XXIV Gliwice Scientific Meetings

Gliwice, November 20-21, 2020

http://gsn.io.gliwice.pl/

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Association for the Support of Cancer Research

Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice Branch

Silesian University of Technology

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Gliwice Scientific Meetings 2020
virtual conference

Scientific Program

Friday, 20th November 2020

9.00 – 9.15 Opening of the conference and welcome addresses

9.15 – 11.30 Session I "**Proteomics and Metabolomics**"

(session co-organized by the Polish Proteomics Society)

Chairman – Piotr Widłak

Piotr Widłak *(NRIO, Gliwice)*: SEMPRA - Serum metabolome profiling in breast cancer risk assessment: introduction into the Polish-Norwegian project.

Guro Giskeødegård *(NTNU, Trondheim)*: Serum metabolomics for risk assessment and treatment monitoring of breast cancer.

May-Britt Tessem *(NTNU, Trondheim)*: Tissue multi-omics - why and how in Prostate Cancer.

Michał Mikuła *(NRIO, Warszawa):* Gut multi-omics profiling in Crohn's disease.

Anna Wojakowska *(IChB-PAN, Poznań)*: Metabolomic and proteomic approach for predicting response to neoadjuvant radiotherapy of colorectal cancer.

Dariusz Rakus *(Wroclaw University)*: Quantitative proteomics to study brain aging and rejuvenation.

Piotr Suder *(University of Science and Technology, Kraków):* ZIKA NS2B-NS3 protease activity studied by iTRAQ labeling with nanoLC-MS/MS analysis.

Wojciech Cypryk *(CMMS-PAN, Łódź):* Proteomic and bioinformatic investigations of extracellular vesicles secreted during inflammatory response.

11.30 – 11.45 Break

11.45 – 14.45 Session II "**RNA in Regulation Processes**"

Chairman – Joanna Rzeszowska

Eric Miska *(University of Cambridge)*: The short- and long-range RNA-RNA interactome of SARS-COV-2.

Fatima Gebauer *(Centre for Genomic Regulation, Barcelona)*: Context-specific roles of the RNA binding protein UNR/CSDE1 in cancer.

Gunter Meister *(University of Regensburg)*: Characterisation of RNA-modifying enzymes and their roles in cancer progression.

Markus Kretz *(University of Regensburg)*: Mechanisms of long non-coding RNA-mediated control of tissue homeostasis.

Daniele Hasler *(University of Regensburg)*: From miRNAs to RNA modifications: emerging functions of the La-related proteins (LARPs) in RNA biology.

Jan Medenbach *(University of Regensburg)*: Cell stress-mediated regulation of translation induces chemoresistance.

Joanna Rzeszowska-Wolny *(Silesian University of Technology, Gliwice):* Role of miRNA in translation of non-targeted transcripts.

14.45 – 15.00 Break

15.00 – 16.45 Session III "**Bioinformatics and Mathematical Modeling**"

Chairman – Marek Kimmel

Philipp Altrock *(Moffitt Cancer Center, Tampa):* Evolutionary dynamics of public goods games in cell populations

Marek Kimmel *(Rice University, Houston):* Modeling of bladder cancer evolution from field effects.

Joanna Polanska *(Silesian University of Technology, Gliwice):* Gene set enrichment for reproducible science.

Tomasz Lipniacki *(IPPT-PAN, Warszawa):* Innate immune response to viral infection at single-cell and population level.

16.45 – 17.00 Break

17.00 – 19.00 Session IV "**Biotechnology in Medicine**"

Chairman – Marek Łos

Dipanjan Chowdhury *(Harvard Medical School, Boston):* DYNLL1-a new player in DNA end resection with implications in the clinic.

Mohsen Akbari *(University of Victoria, Vancouver):* Localized delivery systems for management of glioblastoma.

Anna M. Czarnecka *(NRIO, Warszawa):* Why immunologist should become interested in sarcoma?

Katarzyna Szołtysek *(Princess Maxima Center, Utrecht):* Whole-genome CRISPR screen identifies mechanism of relapse in patient-derived paediatric ALL sample.

Anna Walaszczyk *(Newcastle University):* Therapeutic potential of senolytics in age related cardiovascular diseases.

Saturday, 21st November 2020

9.30 – 12.30 **Virtual Poster Session**

Short presentations of selected posters (4 minutes allocated to each presentation)

[I-1] **Kamila Duś-Szachniewicz**: A protein interaction map for cell-cell and cell-extracellular matrix adhesion in B-cell non-Hodgkin lymphomas.

[I-3] **Łukasz Boguszewicz**: The increased serum lipids and decreased N-acetyl-glycoproteins are positively correlated with a favorable response to induction chemotherapy in men treated for head and neck cancers.

[I-4] **Marta Pałka**: Lamin-associated proteome after heat shock induction – is lamin the novel element in stress granules aggregates?

[I-7] **Agnieszka Skorupa**: Sensitivity of 1H HR MAS NMR technique in detection of breast cancer cells in post-surgical specimens.

[II-1] **Judyta Górka**: RNase activity of the MCPIP1 protein inhibits tumor progression by regulating Wnt/β-catenin signaling pathways and epithelial- mesenchymal transition in clear cell renal cell carcinoma.

[II-2] **Dorota Hudy**: Pre-miRNAs features may influence the specificity of miRNA binding to AGO.

[II-3] **Marta Podralska**: Identification of irradiation-induced ATM-dependent lncRNAs.

[II-6] **Markus Duechler**: Small extracellular vesicles efficiently transport miRNA mimetics into monocytic cells.

[III-3] **Anna Mrukwa**: The impact of socio-economic state of society on COVID-19 development.

[III-4] **Marcin Pacholczyk**: In silico studies suggest T-cell cross-reactivity between SARS-CoV-2 and less dangerous coronaviruses.

[III-5] **Dagmara Blaszczyk**: LCRAnnotationsDB: Database of annotations for low complexity regions in proteins.

[III-6] **Patrycja Rosa**: Comparison of transcriptomes of neurons, glia and non-specific neuroblasts in Drosophila melanogaster larvae

[III-8] **Agata Wilk**: Extended model of RNA interference.

[IV-1] **Damian Kołat**: WWOX may lose ability to regulate pro-tumoral AP-2γ but still possesses synergism with tumor suppressor AP-2α in high-grade bladder cancer.

[IV-5] **Mariusz Hartman**: Encorafenib alters melanoma cell proximity to the apoptotic threshold – implications for complementary therapeutic strategy using selective BH3 mimetics.

[IV-8] **Aleksandra Krystkowska**: Cytotoxicity of styrylquinazoline derivatives and their application in glioblastoma therapy.

[IV-10] **Dagmara Szeliga**: Murine Breast Cancer: cell migration, tumor growth and metastases in mouse model with imaging using power doppler ultrasound and biochemical analysis.

[IV-13] **Barbara Łasut-Szyszka**: How can p53 inhibit the Wnt signaling pathway?

[IV-16] **Małgorzata Adamiec**: HSPA2 deficiency in keratinocytes may augment cytokine-induced psoriasis-like inflammation.

[IV-19] **Patryk Janus**: Heat Shock Factor 1 (HSF1) as a new tethering factor for ESR1 supporting its action in breast cancer.

[IV-22] **Beata Biesaga**: Lack of CD44 overexpression and application of concurrent chemoradiotherapy with cisplatin independently indicate excellent prognosis in patients with HPV-positive oropharyngeal cancer.

[IV-24] **Justyna Czapla**: Cancer-associated fibroblasts in murine models of breast cancer (4T1) and colon carcinoma (CT26).

[IV-25] **Daniel Fochtman**: In ferroptosis-sensitive A549 cells, oxidative stress results in a specific lipid peroxidation death pathway.

[IV-26] **Justyna Odrobińska**: Micellar carriers and conjugates based on grafted or heterografted copolymers - preliminary in vitro evaluation for cosmetic applications.

[IV-27] **Maria Narożna**: Novel oleanolic acid oximes conjugated with ibuprofen and ketoprofen downregulate the NRF2-ARE signaling pathway in human hepatocellular cancer cells in contrast to normal hepatocytes.

[IV-30] **Anastazja Poczta**: New melphalan analogs as compounds with improved antiproliferative properties.

[IV-36] **Ewelina Pilny**: Intramuscular administration of human adipose derived mesenchymal stromal cells accelerate muscle regeneration in a murine model of hindlimb ischemia.

[IV-37] **Anna Sobiepanek**: New melanoma prognostic markers and therapy monitoring by means of label-free methods.

[IV-39] **Marta Cykowiak**: Phytochemicals and their combinations affect the cell cycle, apoptosis and proliferation of HepG2 cells with different efficiency

[IV-40] **Patrycja Przybyłowicz**: Identification of genome wide discordant methylation patterns of the adjacent CpG sites.

[IV-44] **Patryk Chudy**: Detection of G-quadruplexes and heme oxygenase 1 interactions by proximity ligation assay.

[IV-47] **Marcelina Musiałek**: 3D imaging and characterization of the Rb-like protein activity changes in root meristem cells of Vicia faba subjected to replication stress.

[IV-54] **Agata Hadryś**: Effectiveness of Light-encoding oncolytic myxoma virus-loaded ADSCs to destroy murine pancreatic cancer cells.

[IV-62] **Oliwia Kwapisz**: Pathological changes of the liver caused by a high-fat diet.

12.30 – 12.45 Break

12.45 – 13.00 Presentation of awarded posters and closing remarks

Lecture Abstracts

Session I:

Proteomics and Metabolomics

SEMPRA - SERUM METABOLOME PROFILING IN BREAST CANCER RISK ASSESSMENT: INTRODUCTION INTO THE POLISH-NORWEGIAN PROJECT.

Piotr Widłak

Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice Branch, Poland

The SEMPRA project concerns the possibility to estimate the risk of breast cancer based on a novel combination of molecular features with anthropometric and lifestyle-related features. We hypothesize that disease-related features of metabolism could be detected in serum, reflecting cancer-promoting conditions (e.g. chronic inflammation) and/or the existence of early preclinical/symptomless stages of the disease. We hypothesize that such features (metabolites) can be detected in the serum of individuals who were diagnosed with breast cancer a few years after blood sample collection, even though they were considered healthy at that time. Hence, the general hypothesis driving this project states that the combination of a serum metabolome profile and lifestyle-related risk factors will allow building a joint classification model for stratification of breast cancer risk in a healthy population.

Two complementary analytical metabolomics tools based on mass spectrometry and magnetic resonance spectroscopy will be used, which increases the possibility to detect and identify molecular components associated with breast cancer. Sample and data repository from a large population-based study performed in the Trndelag region of Norway (HUNT2) will be involved, which provides a unique opportunity to work with a sufficiently large cohort of individuals where relevant medical, anthropometric and lifestyle data, as well as a long-term follow-up, are available. Furthermore, a reference group of women with actual (clinical) cancer (breast cancer and other solid cancers) will be recruited to the study.

Specific aims of the project include: (i) to identify serum metabolome signature that discriminates individuals who were diagnosed with breast cancer a few years after donating serum from women who remained free of breast cancer; (ii) to compare the serum metabolome signature of a high risk of breast cancer with serum metabolome features of female patients with actual (clinical) breast cancer and other types of malignancies; (iii) to build a classification model for the stratification of breast cancer risk in the population of healthy women, which will combine features of the serum metabolome and features associated with anthropometric and lifestyle-related risk factors.

The SEMPRA project provides a unique possibility to explore the underlying biological mechanisms in early breast cancer development, and possibly identify molecular targets to hinder cancer progression. The project could bring biomarker candidates to complement and enhance breast cancer screening programs helping to reduce over-diagnosis and subsequent over-treatment related to false-positive diagnoses. Hence, the project could contribute to pre-diagnostic management, early diagnosis, and successful treatment of breast cancer, which are issues with high health and socioeconomic impact in both participating countries.

Project funded in the frame of the Norwegian Financial Mechanism for years 2014-2021, Grant no. 2019/34/H/NZ7/00503.

Serum metabolomics for risk assessment and treatment monitoring of breast cancer

Guro Giskeødegård

Norwegian University of Science and Technology

Although the overall prognosis of breast cancer is good, it is highly dependent on the disease stage and subtype at the time of diagnosis. Todays diagnostic procedures cannot accurately differentiate between lethal and non-lethal disease, thus patients may be over-treated and suffer unnecessary late effects. A minimally invasive tool for accurate breast cancer detection would be highly valuable for cancer diagnostics and screening. Further, patient monitoring by repeated blood sampling could be an excellent tool to assess early treatment response and identify patients that do not benefit from the current treatment and should be switched to another treatment. We are currently performing serum metabolomics of future breast cancer patients, newly diagnosed patients, and patients going through cancer treatment, to characterize the metabolic lifespan of breast cancer. The aim is to detect metabolic profiles related to breast cancer risk, diagnosis, treatment response and prognosis.

TISSUE MULTI-OMICS- WHY AND HOW IN PROSTATE CANCER

May-Britt Tessem[1,2]

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Currently available diagnostic methods cannot efficiently differentiate between the aggressive and slow-growing types of prostate cancer. This leads to over-diagnosis of many clinically in-significant cases which may again cause adverse treatment effects, reduced quality of life and a heavy financial burden on world-wide health care providers. The need for good clinical diagnostic and prognostic biomarkers is therefore urgent to prevent over-diagnosis, but at the same time detect cases of rapid-growing life-threatening prostate cancer. We have currently developed a novel multi-omics protocol where several omics levels are included in the analyses of the same tissue sample. The protocol includes methods such as MALDI-MSI (metabolites, peptides), tissue micro-dissected MS, MR spectroscopy, spatial transcriptomics and conventional RNA sequencing, DNA methylation, histology and targeted immunohistochemistry analyses. Most of these methods are usually analyzed separately within targeted research projects, but our aim is to integrate omics results to obtain novel molecular signatures and to understand the entire pathways related to prostate cancer aggressiveness. We are also using this protocol to study tissue heterogeneity, both within a tumor and between tissue types (tumor, stroma, normal epithelial tissue) by obtaining spatial information from many of these methods. Additionally, we have high-quality fresh frozen prostate tissue biobanks available for this project.

