group (CF₃) prepared in the enzymatically catalyzed Kabachnik-Fields reaction. The structure-activity relationship showed inhibitory activity in selected strains of *S. aureus* ATCC 23235 and gram-negative bacterial strains *E.coli* K12 ATCC 25404, R2 ATCC 39544, R3 ATCC 11775, and R4 ATCC 39543. The results showed that the inhibitory activity of the newly synthesized compounds against selected bacteria strains is strongly related to the type of substituents located in the phenyl ring. MIC and MBC studies have shown that the tested compounds at low concentrations have a strong bactericidal effect, similarly to the antibiotics ciprofloxacin, bleomycin and cloxacycline. The collected data also showed that coumarin-based α -aminophosphonates may be potential candidates as antimicrobial drugs against *S. aureus* ATCC 23235 and *E.coli* pathogens and *Acinetobacter baumannii* ATCC 17979, *Pseudomonas aeruginosa* ATCC 15442, *Enterobacter* ATCC 49141, *Porphyromonas gingivalis* ATCC 33277, *Treponema denticola* ATCC 35405 strains. The transfer of coumarins through the BBB/BCSFB was demonstrated by an increase in the CSF concentration of compounds numbered 1-4 (PA116, PA 111, PA098, PA021, PA098), and another fourth numbered 5-8 (revealed PA096, PA097, PA098, PA095) as measured by absorbance and NMR methods to be the most active against studied pathogenic cells, however, compounds 1-4, showed activity similar to the antimicrobial activity of commonly used antibiotics. The value of this research is related to the possibility of using the selected coumarin derivatives in the treatment of CNS infections in animals and humans caused by antibiotic-resistant bacteria.

[VII-13] COMPARISON OF THE EFFECTIVENESS OF SELECTED MACHINE LEARNING ALGORITHMS IN THE CLASSIFICATION OF PATCH-CLAMP SEQUENCES OF BK CHANNELS ACTIVITY

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Classifying nonstationary and nonlinear biological data can pose a challenge to standard classifiers that rely on assumptions of linear separation and constant parameters. In such cases, we should consider using specialized classification methods that are better able to handle this type of data. Standard classifiers are based on the assumption of linear separation of input data. This means that it is possible to draw a line or hyperplane separating one class from another.

There are many machine learning algorithms designed for time series classification. The most popular of them are K-Nearest Neighbors with Dynamic Time Warping (KNN+DTW), Time Series Forest and Random Interval Spectral Ensemble (RISE) algorithms. For example, as artificial intelligence develops, newer algorithms are discovered that achieve better results. These algorithms are often based on reinforcement methods and are characterized by short training and prediction times, as well as high accuracy. It is therefore worth investigating which algorithm is the best choice in terms of time series classification. An important metric for comparing these algorithms is classification accuracy. However, when selecting the appropriate model for classification of sequences, it is also important to take into account the speed of the algorithm measuring training and prediction times. An important aspect is the prediction in each class corresponding to the time series to evaluate. In the case of non-stationary and nonlinear biological data, it is necessary to use more advanced and flexible classification methods.

The BK (Big Potassium) channels are found in most cell types and regulate a wide range of cellular functions. The task of the implemented classifiers was to recognize whether a given time series came from a cellular or mitochondrial BK channel. The investigation of dynamical diversity of BK channels from the inner mitochondrial and cellular membrane can be extremely useful in the channel-oriented therapy or searching for potential modulators of the BK channel proteins.

[VII-14] THREE-DIMENSIONAL SKIN CANCER MODELS: HOW TO RECREATE THE TUMOR MICROENVIRONMENT *IN VITRO*

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Introduction Skin has a complex structure due to its layered anatomy and multiple functions, which are poorly reflected by the widely used 2D *in vitro* models. Reflecting real cell-cell or cell-extracellular matrix (ECM) interactions in healthy tissue without oversimplification poses a challenge for researchers (1). The problem worsens when it comes to skin cancer models. The most popular model in research is the 2D monoculture, which does not recreate characteristics of tumor microenvironment such as hypoxia or hindered mass-transport properties (2,3). Therefore, the results of, for example, the performed drug study on 2D model may not replicate in the human or animal organism requiring additional *in vivo* tests (4). That is why, the aim of this study is to establish and characterize the 3D skin cancer model for usage in the *in vitro* tests.

Methods In this study, to create cell spheres the optimized method of limiting cell adhesion (LCA) was used (1). Four types of skin cells were used: keratinocytes (HaCaT), melanocytes (NHEM M3), fibroblasts (HFF-1) and mast cells (LUVA). Tumor cell spheres were established using melanoma cell lines such as A375, G361, WM115, WM266-4. At first the potential of sphere formation for each cell line was checked in a monoculture, which was followed by establishing their coculture. The dynamics of cell aggregation and sphere morphology was investigated using the cell culture monitoring system. The implementation of melanoma and immune cells to the 3D sphere model was further tested by applying some modifications to the protocol as the addition of ECM proteins to the medium, coculture with other skin cell types or extended time of cell aggregation.

Results: The LCA method assures the creation of stable, round-shaped and repeatable spheres consisting of adherent skin cells. The majority of investigated cell lines were able to form spheres without additional stimulation. The possibility of different cell type introduction to the sphere model is dependent on the properties of a specific cell line. The best way of encouraging cell aggregation turned out to be the coculture of skin cells.

Conclusion: Our results indicate that before using a new cell line in the sphere formation assay, the protocol should be adjusted.

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Keywords: 3D *in vitro* models, spheres, skin cells, skin cancer.

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[VII-15] BENCHMARK DATASET FOR PROTEIN FUNCTION PREDICTION THAT INTEGRATES VARIOUS PROTEIN INFORMATION

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Protein function prediction is currently, among others, the most important challenge in computational biology. The explosion of protein sequence data leads to a huge amount of data that we are unable to analyse experimentally in wet laboratories. Machine learning methods are able to predict various protein annotations, and thus may decrease the number of research scenarios and, consequently, speed up protein confirmation of protein function and reduce its costs. We already have several methods to predict protein functions, but they need improvements and mainly rely only on their amino acid sequences sometimes with some additional information such as protein-protein interactions. We lack, however, a benchmark dataset that integrates predictors which scientists have already developed to predict different protein motifs and properties based on their understanding. Therefore, in this research we want to create such a benchmark dataset.

Our benchmark dataset aims to allow prediction of both GO annotations and UniProtKB keywords. To increase prediction efficiency, we are integrating multiple data sources and predictors. Further, we use feature selection methods to find and evaluate these that are most relevant to protein functions. We also use classical and deep learning methods to initially predict protein functions as a baseline for new methods.

Unlike most methods and benchmark datasets aimed at predicting protein functions, our dataset uses current scientific knowledge and finds data that can help to improve the performance of machine learning models. This is especially useful for classical machine learning approaches, which are unable to build such deep relationships between data as deep learning methods can.



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