GUT MULTI-OMICS PROFILING IN CROHN’S DISEASE

Filip Ambrożkiewicz[3], Jakub Karczmarski[3], Maria Kulecka[1], Agnieszka Paziewska[1], Magdalena Niemira[2], Natalia Zeber-Lubecka[1], Edyta Zagorowicz[1], Adam Kretowski[2], Jerzy Ostrowski[3], Michał Mikula[3]

[1] Department of Gastroenterology, Hepatology and Clinical Oncology, Centre of Postgraduate Medical Education
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[3] Department of Genetics, Maria Sklodowska-Curie National Research Institute of Oncology

Inflammatory bowel diseases are classic polygenic disorders, with genetic loads that reflect immunopathological processes in response to the intestinal microbiota. Herein we performed the multiomics analysis by combining the large scale surveys of gut bacterial community, stool microRNA (miRNA) and short chain fatty acid (SCFA) signatures to correlate their association with the activity of Crohns disease (CD). DNA, miRNA, and metabolites were extracted from stool samples of 15 CD patients, eight with active disease and seven in remission, and nine healthy individuals. Microbial, miRNA and SCFA profiles were assessed using datasets from 16S rRNA sequencing, Nanostring miRNA and GC-MS targeted analysis, respectively. Pairwise comparisons showed that 9 and 23 taxa differed between controls and CD patients with active and inactive disease, respectively. Six taxa were common to both comparisons, whereas four taxa differed in CD patients. -Diversity was lower in both CD groups than in controls. The levels of 4 miRNAs differed (q-value 0.05; FC 1.5) in CD patients and controls. Of six SCFAs, the levels of two differed significantly (p-value 0.05, FC 1.5) in CD patients and controls, and the levels of four differed in patients with active and inactive CD.

METABOLOMIC AND PROTEOMIC APPROACH FOR PREDICTING RESPONSE TO NEOADJUVANT RADIOTHERAPY OF COLORECTAL CANCER

Anna Wojakowska[1], Urszula Strybel[1], Lukasz Marczak[1], Marcin Zeman[2], Mykola Chekan[2], Ewa Zembala-Nozynska[2], Krzysztof Polanski[3], Monika Pietrowska[2]

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[3] *Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridgeshire, UK*

Introduction: Radiotherapy (RT), used either alone or in combination with other treatment modalities, is common modality in the colorectal cancer (CRC) treatment. The major benefit of preoperative RT in CRC is downsizing the tumor to potentially reduce radicalness of surgery, or in some cases, to complete omission of the surgery. Proteomic and metabolomic study of neoplastic tissue can provide valuable information on the tumors response to RT. The aim of this study was to describe the correlation between specific molecular components of tumour and response to the implemented treatment.

Methods: Tumour tissue were taken during surgery from 30 CRC patients after RT. Tissue specimens were characterized by pathologist depending on the response to the treatment: group A tumour with complete response to RT (0% of cancer cells) - sensitive, and group C poor response to RT (40% of cancer cells) - resistant. Metabolites extracted form tumour tissue were analyzed directly after derivatization by GC-MS. LC-MS/MS-based label-free approach was used for proteomic profiling. Metabolomic and proteomic data were subjected to chemometric and functional analysis.

Results: An untargeted GC-MS-based approach allowed the identification of nearly 170 metabolites, including 51 metabolites which levels significantly discriminated (p-value0.05 and fold change 1.5) two groups of tissue specimens. Differentiating metabolites were upregulated in patients with good response (sensitive) to RT. These small molecules were associated with energy metabolism as well as metabolism of lipids, sugars and amino acids. LC-MS/MS-based label-free approach allowed the identification of 3240 proteins, of which 261 were significantly differentiating (p-value 0.05 and fold change2) between two studied groups. Proteins with the most discriminating power were upregulated in patients with poor response (resistant) to RT and connected mainly with mitochondrial translation. Downregulated proteins (C versus A group) were connected with immune system and inflammatory response. Integration of metabolomic and proteomic data showed common overrepresented pathways and processes connected with response to RT of CRC, including tRNA aminoacylation and disorders in transmembrane transporters.

Conclusions: This study reveal a specific pattern of metabolites and proteins characteristic for colorectal tumour which could be used for predicting response to neoadjuvant radiotherapy for CRC. In further study we plan to validate these signatures in plasma and plasma-derived exosomes.

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

QUANTITATIVE PROTEOMICS TO STUDY BRAIN AGING AND REJUVENATION

Dariusz Rakus[2], Jacek Wiśniewski[1], Agnieszka Gizak[2], Dominika Drulis-Fajdasz[2], Przemysław Duda[2]

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[2] Department of Molecular Physiology and Neurobiology, University of Wrocław, Wrocław, Poland

Introduction: Aging is believed to be the result of alterations of protein expression and accumulation of changes in biomolecules. Although there are numerous reports demonstrating changes in protein expression in brain during aging, only few of them describe global changes at the protein level.

Methods: We used the total protein approach (TPA) method to study age-related changes in various structures of mice brain. Our analysis resulted in the deepest quantitative proteomic description of three brain regions, hippocampus, cortex and cerebellum, in mice aged 1 or 12 months.

Results: In all the brain regions, both in young and middle- aged animals, we quantitatively measured over 5,200 proteins. We found that although the total protein expression in middle- aged brain structures is practically unaffected by aging, there are significant differences between young and middle- aged mice in the expression of metabolic proteins as well as some receptors and signaling cascade proteins proven to be significant for learning and memory formation.

The combination of TPA, immunofluorescence, and qPCR demonstrated that aging is associated with reorganization of hippocampal energy metabolism which is manifested by elevated capacity of aging neurons to oxidize glucose in glycolysis and mitochondria, and decreased ability for fatty acids utilization.

Conclusions: Our analysis demonstrates also that the hippocampus is the most variable structure during natural aging and that the first symptoms of weakening of neuronal plasticity may be observed on protein level in middle- aged animals.

ZIKA NS2B-NS3 PROTEASE ACTIVITY STUDIED BY ITRAQ LABELING WITH NANOLC-MS/MS ANALYSIS

Joanna Ner-Kluza[2], Agnieszka Dabrowska[1], Aleksandra Milewska[1], Krzysztof Pyrć[1], Piotr Suder[2]

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ZIKA virus, belonging to *flaviviridae* family, spreads dynamically from Africa to Asia, Europe, and both Americas, along with its carrier organism: *Aedes sp.* mosquito. Up to date, there is no effective therapy or vaccine against this virus. Infection is especially dangerous for pregnant women as viral activity causes microencephaly in human fetuses. In adults, the typical symptoms of infection are usually not dangerous for survival, like elevated temperature and headache, except the so-called, rarely recognized Guillian-Barre syndrome, leading to acute autoimmunological polyneuropathy caused by demyelination of dendrites and later axons.

To broaden the knowledge about ZIKA nonstructural proteins activity in the cells we tried to find cellular protein targets for viral NS2B-NS3 protease. We used two types of cell cultures: 293T cells and human fibroblasts. NS2B-NS3 protein as well as the whole, active virus, were transfected into the cells. After 24 hours cells were harvested. For cellular targets identification, we used protein N-terminal iTRAQ labeling with HPG-ALD resin interactome fraction enhancement followed by routine trypsin digestion. Alternatively, a specific method of labeling without application of the resin was applied. Final samples were analyzed independently by nanoLC-MALDI-TOF/TOF and LC-Orbitrap-MS. Based on the interpretation of the results we were able to select potential cellular targets of the viral enzyme activity.

PROTEOMIC AND BIOINFORMATIC INVESTIGATIONS OF EXTRACELLULAR VESICLES SECRETED DURING INFLAMMATORY RESPONSE

Wojciech Cypryk[1], Liliana Czernek[1], Tuula A. Nyman[2], Sampsa Matikainen[3]

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[2] *University of Oslo and Rikshospitalet Oslo, Norway*
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Extracellular vesicles (EV) is a collective name of a group of heterogenous vesicular entities secreted by virtually all cells. EV carry biological molecules (proteins, lipids and nucleic acids) as cargo and are considered to play essential roles as transmittes of biological information among cells. In past years we have demonstrated that inflammasome activity correlates with increased secretion of EV in models of macrophage-mediated inflammation triggered by various unrelated immune stimuli. Quantitative and qualitative mass spectrometry profiling of the EV proteome indicated that inflammatory macrophages secrete numerous potentially immunomodulatory proteins in EV. Bioinformatic analyses of the global and EV-limited secretome pointed to several processes that may be involved in regulation of EV secretion and consequent modulation of inflammatory responses, as well as indicated potential signaling pathways that are involved in recruitment of proteins to EV and in the secretion process. Among these, activity of calpains - a family of cytosolic calcium-dependent proteases, were shown to be directly regulating the secretion of EV upon canonical inflammasome activation. Recent data demonstrate that non-canonical, caspase-4-dependent inflammatory response also activates EV secretion in human macrophages.

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Session II:

RNA in Regulation Processes

The short- and long-range RNA-RNA Interactome of SARS-CoV-2

Eric Miska

University of Cambridge, United Kingdom

The Coronaviridae is a family of positive-strand RNA viruses that includes SARS-CoV-2, the etiologic agent of the COVID-19 pandemic. Bearing the largest single-stranded RNA genomes in nature, coronaviruses are critically dependent on long-distance RNA-RNA interactions to regulate the viral transcription and replication pathways. Here we experimentally mapped the in vivo RNA-RNA interactome of the full-length SARS-CoV-2 genome and subgenomic mRNAs. We uncovered a network of RNA-RNA interactions spanning tens of thousands of nucleotides. These interactions reveal that the viral genome and subgenomes adopt alternative topologies inside cells, and engage in different interactions with host RNAs. Notably, we discovered a long-range RNA-RNA interaction - the FSE-arch - that encircles the programmed ribosomal frameshifting element. The FSE-arch is conserved in the related MERS-CoV and is under purifying selection. Our findings illuminate RNA structure based mechanisms governing replication, discontinuous transcription, and translation of coronaviruses, and will aid future efforts to develop antiviral strategies.

CONTEXT-SPECIFIC ROLES OF THE RNA BINDING PROTEIN UNR/CSDE1 IN CANCER

Fátima Gebauer

Centre for Genomic Regulation (CRG) and University Pompeu Fabra (UPF), The Barcelona Institute of Technology, Barcelona, Spain

RNA binding proteins (RBPs) are essential players in RNA metabolism, and are gaining great attention in the oncology field for their potential to regulate essentially every hallmark of tumor development. The molecular mechanisms by which RBPs modulate cancer progression are, however, poorly understood. I will focus on CSDE1, also called UNR, a conserved RBP that regulates translation and mRNA stability. We have previously shown that CSDE1 promotes melanoma metastasis by stimulating the expression of critical EMT markers at the level of translation elongation (1). We have now found a surprising role of CSDE1 as a tumor suppressor. CSDE1 promotes oncogene-induced senescence (OIS) in primary mouse keratinocytes that have been challenged by over-expression of H-RasV12. Depletion of CSDE1 leads to cell immortalization, transformation and tumor formation. Using irCLIP-Seq, RNA-Seq and polysome profiling we have uncovered two independent molecular branches by which CSDE1 contributes to OIS. Our results show context-specific functions of CSDE1 in cancer, and highlight the importance of gaining mechanistic understanding for the appropriate design of specific cancer therapies.

(1) Wurth et al., Cancer Cell 30:1-14 (2016).

Characterization of RNA-modifying enzymes and their roles in cancer progression

Gunter Meister

University of Regensburg, Germany

Mitochondria contain a specific translation machinery for the synthesis of respiratory chain components that are encoded on the mitochondrial genome. Mitochondrial tRNAs (mt-tRNAs) are also generated from the mitochondrial genome and, similar to their cytoplasmic counterparts, are processed from precursors and are subsequently modified at various positions. Here, we characterize the RNA methyltransferase Mettl8, which contains a mitochondrial targeting sequence and indeed localizes to mitochondria. Furthermore, using AlkAniline-seq we find that Mettl8 facilitates m^3C methylation at position 32 of mt-$tRNA^{Ser2}$ and mt-$tRNA^{Thr}$. Mettl8 knock out cells suffer from an impaired respiratory chain activity and increase glycolysis and thus lactate production. In specific types of pancreatic cancer, Mettl8 is up regulated and this increase correlates with patient survival. Indeed, Mettl8 up regulation results in an enhanced respiratory chain activity, which is beneficial for tumor growth. This phenomenon can be recapitulated when Mettl8 is over expressed in HEK293 cells suggesting that Mettl8 represents a potential regulatory sensor for respiratory chain activity by tuning translation via m^3C mt-tRNA modification.

MECHANISMS OF LONG NON-CODING RNA-MEDIATED CONTROL OF TISSUE HOMEOSTASIS

Graf Johannes[1], Ziegler Christian[1], Schabenberger Fabian[1], Morgenstern Eva[1], Merkl Rainer[2], Hombach Sonja[1], Kretz Markus[1]

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Long non-coding RNAs (lncRNAs) have emerged as key regulators of gene expression in many different cellular pathways and have been implicated in numerous diseases, including several types of cancer. Using human organotypic epidermis as a model system, we are analyzing how lncRNAs interact with their protein- and RNA- binding partners as well as chromatin and thus help regulating the intricate balance between stem cells undergoing continual regeneration and highly differentiated cells forming the mature tissue environment. We are currently investigating how lncRNAs affect these cellular interactions in normal tissue and during skin cancer progression.

FROM MIRNAS TO RNA MODIFICATIONS: EMERGING FUNCTIONS OF THE LA-RELATED PROTEINS (LARPS) IN RNA BIOLOGY

Daniele Hasler[1], Rajyalakshmi Meduri[2], Maciej Bąk[3], Gerhard Lehmann[1], Leonhard Heizinger[4], Xin Wang[5], Zhi-Tong Li[5], François M. Sement[6], Astrid Bruckmann[1], Anne-Catherine Dock-Bregeon[6], Rainer Merkl[4], Reinhard Kalb[7], Eva Grauer[7], Erdmute Kunstmann[7], Mihaela Zavolan[3], Mo-Fang Liu[5], Utz Fischer[2], Gunter Meister[1]

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The eukaryotic La-related protein (LARP) family comprises members with various functions in RNA metabolism. LARPs typically exploit a combination of a winged helix domain, termed La-motif (LaM), and an adjacent RNA recognition motif (RRM1) to contact their RNA substrates, whereby the LaM and the RRM1 are collectively referred to as La module. Target interactions are nevertheless not restricted to this characteristic structure and additional RNA binding domains are found within LARPs. The name-giving member of the LARP family is the Lupus autoantigen La protein, which binds virtually all primary RNA polymerase III (Pol III) transcripts immediately upon transcription termination, for example pre-tRNAs. Our previous work has revealed that La functions as gatekeeper ensuring correct tRNA maturation and protects pre-tRNAs that might form alternative secondary structures from being processed by the microRNA (miRNA) biogenesis machinery into miRNA-like fragments. However, one specific isoleucine pre-tRNA can partially escape from La bind and is able to produce both a functional tRNA and a miRNA.

Among the other LARPs, La shares most similarities with LARP7 in terms of structural organization, localization and function. LARP7 forms a stable complex with the 7SK small nuclear RNA (snRNA), which is also transcribed by Pol III. This 7SK ribonucleoprotein (RNP) complex is known to regulate RNA polymerase II transcription by sequestering the positive transcription elongation factor b (P-TEFb). Aberrant expression of LARP7 has been implicated in several cancers and mutations in the LARP7 gene have been linked to the Alazami syndrome, a severe developmental disorder characterized by primordial dwarfism and intellectual disability. Here, we report a so far unknown role of this protein in RNA modification. We show that LARP7 physically connects the spliceosomal U6 snRNA with a distinct subset of box C/D small nucleolar RNAs (snoRNAs) guiding the 2-O-methylation of U6. Consistently, these modifications are severely compromised in the absence of LARP7. Although general splicing remains largely unaffected, transcriptome-wide analysis revealed perturbations in alternative splice site usage in LARP7-depleted cells. Importantly, we dentified defects in 2-O-methylation of the U6 snRNA in Alazami syndrome siblings which express a mutated LARP7 variant, suggesting that alterations in splicing fidelity might contribute to the etiology of the Alazami syndrome. Futhermore, our data identify LARP7 as a paradigm for a double-sided RNA binding protein serving as a bridging factor for snoRNA-guided modification of target RNAs.

CELL STRESS-MEDIATED REGULATION OF TRANSLATION INDUCES CHEMORESISTANCE

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Accumulation of unfolded proteins in the endoplasmic reticulum triggers the unfolded protein response (UPR), an adaptive signal transduction pathway aimed at reinstating cellular homeostasis, or, if that fails, at triggering of apoptosis. The UPR plays a key role in a variety of disorders (including diabetes, neurodegenerative disorders, and inflammatory processes) and has been implicated in cancer progression and resistance to chemotherapy. However, the mechanisms and pathways by which the UPR contributes to chemoresistance are only poorly understood.

We have employed a multi-omics approach to monitor changes to gene expression after induction of the UPR with two different compounds, probing in parallel the transcriptome, the proteome, and changes to translation. Stringent filtering reveals the induction of 267 genes (the UPR regulon), many of which have not previously been implicated in stress response pathways. We experimentally demonstrate that UPR-mediated translational re-programming causes an up-regulation of enzymes involved in a pathway that diverts intermediate metabolites from glycolysis to fuel mitochondrial one-carbon metabolism. Concomitantly resistance to treatment with the widely-used folate-based antimetabolite Methotrexate is observed. UPR-driven chemoresistance can be triggered in a variety of different cell types and is driven by a previously unrecognized mechanism that we are currently characterizing in molecular detail to pave the way for novel therapeutic strategies aimed at overcoming resistance .

ROLE OF MIRNA IN TRANSLATION OF NON-TARGETED TRANSCRIPTS

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Regulation of translation is one of the most important steps in achieving the correct levels of proteins in cells, and microRNA-directed regulation plays a very important role in modulation of the translation process. MicroRNAs are short 20-22nt-long RNAs that work in RNA-protein complexes (RISC – RNA-induced silencing complexes) which in human cells include any of four types of Argonaute protein (AGO1-4). The mechanism of translation modulation by microRNAs and RISC is still not completely clear; depending on cell type, conditions, and miRNA-mRNA interactions RISC may have different effects, leading to inhibition of translation initiation and/or formation by mRNA and proteins of high molecular weight complexes visible sometimes as cytoplasmic foci such as P-bodies and stress granules (SG). To understand better the different modes of miRNA action we have studied the expression of luciferase reporter genes transfected into human colon (HCT116) and melanoma (Me45) cancer cells. In different experiments a *Renilla* gene contained sequences targeted by miR-21, miR-24 or Let-7 and was co-transfected with a non-targeted *Firefly* luciferase gene in the same psi-Check-2 plasmid. The presence of sequences targeted by different miRNAs had different effects, specific for miRNA and cell type. In these experiments we noticed that targeting of *Renilla* luciferase transcript by a micro-RNA influenced the expression of the miRNA-nontargeted *Firefly* gene. Both *Renilla* and *Firefly* mRNAs were also found in similar types of complexes which were different in the presence of targeted *Renilla* transcripts, and the presence of an anti-miRNA oligonucleotide inhibitor influenced the expression of both miRNA targeted and non-targeted mRNAs. Here we propose a new model of the translation process which could explain the observed results.

Session III:

Bioinformatics and Mathematical Modeling

EVOLUTIONARY DYNAMICS OF PUBLIC GOODS GAMES IN CELL POPULATIONS

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Evolutionary public good (PG) games capture the essence of production of growth-beneficial factors that are vulnerable to exploitation by free-riders who do not carry the cost of production. PGs play a role in cellular populations, for example in growing bacteria and cancer cells. We study the eco-evolutionary dynamics of a PG in cellular populations that grow in space using evolutionary game theory and adaptive dynamics. In our model, PG-producer cells and free-rider cells exhibit net growth according to birth and death rates that depend on the context of the population. Selection occurs due to public good-driven surplus in the intrinsic growth rates, which comes at a cost to producers. A net growth rate benefit to free-riders leads to the well-known tragedy of the commons in which producers go extinct, but there are also configurations in which this scenario can be avoided, and coexistence is possible. We discuss the nonlinear PG population game, the emergence of producers and free-riders, and the role of space on these scenarios.

MODELING OF BLADDER CANCER EVOLUTION FROM FIELD EFFECTS

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Bladder cancer is a common malignancy and an ideal model of carcinogenesis facilitating studies of field effects. Simple anatomy of the bladder permits mapping of *in situ* preneoplastic conditions across the entire mucosa. Czerniak's laboratory developed an approach referred to as whole-organ histologic and genetic mapping that enables molecular profiling of cancer evolution from occult mucosal field effects to invasive disease. Here we present a mathematical model of cancer mutational profile by a time-continuous Markov branching process. Parameters of cell progression across the transformation process carrying any given mutation on the top of previous mutations were estimated on the basis of variant allele frequencies in different mucosal areas and were related to the parsimony tree. Modeling results and resulting estimates will be discussed.

GENE SET ENRICHMENT FOR REPRODUCIBLE SCIENCE

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Introduction: One of the most common applications of transcriptomic analyses is the identification of differentially expressed genes (DEGs), allowing for the profiling of transcriptomic changes between investigated conditions. However, the number of true discoveries depends on the power of the conducted studies. When the experiment is underpowered due to an insufficient sample size, no DEGs can be observed, even in the event of differences between the conditions. In turn, a large number of DEGs, stemming from an increase in power tied to an overabundance of sampling, can make it challenging to properly interpret findings. As such, it is often better to investigate gene set (GS) co-expression instead of mining the data at a single gene resolution. A number of public GS and molecular interaction repositories are established, including the knowledge-based Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, Molecular Signatures Database or REACTOME as well as a collection of GS related to particular stimulations, e.g. immune system response to vaccination or disease. Enrichment approaches are an established class of algorithms for collapsing a full gene space into a collection of GSs. The first generation of enrichment methods is known as Overrepresentation Analysis (ORA). The main concept of ORA methods is to divide the genes into DEGs and background, as well as identify genes that belong to a particular GS and evaluate the contingency table between these two divisions using an appropriate statistical test. The second generation of enrichment methods are Functional Class Sorting (FCS) techniques. They use information about all of the captured genes in the form of a gene list, captured via a collection of metrics. Moreover, some methods incorporate information about the effect size of the difference in gene expression levels between the groups when transforming the data from gene to pathway level. As in ORA, each GS is analyzed independently. A number of FCS methods have been proposed, including Gene Set Enrichment Analysis (GSEA), Pathway Level Analysis of Gene Expression (PLAGE), Pathway Analysis with Down-weighting of Overlapping Genes (PADOG), LEGO, singscore and hybrid approaches like EGSEA. Finally, Pathway Topology (PT)-based approaches are the third generation of enrichment methods. PT-based approaches are similar to FCS, but they incorporate the known signal cascade to compute gene-level statistics. This group includes methods such as NetGSEA, CePa and hybrid approaches like SPIA, EnrichmentBrowser. While the third generation methods capture the complexity of molecular biology in a convenient way, they still have nontrivial drawbacks. Here, we complement the established evaluation metrics of GS enrichment algorithms with a novel approach to assess the reproducibility of scientific results obtained from GS enrichment tests when applied to related data from different studies.

Data: All algorithms were tested using two publicly available microarray dataset collections. In total, the data comprised 38 benchmark microarray datasets, each with an average of 32 samples; the smallest dataset consisted of 8 samples, and the largest had 153 samples. For each dataset and the corresponding disease, the target pathway (true positive) from KEGGs was matched to measure the efficiency of the evaluated methods (299 different pathways in total).

Results: We compared eight established and one novel algorithm for reproducibility, sensitivity, prioritization, false positive rate and computational time. In addition to the published methodology, we included Coincident Extreme Ranks in Numerical Observations (CERNO), a flexible and fast algorithm based on modified Fisher P-value integration. Using real-world datasets, we show that CERNO is robust to ranking metrics, as well as sample and GS size. CERNO had the highest reproducibility while remaining sensitive, specific and fast. PADOG, CERNO and over-representation analysis performed best overall, while CERNO and GeneSetTest scored high in terms of reproducibility.

Availability and implementation: tmod package implementing the CERNO algorithm is available from CRAN (cran.r-project.org/web/packages/tmod/index.html) and an online implementation can be found at http://tmod.online/. The datasets analyzed in this study are widely available in the KEGGdzPathwaysGEO, KEGGandMetacoreDzPathwaysGEO R package and GEO repository.

INNATE IMMUNE RESPONSE TO VIRAL INFECTION AT SINGLE-CELL AND POPULATION LEVEL

Zbigniew Korwek, Maciej Czerkies, Wiktor Prus, Marek Kochańczyk, Joanna Jaruszewicz –Błońska, Frederic Grabowski, Tomasz Lipniacki

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Cell monolayer infected by respiratory syncytial virus (RSV) exhibits high heterogeneity as visualized by immunostaining and RNA FISH in the figure below, and should be investigated using single cell techniques.

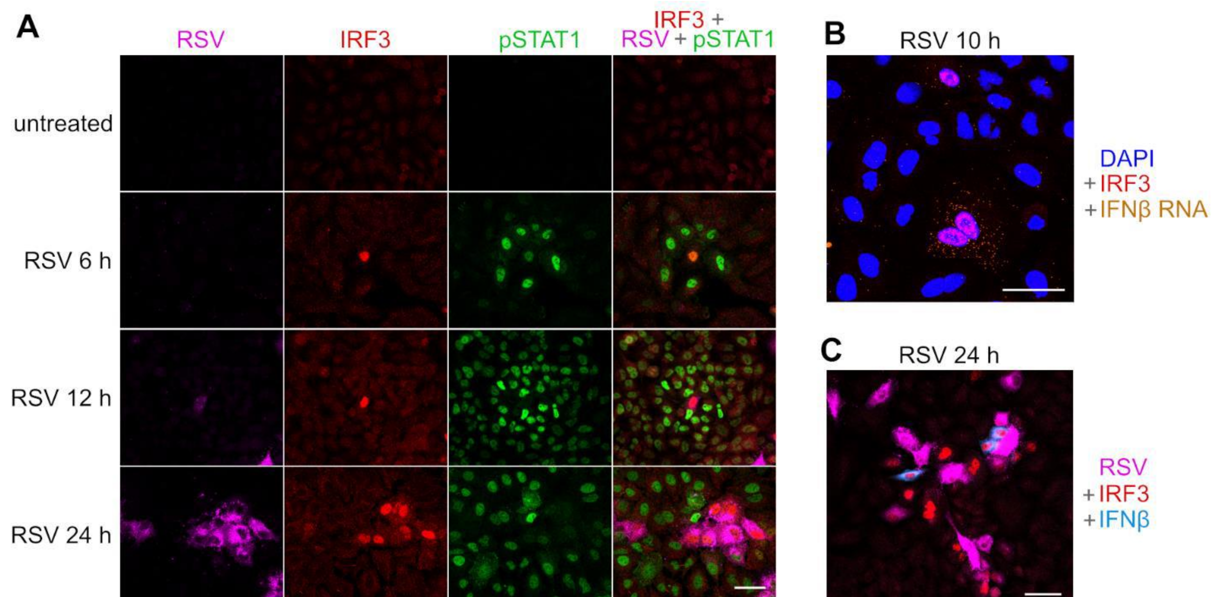

Figure A549 cells infected with RSV at MOI = 0.01 stained for **A**: IRF3, p-STAT1, and RSV protein F; **B**: IRF3, IFNβ (RNA FISH), and Dapi (merge). **C**: RSV protein F, IRF3, and IFNβ (merge); Scale bar: 50 μm.

Innate immune responses are initiated after recognition of viral RNA, which in some cells triggers activation of transcription factors IRF3 (red) and NF-κB, leading to synthesis and secretion of IFNβ (blue). IFNβ works as messenger that tells surrounding cells to prepare for viral infection; it activates STAT1/STAT2 (green) transcription factors regulating hundreds of antiviral genes. In turn RSV, using its nonstructural proteins NS1 and NS2 tries to block innate immune responses. Such mutual inhibition leads to bistability, observed at a single cell level in Panel C: three infected cells translating IFNβ (blue) are not translating viral protein F (magenta), while all cells translating protein F, are not translating IFNβ. We attempt to elucidate mechanisms leading to this bistability combining immunostaining ad live confocal imaging with stochastic modeling of viral propagation on cell monolayer.

Session IV:

Biotechnology in Medicine

DYNLL1-A NEW PLAYER IN DNA END RESECTION WITH IMPLICATIONS IN THE CLINIC

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Double stranded DNA break (DSB)s are repaired by two major mechanistically distinct pathways, homologous recombination (HR) and non-homologous end joining (NHEJ). The relative contribution of the competing DSB repair pathways differ in the different cell types and in different phases of the cell cycle, and this balance is critical for maintaining genomic stability. A decisive factor in the choice between DSB repair pathways is in the competition between DNA end protection (necessary for NHEJ) and DNA end resection (necessary for HR). The balance of HR and NHEJ proteins involved in early steps of the two repair pathways are critical for pathway choice and the cell-cycle phase specific regulation of each pathway. The clinical relevance of DSB repair pathways has been exemplified by use of Poly(ADP-ribose) polymerase inhibitor (PARPi)s to selectively target BRCA1-deficient tumors that have a defect in HR. The synthetic lethal interaction of BRCA1-mutations with PARPi is being exploited therapeutically in diverse clinical contexts and most notably in ovarian cancer where the PARPis olaparib, rucaparib, and niraparib have recently received FDA approval. However, PARPi resistance has already emerged as an important clinical problem for the treatment of BRCA1/2 deficient carcinomas. The bacterial clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system for genome editing has greatly expanded the toolbox for mammalian genetics, enabling the rapid generation of isogenic cell lines with disrupted genes. We utilized this elegant system in the form of a genome-scale lenti-viral CRISPR-Cas9 knockout (GeCKO) library targeting 18,080 genes with 64,751 unique guide sequences to screen a panel of patient-derived BRCA1-deficient ovarian cancer lines for resistance to clinical grade PARPi. The goal was to identify specific genes and signaling pathways that influence the process of resistance in these tumors. We identified DYNLL1 as a negative regulator of DNA end resection in BRCA1-mutant ovarian carcinoma cells. Loss of DYNLL1 allows DNA end resection and restores HR in BRCA1-mutant cells, thereby inducing resistance to platinum drugs and PARP inhibitors. In primary ovarian carcinomas low BRCA1 expression correlates with increased chromosomal aberrations, and the junction sequences of somatic structural variants indicate the loss of HR. Concurrent decrease in DYNLL1 expression in BRCA1 low ovarian cancers ‘rescued’ this phenotype with reduced genomic alterations and increased homology at putative lesions. DYNLL1 limits nucleolytic degradation of DNA ends by interacting with the DNA end resection machinery (MRN complex, BLM helicase and DNA2) in cells. The impact of DYNLL1 on end resection can be re-capitulated *in vitro* and this is dependent on direct interaction with MRE11. In the absence of exogenous stress, depletion of DYNLL1 slows DNA replication fork progression due to ectopic activity of MRE11. As the logical next step we have now asked what is the function of DYNLL1 in ‘normal’ cells and also identified upstream factors that regulate DYNLL1 recruitment to DSBs and MRE11.

LOCALIZED DELIVERY SYSTEMS FOR MANAGEMENT OF GLIOBLASTOMA

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Glioblastoma (GBM) is the most prevalent and devastating adult primary brain tumor. Current treatment strategies for GBM – including surgery, radiotherapy, and chemotherapy with oral administration of temozolomide (TMZ) – still lead to poor survival rates, leaving the management of GBM a major challenge. Therefore, the development of more effective treatment strategies for GBM is an urgent need. Localized delivery of in a sustained manner using biodegradable polymeric substrates is a promising approach as it allows delivering higher doses of the drugs with a reduced adverse effect on other organs. Additionally, since the drug is delivered directly to the tumor site, the blood-brain-barrier and diffusion-limited drug distribution will no longer be a concern, thus increasing therapeutic choices and allowing for greater personalization of treatments. In this talk, I will summarize our efforts in developing localized delivery systems for the management of GBM. In particular, I will describe the use of smart polymeric microparticles and hydrogel-based drug depots as promising platforms for post-surgical delivery of therapeutics to the tumor site

WHY IMMUNOLOGIST SHOULD BECOME INTERESTED IN SARCOMA?

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Research on CD3, CD4 (helper T cells), CD8 (cytotoxic T cells), foxp3 (regulatory T cells), as well as PD-1 and PD-L1 signaling has been extensively developed in sarcomas over the last five years. In 2017 SARC028: A Phase II Study of the Anti-PD1 Antibody Pembrolizumab (MK-3475) in Patients With Advanced Sarcomas results were first presented. In this nonrandomized multi-cohort study, 40 patients with LMS, dedifferentiated LPS, UPS, and synovial sarcoma were treated. At a median follow-up of 17.8 months, 18% achieved an objective response, including one patient with a complete response. Efficacy of anti-PD antibodies was also evaluated in bone sarcomas, including osteosarcoma, Ewing sarcoma, and chondrosarcoma. Chondrosarcoma accounts for up to 20% of primary bone tumors in adults. Among those conventional chondrosarcoma represents around 80-85%, while dedifferentiated chondrosarcoma is diagnosed in up to 10% of patients. In patients with advanced chondrosarcoma, the efficacy of conventional chemotherapy, as well as radiotherapy, is minimal. As a result, novel therapies are urgently needed for this group of patients. Immune infiltrates composed of TILs (CD3+, $CD4^{+}$, CD8+ cells) and TAMs (CD68+, CD163+ cells) were detected in the peripheral area of the tumors as well as in the peri-tumoral area of conventional chondrosarcoma. Immune infiltrates cell composition was shown to correlate with chondrosarcoma grade, invasiveness, and size. In fact, macrophages were shown to be the main population in chondrosarcoma immune infiltrates. In dedifferentiated chondrosarcoma high CD68+/CD8+ ratio is an independent poor prognostic factor of survival. At the same time, high CD68+ levels are associated with the presence of metastases at initial diagnosis. Patients with an increased number of $CD8^{+}$, $CD4^{+,}$ and $CD3^{+}$ cells within the tumor have more prolonged overall survival. In animal models, depletion of T lymphocytes from chondrosarcoma tumors results in a tumor accelerated growth. On the contrary, depletion of $CD163^{+}$ macrophages slows down tumor progression. The interplay between all immune cells in chondrosarcoma is not known. In fact, it is expected that the tumor microenvironment plays a crucial role in sarcoma invasiveness and in the drug-resistance. Because more effective therapies are urgently needed in the sarcoma field, a deep understanding of sarcoma immunobiology is vital to select new therapeutic targets.

Whole-genome CRISPR screen identifies mechanism of relapse in patient-derived paediatric ALL sample

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Acute lymphoblastic leukaemia (ALL) is the most common type of childhood leukaemia with response to glucocorticoids being one of the most important prognostic indicators of treatment outcome. Despite extended survival for the majority of ALL cases, observed steroid resistance and subsequent relapse is currently one of the major clinical challenges in this group of patients.

Here we performed genome-wide CRISPR screens in t(17;19)-positive patient-derived xenotransplant (PDX) material obtained at both diagnosis and relapse stage of the disease. Corresponding PDX samples were generated, lentivirally transduced with the CRISPR knockout pooled Brunello library and, in order to investigate the mechanism of steroid resistance, subjected to glucocorticoid treatment in both *ex vivo* and *in vivo* conditions. For the *in vivo* screen, NSG mice engrafted with CRISPR-modified cells were treated with either dexamethasone or vehicle for 4 weeks. For performed in parallel *ex vivo* arm, PDX cells co-cultured with iPSC-engineered human bone marrow stromal cells, were subjected to a short dexamethasone treatment followed by 5 weeks of recovery.

Screen identified the glucocorticoid receptor gene *NR3C1* as the main driver of chemoresistance-mediated relapse. Notably, we have found that *NR3C1* was homozygously deleted in the corresponding matched relapse sample analyzed in primary cells. Validation experiments, with knockout of *NR3C1* in diagnostic PDX material, confirmed dexamethasone resistance as a main consequence of *NR3C1* loss.

The CRISPR screen in *NR3C1*-deficient relapse identified few pro-survival members of BCL2 protein family and several negative regulators of mTOR pathway as essential mediators of leukemic propagation in this PDX sample. To explore a potential synergism between BCL2 and mTOR pathway inhibition, we analyzed the influence of a BCL2 inhibitor (ABT-199) and several mTORC1 inhibitors (rapamycin and its derivatives) on the cell proliferation/cell death induction in both presentation and relapse samples. Dexamethasone-resistant relapse sample was significantly more sensitive to BCL2i (ABT-199) than its matched diagnostic pair. Moreover, in both samples high synergy was observed at low nanomolar concentrations of BCL2 and mTOR inhibitors.

In conclusion, in the model of currently incurable t(17;19)-positive ALL, we identified novel non-genotoxic drug combinations, effective in chemo-resistant relapsed disease, for further preclinical evaluation. Furthermore, our results showed for the first time that genome-wide CRISPR screens can be successfully performed in patient-derived leukemic material, both *ex vivo* and *in vivo*. Finally, our data indicate that this approach has the potential to predict clinically relevant escape mechanisms as functional response to drug-exerted selection pressure.

THERAPEUTIC POTENTIAL OF SENOLYTICS IN AGE RELATED CARDIOVASCULAR DISEASES

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Cardiovascular disease (CVD) is the leading cause of death in individuals over 60 years old. Aging is associated with an increased prevalence of coronary artery disease and a poorer prognosis following of acute myocardial infarction (MI). We have demonstrated that oxidative stress induces myocardial senescence, which is a major contributor to impaired function. In aged mice, prophylactic treatment with the senolytics drug navitoclax reduces senescence and the expression of SASP associated proteins resulting in attenuated age-related myocardial remodelling and improved survival and functional outcome following MI. In young animals, navitoclax treatment following MI with reperfusion improved left ventricular function, increased myocardial vascularization, and decreased scar size. Proteomics revealed that elimination of senescent cells attenuated biological processes associated with maladaptive remodelling including fibrosis and inflammation. Cytokine array demonstrated navitoclax reduced expression of proinflammatory, profibrotic and anti-angiogenic cytokines, including interferon gamma-induced protein-10, TGF-3, interleukin-11, interleukin-16 and fractalkine. Together our studies provide proof-of-concept evidence that cellular senescence and the proinflammatory SASP contribute to impaired heart function in multiple CVDs by promoting myocardial remodelling. Subsequently, senolytic treatment represents a potential novel therapeutic avenue to improve patient outcome for these CVDs.

Poster sessions

I: Proteomics and Metabolomics

II: RNA in Regulation Processes

III: Bioinformatics and Mathematical Modeling

IV: Basic Research and Medical Biotechnology

Poster Session I:

Proteomics and Metabolomics

[I-1] A PROTEIN INTERACTION MAP FOR CELL-CELL AND CELL-EXTRACELLULAR MATRIX ADHESION IN B-CELL NON-HODGKIN LYMPHOMAS

Kamila Duś-Szachniewicz[1], Grzegorz Rymkiewicz[2], Jacek R Wiśniewski[3]

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INTRODUCTION. Cell-cell and cell-extracellular matrix (ECM) adhesions are central for development and maintenance of tissue structure and function. Abnormalities of the matrix or cell adhesion dynamics are becoming associated with a wide range of malignant disorders, however their role in the diseases with haematopoietic origins remain elusive. Here, we identified 51 cellular adhesion molecules (CAMs) and proteins involved in EMC-receptor pathway in B-NHL lymphomas using the high throughput proteomic tools.

METHODS. Cells were obtained from B-NHL patients by fine needle aspiration biopsies. B-cells were isolated by positive selection using the Miltenyi CD19 Positive Isolation Kit and the purity of samples was confirmed by flow cytometry. Next, cells were lysed and processed in accordance with MED-FASP protocole (Multi-Enzyme Digestion Filter Aided Sample Preparation) and analyzed using a QExactive HF mass spectrometer. The spectra were searched by MaxQuant software and specific protein concentrations were calculated by the Total Protein Approach (TPA). Statistical analysis of proteomic data was performed by Perseus software. Additionally, we used ClueGO, STRING and cytoHubba Cytoscape pluggins to generate pathways based on the Gene Ontology, KEGG, Reactome and WikiPathways databases and the hub proteins in networks were identified.

RESULTS. Almost 50% of proteins involved in ECM-receptor interactions and 33% of CAMs were found to be differentially expressed in lymphoma cells compared to normal B-cells, including: ICAM1, CDH1, CD86 and ITGB1. Using a protein interaction mapping strategy, we identified linkages between the cell-cell adhesion proteins and explored the several novel interactions between differentially aboundant proteins (DAPs) and other well-known cell-cell adhesion proteins.

NOVEL ASPECTS. Bioinformatic analyses identified biological pathways associated with adhesion as being particularly perturbed in lymphoma cells compared to normal B-cells. The dysregulated signaling pathways and their hub proteins are quickly becoming mainstream in the translational cancer research, thus our results provide novel insight into the molecular mechanisms of B-cell non-Hodgkin lymphomas. Moreover, we provided strong evidences that the CAMs and EMC-receptor interaction pathways contribute to the lymphomagenesis.

[I-2] COMPARATIVE ANALYSIS OF PROTEIN-LINKED OLIGOSACCHARIDES IN MELANOCYTES AND MELANOMA CELLS

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Changes in the pattern of protein glycosylation during oncogenic transformation result in the emergence of diagnostic and prognostic markers of disease progression. The aim of this study was to determine and compare the N-glycosylation profile of proteins in melanocytes and melanoma cells. The study was performed using human cell lines HEMa-LP (melanocytes) and WM266-4 (metastatic skin melanoma). N-glycans released from proteins were analyzed in terms of their structure using high performance liquid chromatography based on hydrophilic interactions (HILIC HPLC) with simultaneous sequencing of oligosaccharides using exoglycosidase arrays. It was found that N-glycans released from glycoproteins contained in melanocytes were most frequently represented by oligomannose structures, whereas among complex N-glycans the most common were biantennary structures. Metastatic melanoma cells had fewer high-mannose-type oligosaccharides compared to melanocytes, whereas complex N-glycans were most represented by 1-4-branched triantennary structures. Among the N-glycans released from proteins of the WM266-4 cell line, structures containing 1-3-linked galactose constituted a large proportion. The study shows that changes in protein glycosylation during the transformation into metastatic melanoma are mainly quantitative, so further research is needed to improve the methods of assessing the content of individual N-glycans, as well as precisely determining the profile of N-glycans in different stages of melanoma development, in order to determine markers of cancer progression.

This work was supported by a grant from the National Science Centre, Poland (2016/21/B/NZ3/00348)

[I-3] THE INCREASED SERUM LIPIDS AND DECREASED N-ACETYL-GLYCOPROTEINS ARE POSITIVELY CORRELATED WITH A FAVORABLE RESPONSE TO INDUCTION CHEMOTHERAPY IN MEN TREATED FOR HEAD AND NECK CANCERS

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Background: Induction chemotherapy - iCHT has been investigated as a strategy to shrink or downstage locally advanced head and neck squamous cell carcinomas (LA-HNSCC), increase organ preservation rates, and/or reduce the risk of locoregional and/or distant recurrence. That is why iCHT making a subsequent treatment, as surgery, radiotherapy (RT) or chemoradiotherapy (CHRT), more effective. The aim of this prospective study is to identify the biomarkers associated with iCHT toxicity and favorable response to the treatment.

Methods: The studied group consisted of 53 LA-HNSCC patients (35 men and 18 women, all Caucasians, at the median age of 57 (22-74) years) treated with iCHT. The treatment tolerance was measured by the Common Terminology Criteria for Adverse Events (CTCAE). The response to iCHT was evaluated by clinical, fiberoptic and radiological examinations made before and after iCHT (the TNM Classification of Malignant Tumors was used for classifying the extent of cancer spread). The blood samples were collected before and after iCHT. 1H-NMR serum spectra were acquired with 400 MHz spectrometer and analyzed using multivariate and univariate statistical methods.

Results: We identified different metabolic response to iCHT between males and females. Both sexes showed significant postCHT increase of serum lipids. In addition, males had significantly decreased postCHT N-acetyl-glycoprotein (NAG), glucose and alanine. However, the reduction of glucose and alanine was presumably due to the increased pretreatment levels of these metabolites as compared to women. In males, the magnitude of iCHT induced changes in lipid signals and NAG significantly correlated with regression of the primary tumor. Furthermore, the multivariate model identified two subgroups of patients with weaker metabolic and clinical response. One of this subgroup consisted of patients with significantly lower initial nodal stage. The second subgroup showed no differences in initial clinical and metabolic status.

Conclusions: Presented results show a promising approach to the monitoring of efficacy of induction chemotherapy, as well as bring forth question about the gender specific response to chemotherapy. Identification of a subgroup of patients with weaker metabolic and clinical response without any relevant initial differences makes it justified to conduct further studies taking into account more factors (e.g. genetic profile, broader clinical monitoring).

The work has been funded by National Science Centre, Poland (Grant 2015/17/B/NZ5/01387)

[I-4] LAMIN-ASSOCIATED PROTEOME AFTER HEAT SHOCK INDUCTION – IS LAMIN THE NOVEL ELEMENT IN STRESS GRANULES AGGREGATES?

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Lamins (V- type intermediate filaments) are major karyoskeletal proteins localized in the nuclear periphery called the lamina. They are present in almost all living organisms (except plants or fungi) and can be divided into two categories: A-type (expressed in differentiated cells) or B-type lamins (present in every cell). Lamins fulfill structural and regulatory functions in the cell nucleus. Moreover, they are involved in DNA replication, transcription, and chromatin organization. Chromatin decondensation occurs during the so-called heat shock response (HSR). After stress induction, the heat shock transcription factor (HSF) binds to the promoters of heat shock proteins genes resulting in activation of heat-inducible genes and a global down-regulation of transcription. This project aimed to investigate the hypothesis that lamin together with interacting proteins play a role in chromatin remodeling during HSR and take part in epigenetic regulation of heat shock-induced gene expression.

For this purpose, the *Drosophila melanogaster* model system was chosen as one of the simplest due to the presence of only two single genes encoding for lamins. In this particular work, we focused on investigating changes between lamin-associated proteome before and after heat shock induction. As a research method in this experiment, we used cross-linking co-immunoprecipitation followed by LC-MS/MS analysis. Mass spectrometry data were processed using the Mascot searching engine and then proceeded with bioinformatics tools as FlyEnrichr (qualitative analysis), Diffprot (quantitative analysis), and STRING (interaction network analysis).

We noticed significant differences between studied groups. We observed a considerable increase in the number of proteins identified in MS after heat shock (almost 70 more interactions were identified in comparison to control). Grouped in functions, the proteins differed between conditions. After heat shock induction clustered identifications were involved in nucleic acid binding (both DNA and RNA), transcriptional factor activity, and regulation of transcription. Statistically significant quantitative analysis showed that after heat shock induction the number of interactions increased in a group of proteins involved in RNA interference, gene silencing mediated by RNA, and RNA splicing.

All of those analyses taken together with available literature data suggest that lamin might be part of the stress granules (SGs) complexes formed in response to thermal stress. The described functions of the proteins involved in these structures match those identified in our experiment. Besides many specific markers for SGs were identified in our samples. Summarizing, identification of those proteins may suggest that indeed lamins may play a role in the epigenetic shutdown of transcription after heat shock induction together with other components of a protein complex involved.

[I-5] UROTHELIAL BLADDER CARCINOMA-DERIVED ECTOSOMES PRESENTS ABILITY TO BOOST VIABILITY AND MOTILITY OF RECIPIENT CELLS THROUGH TRANSFER OF CANCER-RELATED MOLECULES

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Introduction/Rationale: Urothelial bladder carcinoma (UBC)-derived exosomes have been shown to directly modulate various processes during carcinogenesis including cancer cell migration, epithelial-mesenchymal transition, angiogenesis, and inhibition of apoptosis. Besides the functional effects, higher concentration exosomes has also been observed in urine of UBC patients. Proteomic strategies that have been applied to profile the very dynamic range of proteins present in UBC-derived exosomes, have identified multiple, potential protein markers of UBC, though their clinical relevance is yet to be determined. The aim of this study was to conduct similar studies on the latter population of EVs ectosomes.

Methods: In the present study cancer-promoting effects exerted by UBC-derived ectosomes on non-invasive cells in terms of cell proliferation (Alamar Blue cell viability assay) and migratory properties (wound healing assay) were assessed. A liquid chromatography coupled with mass spectrometry-based (nanoLCMS) proteomic approach was used to investigate the protein content of ectosomes released in vitro by T-24 UBC and HCV-29 normal ureter epithelial cells.

Results: Changes in cell viability and motility were evaluated after 18 hours of incubation of normal and UBC cells with T-24-derived ectosomes and HCV-29-derived ectosomes as a control. In wound healing assay, T-24-derived ectosomes increased the motility of recipient T-24 and HCV-29 cells. On the contrary, ectosomes derived from normal HCV-29 cells did not induced significant response in either of the recipient cell lines. In Alamar Blue cell viability assay approximately two-fold increase in fluorescence intensity was observed after addition of T-24-derived ectosomes to either T-24 or HCV-29 cells. Also, higher dose of HCV-29-derived ectosomes increased the viability of HCV-29 cell, but did not affect T-24 cells. Protein content of T-24-derived and HCV-29-derived ectosomes was then profiled using shotgun nanoLCMS/MS. A total of 1158 proteins was identified in T-24-derived ectosomes, while HCV-29-derived ectosomes contained a significantly lower number of 259 identified proteins. The bioinformatic analysis of 938 proteins identified uniquely in T-24 ectosomes reflected their cancerous origin. They included proteins involved in cancer cell proliferation, adhesion and migration, angiogenesis as well as immune and drug response.

Conclusions/Novel aspect: The present study was the first to demonstrate that ectosomes derived from cancerous T-24 cells displayed higher ability to boost viability and motility of both recipient cell lines in comparison to ectosomes derived from non-transformed HCV-29 cells. Therefore, tumor-derived ectosomes and their protein cargo should be considered as one of the factors promoting the progression of existing tumors. To some degree they may also affect the function of normal epithelial cells within the tumor microenvironment.

[I-6] NOVEL PROTEIN BINDING PARTNERS OF PHLDA1 IN HUMAN NEUROBLASTOMA CELLS

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Introduction: Neuroblastoma is the most common solid extracranial cancer in children. One of the available treatment method is therapy based on anti-GD2 ganglioside antibodies (ch14.18/CHO). Previously we found that PHLDA1 (pleckstrin homology-like domain family A member 1) is up-regulated in a human neuroblastoma cell line IMR-32, treated with anti-GD2 mouse monoclonal antibody 14G2a. Silencing of PHLDA1 in human neuroblastoma cell lines is leading to up-regulation of Aurora A kinase and down-regulation of apoptotic potential of the cells. We aim to unravel molecular networks of PHLDA1 and identify its protein partners in neuroblastoma cells treated with therapeutic antibodies what will broaden our knowledge on cellular roles of the protein and might point to new therapeutic targets and expand current treatment strategy.

Methods: IMR-32 cells were treated with anti-GD2 antibodies (mouse monoclonal 14G2a or chimeric ch14.18/CHO). To evaluate cell viability we measured level of cellular ATP. PHLDA1 binding partners were identified by immunoprecipitation, using anti-PHLDA1-specific antibody, followed by mass spectrometry and verified by western blot analysis.

Results: Treatment of IMR-32 cells with anti-GD2 antibodies (14G2a and ch14.18/CHO) results in significant down-regulation of cellular ATP level in comparison to control cells. Mass spectrometry analysis revealed five PHLDA1 binding partners in both control cells and treated with ch14.18/CHO antibodies: calcium/calmodulin-dependent protein kinase type II gamma (CaMKIIgamma), calcium/calmodulin-dependent protein kinase type II delta (CaMKIIdelta), DDB1- and CUL4-associated factor 7 (DCAF7), leucocyte receptor cluster member 9 (LENG9) and secretogranin-2 (SCG2). Autism susceptibility gene 2 protein (AUTS2) was identified only in control cells and RNA-binding protein 14 (RBM14) in cells treated with ch14.18/CHO antibody. Interaction of PHLDA1 with DCAF7 was further confirmed by co-immunoprecipitation and immunoblotting.

Conclusion: Our findings expand our knowledge of possible proteins involved with PHLDA1 in investigated by us cytotoxic impact of therapeutic anti-GD2 antibodies on neuroblastoma cells correlated with up-regulation of PHLDA1 protein. We confirmed that DCAF7, a protein known to be involved in protein ubiquitination, neuronal stem cells differentiation and craniofacial development, can bind PHLDA1 in human neuroblastoma cells. We continue our studies aimed at identification of other previously unknown PHLDA1 binding partners.

[I-7] SENSITIVITY OF 1H HR MAS NMR TECHNIQUE IN DETECTION OF BREAST CANCER CELLS IN POST-SURGICAL SPECIMENS

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Introduction/Rationale: 1H HR MAS NMR (proton High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance) spectroscopy allows measurement of metabolic profiles of intact tissues. In this work we investigated the sensitivity of this technique in detection of cancer cells in the post-surgical specimens obtained from breast cancer patients.

Methods: The studied group consisted of 51 breast cancer patients operated in Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice Branch. The tissue samples (from the tumor center, tumor border, macroscopically normal tissue at distances below 1 cm, equal to 1 cm and above 1 cm from the tumor border) were resected for metabolic profiling. 1H HR MAS CPMG NMR spectra of these specimens were acquired on Bruker Avance III 400 MHz spectrometer. After the NMR measurements the samples were frozen for a histopathological evaluation. For this analysis the specimens were fixed in 10% formalin and embedded in paraffin. 5 m tissue sections were stained with haematoxylin and eosin and examined microscopically. Multivariate PCA, PLS-DA and OPLS-DA analyses were used to find the relation between metabolic profiles and the sample content.

Results: PCA analysis showed that the directions of the largest variance in the whole dataset were related to the percentage content of cancer cells, connective tissue and fat tissue. OPLS-DA analysis showed the higher level of creatine, phosphocholine, taurine, glycine, lactate and ascorbate and the lower level of lipids in cancer cells than in fat tissue. Similar results were obtained from the comparison of metabolite levels in cancer cells to the levels in the samples containing mainly connective tissue. Additionally, the latter tissue type was characterized by the high glucose concentration. The multivariate PLS-DA model constructed based on the samples characterized by the cancer cells content 25% and the samples of the non-transformed tissue was succesful in the correct classification of 21/25 specimens characterized by the cancer cell content 25%.

Conclusions/Novel aspect: 1H HR MAS NMR technique allows classification of cancer and non-transformed breast tissue samples and could be helpful in metabolic charaterization of the resection margins.

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Poster Session II:

RNA in Regulation Processes

[II-1] RNase activity of the MCPIP1 protein inhibits tumor progression by regulating Wnt/β-catenin signaling pathways and epithelial- mesenchymal transition in clear cell renal cell carcinoma

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Introduction: The MCPIP1 (Monocyte Chemoattractant Protein-1 Induced Protein) protein is important regulator of inflammation and homeostasis of the immune system. MCPIP1 thanks to its RNase activity specifically digests a number of pre-miRNAs, including miR-21 and miR-135b and regulates gene expression. MCPIP1, acting antagonistically to the DICER enzyme complex, is responsible for the formation of mature miRNA. Although some pre-miRNAs are known to be degraded by MCPIP1, the long-term biological effect remains unknown. Changes in the miRNA levels may be associated with the increased resistance of cancer cells to treatment and the acquisition of migratory and invasive properties leading to metastasis. The aim of our research was to identify miRNAs regulated by MCPIP1 in ccRCC and critical in the EMT process and WNT pathway.

Materials and method: The level and distribution of genes and proteins were studied by real-time PCR, western blot, immunofluorescence staining. Microarray analysis was conducted on samples from patients suffering from ccRCC. The level of miRNAs was conducted by Next Generation Sequencing and real-time PCR. 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 and discussion: Our results showed that in patients with ccRCC, the level of -catenin increases together with a decrease of MCPIP1. Furthermore, in a xenotransplant model of ccRCC established in NOD-SCID mice, the transcript level of -catenin was increased in tumors with the mutant form of MCPIP1 lacking RNase activity. Regulation of -catenin activity occurs via its phosphorylation, which is crucial for the degradation process. We observed that MCPIP1 regulates the activity and phosphorylation status of -catenin in ccRCC cells. The active -catenin (non-P S45) and stabilized form of -catenin (S552) were increased in the cytoplasmic and nuclear fractions of MCPIP1-D141N cells. Moreover, MCPIP1 regulates the expression of inhibitors of the WNT pathway through its RNase activity. We identified several miRNAs dependent on the RNase activity of MCPIP1. Based on a database, we identified several target genes, which act as negative regulators of WNT signaling pathways and reduce the level of active -catenin.

Conclusion: Based on the obtained results, we propose a possible mechanism for the regulation of the EMT process and -catenin level by MCPIP1. MCPIP1 degrades miRNA, thereby actively influencing the levels of genes which inhibiting the WNT pathway by inactivating -catenin. In the case of the MCPIP1-D141N mutation, there is no MCPIP1 RNase activity and miRNAs mature and inhibit selected genes, thus activating the WNT pathway and EMT process. Our results may contribute to a better understanding of ccRCC progression, which may help in the development of more effective therapy in the future.

[II-2] PRE-MIRNAS FEATURES MAY INFLUENCE THE SPECIFICITY OF MIRNA BINDING TO AGO

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MicroRNAs are short 20-22nt-long RNAs that modulate a wide variety of mRNAs expression at the post-transcriptional level. These small RNAs work in RNA-protein complexes (RISC RNA-induced silencing complexes) which in human cells include any of four types of Argonaute protein (AGO1-4). Some published works suggest that different AGOs do not show specificity with respect to miRNAs. However, in our experiments we observed that regulation of expression of the same reporter gene but targeted by different miRNAs and transfected into the same cell types was different, which could be explained by the presence of different AGO proteins in RISC complexes. We hypothesized that some pre-miRNA features may influence the process of miRNA specific segregation to different AGOs.

To check this hypothesis we analyzed the structure of pre-miRNAs of miRNAs bound to different AGOs. The miRNAs for the analysis were obtained from two publications using AGO immunoprecipitation (Dueck et al. 2012, Turchinovich and Burvinkel 2012). Pre-miRNA features suvh as hairpin and mature miRNA lengths, size of hairpin loop, numbers of unpaired bubbles, A-G mispairing, and length of double stranded fragments of pre-miRNA were calculated for all miRNAs on the basis of data from miRBase. In order to detect the features most strongly influencing the loading of miRNAs into AGO, those miRNAs that most deviate from the median obtained for all miRNAs were selected for further analyzes and the frequency of their occurrence in complexes with individual AGO proteins was examined. The relationship between the number of miRNA molecules in complexes with individual AGO proteins and the presence and number of individual features was determined using the Spearman correlation coefficient. Statistically significant results obtained for a few features showed that: AGO2 has preference for miRNAs whose pre-miRNAs show shorter double stranded regions while AGO3 prefers pre-miRNAs with larger hairpin loop and with unpaired bubbles. AGO4 had higher affinity for miRNA that had many A-G mismatches and shorter double stranded regions in pre-miRNA, but this set of miRNA was rather small and the data should be further investigated. AGO1 had no distinctive preferences.

Conclusions: Our results from correlation analysis suggest that there could be a preference in AGO -miRNA binding dependent on pre-miRNA features.

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[II-3] Identification of irradiation-induced ATM-dependent lncRNAs

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DNA damage response (DDR) is a complex process, essential for cell survival. Especially deleterious types of DNA damage are DNA double-strand breaks (DSB), which can lead to genomic instability and malignant transformation if not repaired correctly. The central player in DSB detection and repair is the ATM kinase which orchestrates the action of several downstream factors. Despite substantial knowledge of DNA repair processes still several aspects of DNA damage detection and signalling are not fully understood. Recent studies have suggested that long non-coding RNAs (lncRNAs) are involved in DDR. Here, we aimed to verify the hypothesis that ATM-dependent lncRNAs are essential players involved in the DDR.

DNA damage was induced by ionizing radiation (IR) in immortalized lymphoblastoid cell lines (LCLs) derived from 4 patients with ataxia-telangiectasia (AT) and 4 healthy donors. Cells were collected 1h and 8h after IR to allow identification of lncRNAs involved in the early and late response to DNA damage. A strand-specific RNA sequencing approach was applied to identify IR-induced lncRNAs and mRNAs. The induction and dynamics of selected lncRNAs were verified by RT-qPCR at several time-points after IR on a larger cohort of 12 AT patients and 8 healthy donors. To further prove that ATM is involved in regulation of selected lncRNAs we utilized a specific ATM inhibitor (KU-60019).

7 mRNAs and 10 lncRNAs were significantly induced 1h after IR in healthy donors, whereas none in AT patients. 447 mRNAs and 149 lncRNAs were induced 8h after IR in the control group, while only 100 mRNAs and 3 lncRNAs in AT patients. The overlap between the control and AT patient groups was limited (19% and 6% for mRNAs and lncRNAs, respectively). Among IR-induced mRNAs, we found several genes with well-known functions in DDR, such as *CDKN1A*, *BBC3*, and *GADD45A*. Gene Set Enrichment Analysis revealed delayed induction of key DDR pathways in AT patients compared to controls. Based on Transcription Factor ChIP-seq ENCODE data, we found 71 TFs with binding sites within 1kb from differentially expressed lncRNAs. The majority of TFs are involved in pathways connected with DNA repair, p53, and cell cycle regulation, which supports the involvement of lncRNAs in DNA damage response. RT-qPCR validation confirmed the ATM-dependent induction of 10 selected lncRNAs. Inhibition of ATM with KU-60019 proved that those lncRNAs are dependent on ATM. Some of the detected lncRNAs are localized next to protein-coding genes involved in DDR (*XPC*, *IER5*, *TNFRSF10B*, *TNFRSF10C*, *MYC*). We observed that induction of lncRNAs after IR preceded changes in expression of adjacent genes. This indicates that IR-induced lncRNAs may regulate the transcription of nearby genes and thus affect DDR.

In conclusion, we identified lncRNAs induced in response to DNA damage in an ATM-dependent manner. The role of selected lncRNAs in DDR is currently under investigation.

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[II-4] THE MURINE BFAR ISOFORM 3 IS INVOLVED IN THE METABOLISM OF RNA

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Here we aimed to characterize the mouse *3110001I22Rik* gene, which is located in the first intron of *Bfar*, therefore it is considered an additional *Bfar* variant coding for the BFARv3 protein. However, it differs from other BFAR isoforms and resembles PPHLN1 due to its two conserved domains, Lge1 and serine-rich. In somatic cells, BFARv3 and its Lge1- or Ser-rich-deficient mutants were preferentially localized in the nucleus. To identify BFARv3 partners, we analyzed by the LC/MS-based proteomics the BFARv3/EGFP interactome. BFARv3 co-immunoprecipitated with 40S ribosomal proteins (RPS3, RPS14, RPS19, RPS25, RPS27), histones (H1.2, H1.4, H3.3C), proteins involved in RNA processing and splicing (SFPQ, SNRPA1, HNRNPA3, NONO, KHDRBS3), calcium signaling (HPCAL1, PTK2B), as well as HSD17B4, GRB14, POSTN, and MYO10. Both Lge1 and Ser-rich domains of BFARv3 were necessary for binding to RNA-interacting factors NONO and SFPQ, which was confirmed by co-immunoprecipitation. We showed that although *Bfar v3* is expressed ubiquitously in mouse tissues, its expression is the highest in metaphase II oocytes. The BFARv3 interactome suggests its role in RNA metabolism, which function is critical for the transcriptionally silent MII oocyte. BFARv3 has no ortholog in the human genome, thus it may contribute to species-specific features during oocyte maturation and early embryonic development.

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[II-5] CRISPRi tiling screen identifies regions in IGH enhancers crucial for Burkitt lymphoma cell growth

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Immunoglobulin heavy chain (IGH) locus undergoes several complex rearrangements during B cell development which result in expression of high affinity antibodies. The intermediates of those rearrangements are DNA double-strand breaks which pose a risk of illegitimate DNA recombinations. Indeed, translocations of oncogenes into the IGH locus are hallmarks of B-cell lymphomas; e. g. MYC/IGH (t(8;14)(q24;q32)) is common in Burkitt lymphoma (BL) and IGH/BCL2 (t(14;18)(q32;q21)) occurs in follicular lymphoma (FL) and diffuse large B cell lymphoma (DLBCL). As a result, oncogenes are juxtaposed to IGH enhancers: E, 3RR1 and 3RR2, which drive their expression and promote lymphomagenesis. However, it is still unclear which exact elements in the IGH enhancers are essential for regulating oncogene expression and for cancer cell growth.

Here, we performed a tiling CRISPR interference screen to identify crucial regions in IGH enhancers. We designed a CRISPR-IGH library of almost 7 000 sgRNAs densely covering the E, 3RR1 and 3RR2 enhancers. Simultaneously, we established Burkitt lymphoma cell lines (BL41 and DG75) with stable expression of dCas9-KRAB protein, which were subsequently transduced in duplicate with the CRISPR-IGH library. Cells were harvested on day 5 post transduction (T0) and further cultured for 20 population doublings (T1). Changes in abundance of sgRNAs in the cell pool at T1 vs T0 were determined by NGS. We identified 680 sgRNAs at least two-fold depleted in DG75 and 279 sgRNAs in BL41. Sliding window analysis revealed one region in the E and two in the 3RR enhancers whose targeting with CRISPR profoundly inhibited BL cell growth. Those regions harbor motifs recognized by several transcription factors with a role in normal and malignant B cells, such as PAX5, RUNX3, ZFX, STAT6, ZBTB family. Moreover, we confirmed with RT-qPCR that these regions are transcriptionally active in lymphoma cells, possibly giving rise to enhancer RNAs.

In conclusion, our study pinpointed three regions in IGH enhancers which are crucial for BL cells. Screens in DLBCL cell lines are ongoing. The role of these regions in lymphoma cell survival and oncogene expression will be further validated. The results will be also intersected with the data on enhancer RNA expression from chromRNA-seq experiments. Altogether, our study will provide novel insights into the function of IGH enhancers in B-cell lymphoma.

[II-6] SMALL EXTRACELLULAR VESICLES EFFICIENTLY TRANSPORT MIRNA MIMETICS INTO MONOCYTIC CELLS

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Introduction/Rationale: Utilization of the gene regulatory potential of miRNA mimetics for therapeutic purposes depends on efficient delivery methods into target cells. We tried to employ small extracellular vesicles (sEVs) for the transfer of miR-494 to THP-1 monocytic cells.

Methods: sEVs were isolated from THP-1 cell culture supernatants by differential centrifugations. The vesicles were loaded with synthetic double stranded RNA mimicking miR-494 by electroporation (EP). Possible damage caused by the EP was monitored by electron microscopy. THP-1 cells were also used as target cells. miR-494 was quantified in RNA samples isolated from sEVs and THP-1 cells by qRT-PCR (TaqMan Small RNA Assays).

Results: EP resulted in an increase of the number of miR-494 molecules in sEVs by several orders of magnitude. Similar relative increases were observed in THP-1 cells after incubation with electroporated sEVs for 72 hours. No significant cell damage was observed in sEVs after EP.

Conclusions/Novel aspect: Electroporation is a very efficient method for loading sEVs with miR-494 mimics without damaging the vesicles. By quantifying both strands of the RNA duplex, we were able to measure the increase of miR-494-3p (already present in the target cells), and the absolute number of transferred miR-494-5p that does not naturally exist in the cells in measurable amounts.

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[II-7] EFFECT OF IGH ENHANCERS INHIBITION WITH COMPOUND 30666 ON B-CELL LYMPHOMA SURVIVAL AND ONCOGENE EXPRESSION

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Translocations juxtaposing immunoglobulin heavy chain (IGH) regulatory regions with oncogenes such as MYC, BCL2, BCL6, CCND1 are characteristic features of B cell non-Hodgkin lymphomas (NHL) and multiple myeloma (MM). IGH enhancers: E, 3RR1 and 3RR2 drive expression of translocated oncogenes and promote proliferation and survival of cancer cells. Therefore, targeting IGH enhancers could offer a therapeutic strategy for IGH translocation-positive malignancies. Recently, a small molecule - 7-[[(4-methyl-2-pyridinyl)amino](2-pyridinyl)methyl]-8-quinolinol (compound 30666) was shown to inhibit activity of the E enhancer, resulting in reduced oncogene expression and decreased cell survival of MM and some NHL cell lines (Dolloff, Cancer Biology Therapy, 2018).

Here, we aimed to validate the inhibitory effect of compound 30666 in diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) cells carrying the t(14;18) IGH/BCL2 and t(8;14) MYC/IGH translocations, respectively. Lymphoma cells as well as IGH translocation-negative B cells and non-B cells were treated with 1.25-100 M of compound 30666 or DMSO and surviving cell fraction was measured after 48 hours. In addition, we checked the effect of 30666 on the transcriptional activity of E, 3RR1 and 3RR2 enhancers and on the expression of MYC and BCL2. Indeed, compound 30666 inhibited survival of DLBCL and BL cells with IC50 values 1-5 M. In agreement with N. G. Dolloff we observed decreased expression of MYC in BL cell lines, which was accompanied by decreased expression of E eRNAs. On the other hand, in DLBCL cells expression of BCL2 was not affected. 3RR1 and 3RR2 enhancers responded differently to 30666; 3RR1 was upregulated while 3RR2 was downregulated consistently in all cell lines. We also tested survival of several IGH translocation-negative B cells as well as non B cells where IGH enhancers are not active. Our results show that majority of them are susceptible to compound 30666 at similar doses as lymphoma cells bearing IGH translocations. There were no statistically significant differences between t(+) lymphomas, t(-) B cells and t(-) non-B cell groups. However we identified two outliers in t(-) non-B cell group K562 and HepG2 cells which were more resistant to 30666 (IC50 of 10 and 50 M). Our findings suggest that 30666 inhibitor might not be as specific for IGH enhancers as previously reported and that further research regarding its mechanism of action is necessary.

Poster Session III:

Bioinformatics and Mathematical Modeling

[III-1] Radiomics in prediction of radiation-induced hypothyroidism in patients with oropharyngeal cancer treated with Intensity-Modulated Radiation Therapy

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Introduction. The main goal of this project was to use data mining technique on image data (radiomics) to develop a radiomic normal tissue complication probability (NTCP) model for radiation-induced hypothyroidism (RIHT). RIHT commonly develops in cancer survivors that receive radiation therapy (RT) for oropharyngeal cancer (OPC). In a long follow-up (10 years), more than 50% of patients experience RIHT. The state-of-art NTCP models do not take into account more complex individual anatomical variations such as shape characteristics or more subtle image patterns, which we address.

Material and methods. We collected CT images and clinical data from 98 patients with OPC treated in 3 oncology centers (38 from Lodz, 48 from Gliwice, 12 from Radom), who received intensity-modulated radiation therapy with a planned total dose of 69.9670.0 Gy in 3335 fractions, concomitant systemic treatment was allowed. The group was monitored during a follow-up after a median of 28 months (IQR 2138 months), 27 (27.6%) patients developed RIHT. For each patient, we extracted 1316 radiomic features from original and transformed (Laplacian of Gaussian, wavelet, square, square root, logarithm, exponential, Gradient Magnitude, Local Binary Pattern filters) images using thyroid masks obtained by manual segmentation and pyradiomics Python package. Those features were then filtered in order to remove redundant variables, ones that were deemed unstable with respect to organ delineation inaccuracy, and those which did not discriminate the outcome in univariate analysis. We removed the observed batch effect (likely due to images source heterogeneity) using ComBat. Batch-corrected values, together with clinical variables (sex, age, dosimetric data) were entered into a logistic regression model, with sequential forward variable selection. The predicted outcome was the occurrence of RIHT during follow-up. Data from Lodz was used to derive the model while those from the other 2 centers served as a validation group.

Results and conclusions. The predictors selected during the model training were: coarseness from NGTDM on log-transformed image, median (D50) and maximal (D max) dose to thyroid. The accuracy on training data equaled 89.5% (95%CI: 79.7-99.2) with AUC=0.91 (95%: CI 0.78-1.0) and, for finally chosen threshold, sensitivity=0.70 (95% CI: 0.40-0.89) and specificity=0.96 (0.82-0.99). Meanwhile, the results for validation sets were: accuracy=91.7% (95%CI: 76-100), sensitivity=1.0 (95%CI: 0.44-1.0), specificity=0.88 (95%CI: 0.57-0.98) and accuracy=83.3% (95%CI 72.8-93.9), sensitivity=0.57 (95%CI: 0.33-0.79), specificity=0.94 (95%CI: 0.80-0.98), respectively for Radom and Gliwice.

Our model reaches accuracy comparable with previously presented non-radiomic NTCP models. It is characterized by high specificity and moderate sensitivity that in practical application would allow the identification of patients who particularly require monitoring of thyroid function due to elevated risk of RIHT.

[III-2] Logistic regression method in search of radiation responsive genes

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Logistic regression methods, applied to single-cell RNAseq data, can be utilized to detect gene expression differences. Such an approach works also very well in the problem of binary classification of control and irradiated cells for scRNAseq data. While analyzing this kind of data for classification purposes, once have to be very careful about the low-level analysis, because this step could strongly affect the results. This work focuses on the analysis of Peripheral Blood Mononuclear Cells (PBMCs) from one donor control and irradiated with 1Gy dose samples. PBMCs give a selective response to the immune system and are one of the major cells in the human body immunity system. They contain several types of cells such as lymphocytes, monocytes, and macrophages. Because of the fact that exposure to chemicals, radiation, or other harmful factors occurs in the peripheral blood, PBMCs are very prone to be influenced by those factors and this is why PBMCs are very important while analyzing the impact of radiation exposure.

In the presented study, after the low-level analysis, data were log-transformed to better present the dynamics of mean expression per cell changes for the purposes of further analysis. In order to check, if there can be an expected strict radiation response pattern, we utilized unsupervised methods to visualize control and irradiated samples together. Results showed that there is another very specific, strong pattern showing that cell heterogeneity over-dominate the radiation response pattern. We wanted to find some genes that differ among control and irradiated sample - we used effect size measure, which is a quantitative measure of the magnitude of, in this case, the radiation response, to check if we can expect that there are some genes with specific radiation response pattern. As a result, we obtained 103 from overall 368 genes to have an effect size at at least a very small level (10 of them had the effect size at at least a small level). What is interesting here, we found genes that are connected with apoptosis, DNA repair process, cytotoxicity, cell growth and differentiation, or T and B cells regulation. As the last part, we prepared our own implementation of the control and irradiated cells classifier, based on logistic regression methods, utilizing those previously found 103 genes with at least a very small effect size. Our main aim was to find genes that could be used as factors that indicate if the blood sample was irradiated or not. Created classifier enabled building model that could be used for control and irradiated cells classification. It included the following genes: BAX, GAPDH, PCNA, PTPRC, MYC, HAVCR2, TNFSF8, STAT5A, IL2RB and CD4. What is more, those genes that were found as radiation responsive ones are confirmed to be connected with radiation response by a number of literature reports. Using our approach we are able to capture those genes that are important from the point of view of radiation response patterns.

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[III-3] THE IMPACT OF SOCIO-ECONOMIC STATE OF SOCIETY ON COVID-19 DEVELOPMENT

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Aim: The aim of the study was to investigate the influence of socio-economic factors on the progress and national prevention of COVID-19 in countries across the world.

Materials and Methods: The material under investigation consists of information about the disease situation of 192 regions or countries from early October, provided by Worldometers.info. The dataset was enhanced by the data gathered by CSSE at Johns Hopkins University for the same period of time in order to fill missing data, such as WHO region or number of recovered patients for some of the countries. Next, the samples were augmented with the following variables: Recovered/1M population to complete the collection of other affected per million population: performed tests, confirmed cases and deaths. To obtain more informative normalized data, confirmed cases in 1M tests and deaths as well as recovered in 1M cases were added. The source of socio-economic factors was the World Happiness Report for the year 2019. The components contributing to the Happiness Score were: GDP, life expectancy, social support, freedom to make own choices, generosity and trust toward government. The countries without specified numbers of conducted tests or not studied in the World Happiness Report were removed and the final number of countries taken into consideration equaled 137. Then, using Pearson correlation coefficients and their corresponding p-values, correlations for the whole world, Europe, Africa and Americas were calculated. Moreover, in order to distinguish the influence of socio-economic on the situation of the country, the 20 most and 20 least happy countries were selected for additional analysis. Their normalised reports were compared by the Students t-test.

Results: Based on the data from 137 countries, the strongest, positive correlation occurs between the number of tests performed for 1 million of citizens and both GDP and social support in a particular country. This phenomenon is also observed in Europe and Africa separately. Correlation analysis shows that the higher life expectancy the larger level of deaths are observed. In Europe there is also a significant negative correlation between GDP and confirmed cases for 1 M tests (higher GDP lower number of confirmed cases) also longer life expectancy give lower estimation of recovered cases and increased deaths. The relation between GDP and number of tests, after depicting scatter plot, showed distinction of the WHO regions in groups. Also, the Happiness Score divided countries showed differences in means of testing categories.

Conclusion: The chosen socio-economic factors appear to differentiate the pandemic situation. GDP and life expectancy, strongly regulating Happiness Score, occur to have the greatest impact for COVID-19 strategies.

Acknowledgements: This work is supported by the Silesian University of Technology grant for Support and Development of Research Potential.

[III-4] In silico studies suggest T-cell cross-reactivity between SARS-CoV-2 and less dangerous coronaviruses

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So far, it is impossible to explain the diverse individual and population responses to SARS-CoV-2 infection. Many factors may be involved, including genetics, diet, vaccinations, the innate immune response, viral load, and other phenomena. Further, immune responses raised against pathogens other than SARS-CoV-2 (cross-reactivity) may also be involved. In this work, we analyzed the potential for T-cell cross-reactivity between less contagious coronaviruses (HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63) and SARS-CoV-2. *In silico* research suggests that SARS-CoV-2 and less dangerous coronaviruses share identical peptides, which can be presented on MHC class I molecules. Those T-cells epitopes belong to several coronavirus proteins localized inside the viral envelope, including helicase, RNA polymerase, proofreading exoribonuclease, and 2-O-methyltransferase. Our data suggest that a milder course of COVID-19, in some populations, may be related to the cross-reactivity of T cells.

[III-5] LCRAnnotationsDB: Database of annotations for low complexity regions in proteins

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Low Complexity Regions (LCRs) are fragments of proteins characterized by low diversity of amino acid composition. These regions are mostly intrinsically disordered. Knowledge about LCRs is not well established. However, some research shed light on LCRs and revealed some of their functions. LCRs are frequently responsible for binding to other proteins, RNA, DNA and metal ions. These regions may also initialize signal transduction or perform the function of processes regulations that occur in cells. The result of the project is LCRAnnotationsDB database that contains proteins, LCRs and annotations that overlap with LCRs. Annotations were found in different, open access data sources.

[III-6] COMPARISON OF TRANSCRIPTOMES OF NEURONS, GLIA AND NON-SPECIFIC NEUROBLASTS IN DROSOPHILA MELANOGASTER LARVAE

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The *Drosophila melanogaster* organism is very small, but serves as a useful model for many biological processes occurring inside the body. Three-staged, relatively short life is a suitable situation to observe how cells differentiate and from what progenitor cells they are developed. This organism is one of the best models for neurosciences research. Inside *Drosophila*s brain there is around 100,000 cells, of which about 85% are neurons.

The main purpose of this work was to compare gene expression profiles of neurons, glia and non-specific neuroblasts in *Drosophila melanogaster* larvae. The comparison allowed to find the genes characterising the development of neurons and glia, which in turn is important to find the solution of the problem how the neural and glial cells differentiate from their progenitor cells (neuroblasts). For this purpose, published RNA sequencing data from the study of Yang et al. Transcriptomes of lineage-specific *Drosophila* neuroblasts profiled by genetic targeting and robotic sorting (Development 2016, 143: 411-421) were analysed.

Published high-throughput molecular biology data from a next generation sequencing technique, RNA-seq, were used in the form of count matrices for eight different cell types. The data were subjected to pre-processing, filtering and normalization. Linear (Principal Component Analysis) and non-linear (Uniform Manifold Approximation and Projection) dimensionality reduction methods were applied. The statistical analysis was based on the negative binomial regression, with empirical Bayes parameter estimation and quasi-likelihood F-test. Multiple testing problem was addressed by using Benjamini-Hochberg correction method. Data were processed in the R programming environment, using libraries such as: edgeR for normalization, scaling counts and statistical analysis, ggplot2, umap, VennDiagram, pheatmap and RColorBrewer for data visualisation.

The statistical models of transcript abundance for derived cells were fitted to the given data properly. Differentially expressed genes in comparisons between neurons, glia and non-specific neuroblasts were identified, with a count number of about 1,600 genes for each contrast. Genes characterising the development of neuronal and glial cells were found.

[III-7] SEGMENTATION AND DETECTION OF ANEURYSM FROM MAGNETIC RESONANCE ANGIOGRAPHY

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Early detection of the aneurysm is very important due to severe complications and a very high mortality rate in case of aneurysm rupture, especially when it concerns the cerebrum. The aim of this work was to process the images coming from magnetic resonance angiography (MRA) in order to find the vasculature, and the rules for aneurysm detection.

Both synthetic and non-contrast Time of Flight MRA data ware analyzed. Processing of the images included filtering, segmentation of the blood vessels based on the region growth method, skeletonization, detection of a branch- and end-points in the skeleton. Collected data allowed for the creation of a three-dimension model of the vascular network/tree as well as quantification of each branch of the network/tree.

The worked-out algorithm allowed the creation of a three-dimensional model representing the structure and features of the indicated vascular network. The analysis of the geometric features of the processed vessels permitted for the development of a method of aneurysm detection, however, it has to be further verified.

The developed algorithm has potential both in clinical applications as well as in scientific works.

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[III-8] EXTENDED MODEL OF RNA INTERFERENCE

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Rationale. MicroRNAs are small, non-coding RNAs playing a major role in gene expression regulation, acting as a sequence-specific component of RISC complex which neutralizes or degrades mRNA molecules in a process known as RNA interference (RNAi). The 3-UTR region may contain multiple recognized motifs, making mRNA-miRNA interactions a many-to-many relationship. Thus approximately 2000 unique human miRNAs target over 60% of all transcripts, indicating RNAi as a promising basis for modeling of gene expression. In their 2019 article [1], Mura et al. proposed a mathematical model for predicting ionizing radiation-induced transcriptome changes. The model describes transcript level change as a sum of interactions between distinct mRNA-miRNA pairs, linking them to amount of miRNA (treated as constant), number of binding sites, and a proportionality factor which may be interpreted as specific miRNA activity (influenced by oxidative stress). We present an extended model, in which expression changes in irradiated cells are the result of differences both in miRNA amount and activity.

Methods. Two cell lines, HCT116 and Me45, were treated with 4 Gy X-ray. 12 hours after irradiation their RNA levels were measured: mRNA on HGU133A microarrays, and miRNA utilizing Illumina Next Generation Sequencing technology. Numbers of miRNA binding sites were calculated as a weighted average of values given by three commonly used dedicated tools, miRanda, MiRDB and TargetScan. Proportionality factors were estimated from the data with linear least squares approach. Most significant miRNAs were identified by means of sequential selection, with Spearmans correlation coefficient as model quality measure. Gene set analysis against KEGG pathways was performed with DIANA-miRPath which combines target prediction with overrepresentation analysis.

Results. Although ionizing radiation response differed between cell lines, the extended model was capable of predicting expression changes with moderate accuracy. Furthermore, it was possible to considerably reduce the number of miRNAs with only a small decrease in predictive potential as few as 20 miRNAs could be used to construct a model achieving over 80% of maximum obtained correlation. High ranks of those miRNAs largely coincided with their relative abundance of binding sites. Gene set analysis revealed as significantly overrepresented signaling pathways connected with growth factors, cell signaling, biological clock and extracellular matrix, as well as some well known oncogenic signatures.

Conclusions. MiRNAs are one of key elements in oxidative stress response, regulating many vital processes, such as proliferation, signaling, adhesion and motility, as well as pathways associated with cancer, which might make them potential tumor markers. Through mathematical modeling, miRNAs can also be successfully utilized to predict gene expression changes.

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[III-9] SEARCHING FOR THE SARS-CoV-2 MAIN PROTEASE INHIBITORS

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the pandemic coronavirus disease 2019 (COVID-19), which is threatening global health since late 2019. The virus main protease, SARS-CoV-2 Mpro, is considered an attractive drug target. SARS-CoV-2 Mpro is one of the key enzymes in the viral life cycle, because of its role in processing the polyproteins translated from the viral RNA.

The research objective of this project is to discover and develop small-molecule inhibitors against SARS-CoV-2 main protease. We performed an analysis of thiocarbamate derivatives as potent inhibitors. Using a combination of advanced synthetic methods supported by computational chemistry and biochemistry it is planned to obtain a set of compounds that binds selectively to the Mpro protein. Molecular modeling and *in silico* ligand optimization procedure support experimental part of the project.

The method of ligand synthesis, thiocarbamate derivatives, was developed in our laboratory. Compounds selected from a library of 45 structures were examined *in vitro* on the SARS-CoV-2 main protease.

For *in silico* part of the study we selected a crystal structure of SARS-CoV-2 main protease (PDB ID: 6LU7). The protein structure with and without water molecules was used as a receptor for the docking of the potential inhibitors using AutoDock Vina software. Water molecules were included because of its catalytic function in binding cavity of Mpro. Additionally, AQUA-DUCT was used to search for hotspots places within the protein, where water molecules spent most of the simulation time. The hotspots were then compared with docked ligands to provide feedback on dynamic changes occurring during molecular dynamics simulations.

Some of the designed molecules exhibit in experimental part interactions with SARS-CoV-2 main protease. Using computational methods we investigated the binding mechanism of the ligands and based on those results we are now able to propose a set of structure modifications of ligands which can then be used as more sufficient inhibitors. Our results may serve as the basis for the development of new therapeutic strategies against COVID-19 disease in which the main protease plays a pivotal role.

The computational part of the work is supported by the Plastic Omnium Auto Sp. z o.o.

[III-10] Classification of Thyroid Tumors Based on Mass Spectrometry Imaging of Tissue Microarrays; a Single-Pixel Approach

Agata Kurczyk[1], Marta Gawin[1], Mykola Chekan[1], Agata Wilk[2], Krzysztof Łakomiec[2], Grzegorz Mrukwa[2], Katarzyna Frątczak[2], Joanna Polanska[2], Krzysztof Fujarewicz[2], Monika Pietrowska[1], Piotr Widlak[1]

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The primary diagnosis of thyroid tumors based on histopathological patterns can be ambiguous in some cases, so proper classification of thyroid diseases might be improved if molecular biomarkers support cytological and histological assessment. In this work, tissue microarrays representative for major types of thyroid malignanciespapillary thyroid cancer (classical and follicular variant), follicular thyroid cancer, anaplastic thyroid cancer, and medullary thyroid cancerand benign thyroid follicular adenoma and normal thyroid were analyzed by mass spectrometry imaging (MSI), and then different computation approaches were implemented to test the suitability of the registered profiles of tryptic peptides for tumor classification. Molecular similarity among all seven types of thyroid specimens was estimated, and multicomponent classifiers were built for sample classification using individual MSI spectra that corresponded to small clusters of cells. Moreover, MSI components showing the most significant differences in abundance between the compared types of tissues were detected and their putative identity were established by annotation with fragments of proteins identified by liquid chromatography-tandem mass spectrometry in corresponding tissue lysates. In general, high accuracy of sample classification was associated with low inter-tissue similarity index and a high number of components with significant differences in abundance between the tissues. Particularly, high molecular similarity was noted between three types of tumors with follicular morphology (adenoma, follicular cancer, and follicular variant of papillary cancer), whose differentiation represented the major classification problem in our dataset. However, low level of the intra-tissue heterogeneity increased the accuracy of classification despite high inter-tissue similarity (which was exemplified by normal thyroid and benign adenoma). We compared classifiers based on all detected MSI components (n = 1536) and the subset of the most abundant components (n = 147). Despite relatively higher contribution of components with significantly different abundance and lower overall inter-tissue similarity in the latter case, the precision of classification was generally higher using all MSI components. Moreover, the classification model based on individual spectra (a single-pixel approach) outperformed the model based on mean spectra of tissue cores. Our result confirmed the high feasibility of MSI-based approaches to multi-class detection of cancer types and proved the good performance of sample classification based on individual spectra (molecular image pixels) that overcame problems related to small amounts of heterogeneous material, which limit the applicability of classical proteomics.

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[III-11] MODELING AND PREDICTING THE DEVELOPMENT OF A VIRAL EPIDEMIC ON THE EXAMPLE OF THE SARS-COV-2 PANDEMIC

Agnieszka Baluch[1], Paulina Kądziołka[1], Agnieszka Morel[1], Jarosław Czerniak[1], Adam Jagoda[1], Piotr Paczuła[1], Marek Żabiałowicz[1], Sebastian Student[1], Krzysztof Fujarewicz[1], Dariusz Mrozek[1], Krzysztof Psiuk-Maksymowicz[1]

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31 December 2019 was the day when Wuhan Municipal Health Commission reported a cluster of cases of pneumonia, the day when novel coronavirus named *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) was identified. Since that day, a new virus has been in the spotlight not only for scientists and politics but also for a huge part of the human population affected by the pandemic. One of the most important research topics was to create a model of pandemics which would be a useful tool to predict possible scenarios of its spread and might also verify the impact of different efforts stepped up by the governments to bring it under control.

Many different approaches have been used to model -coronavirus spread. One of them was to use compartmental models. One of the most popular of them is the SIR (Susceptible-Infected-Recovered) model that has 3 compartments: S(t) as suspected individuals that are not infected, but could become infected, I(t) as infected individuals that have already been infected by the virus and can transmit it to those individuals who are susceptible, R(t) as recovered individuals that are those individuals who have recovered from the virus and are assumed to be immune or have died (Rm(t) and D(t)). The model works with the assumption, which says we can neglect births and deaths other than deaths caused by the virus since the time scale of the SIR model is short enough [1]. Another popular method used to model coronavirus pandemics are discrete time series models like ARIMA and ARIMAX.

The goal of our project is to check different approaches for SARS-CoV-2 modeling containing various sets of predictors in order to evaluate their performances in predicting the number of coronavirus cases for different countries in a specified forecast period. The project aims at creating model-driven application enabling users to easily make predictions for simulation parameters set by them and visualize the prediction results.

Acknowledgments. The research was supported by the Silesian University of Technology as a Center of Modern Teaching based on research and innovations program. The program is co-financed by the EU within the European Social Fund.

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Poster Session IV:

Basic Research and Medical Biotechnology

[IV-1] WWOX MAY LOSE ABILITY TO REGULATE PRO-TUMORAL AP-2Γ BUT STILL POSSESSES SYNERGISM WITH TUMOR SUPPRESSOR AP-2A IN HIGH-GRADE BLADDER CANCER.

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Introduction: The WW Domain Containing Oxidoreductase (*WWOX*) is important gene regarding bladder carcinogenesis since it spans the common fragile site FRA16D whose chromosomal breakage is more common in bladder cancer patients who are tobacco smokers. *WWOX* encodes protein containing two tryptophan domains, the first of which recognizes motifs of other proteins such as Activating Enhancer-Binding Protein 2 (AP-2) or AP-2 transcription factors (encoded by *TFAP2A* or *TFAP2C* gene, respectively). It is known that the role of both AP-2 transcription factors is meaningful in terms of bladder cancer, yet their character is divergent. Literature data summarize *TFAP2C* as oncogene, while *TFAP2A* mainly possess tumor suppressor properties. Since the interaction between WWOX and two transcription factors regulates thousands of genes, the present study aimed to determine WWOX, AP-2 and AP-2 function in regulating biological processes of high-grade bladder cancer.

Materials and Methods: HT-1376 cell line was subjected to two stable lentiviral transductions. Overall, this allowed the development of six *in vitro* variants to examine distinct WWOX, AP-2 or AP-2 functionality as well as WWOX in collaboration with a particular transcription factor. Cellular models were compared in terms of differences in biological processes using assays investigating clonogenicity, proliferation, adhesion and cell viability.

Results: *WWOX* overexpression decreased colony formation, proliferation and adhesion to collagen I and IV but also laminin. *TFAP2A* overexpression led to identical direction of changes in biological processes with additional decrease in adhesion to fibronectin. *TFAP2C* overexpression increased clonogenicity, proliferation and adhesion to all examined extracellular matrix proteins. Simultaneous *WWOX* and *TFAP2A* overexpression intensified the anti-tumor effect of each individual through potent decrease of clonogenicity and proliferation. Overexpression of both *WWOX* and *TFAP2C* increased proliferation and ability of a single cell to grow into colony. Although the results of cell viability assay are vague, *TFAP2C* overexpression presumably increased mitochondrial redox potential while *WWOX* overexpression decreased it.

Conclusion: Our findings confirm the tumor suppressor character of *WWOX* or *TFAP2A* but the oncogenic properties of *TFAP2C*. However, simultaneous overexpression of *WWOX* and particular transcription factor caused dissimilar biological effect. The results for both *WWOX* and *TFAP2A* overexpression suggest a synergistic suppressor effect, while in the variant with overexpressed *WWOX* and *TFAP2C*, no inhibition of the oncogenic nature of *TFAP2C* is observed. This indicates that WWOX may lose its ability to regulate the oncogenic transcription factor in high grade bladder cancer despite ability to do so at lower tumor grade. Further studies are clearly needed to investigate other aspects of bladder cancer.

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[IV-2] The *WWOX* gene inhibits the tumor progression of glioblastoma multiforme – in vitro study.

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The WW domain-containing oxidoreductase (*WWOX*) gene locus is positioned on chromosome 16 in a common fragile site FRA16D (16q23.1-q23.2) and it has a broad impact on cell function. The influence of the *WWOX* on many biological processes was found, regardless of the type of tissue. Of all cancers, little is known about the role of this gene in brain tumors. So far it has been established that approximately 20% of *WWOX* gene alternations are present in glioblastoma multiforme (GBM).

Based on current state of literature, we aimed our study to determine the effect of the *WWOX* gene overexpression on processes associated with signal transduction and adhesion via performing biological assays.

To conduct *in vitro* assays, firstly the stable transduction with lentiviral system was performed on glioblastoma multiforme cells (U251MG and U87MG) to obtain cell models with overexpression of *WWOX* gene and control. Subsequently, properties such as clonogenicity, ability to grow in suspension, adhesion to five extracellular matrix proteins, 3D growth, metalloproteinases (MMPs) activity or migration potential were evaluated.

We revealed the decrease of clonogenicity in U251MG and U87MG cell line variants with high *WWOX* gene expression. Interestingly, both U251MG and U87MG were not able to grow in suspension, unlike the previously tested T98G and DBTRG-05MG cell lines of GBM. Moreover, *WWOX* overexpressing cells demonstrated lower capacity for invasive migration and activity of MMP-2 and MMP-9 in comparison to control. Furthermore, *WWOX* overexpression lowered the ability to adhere to collagen IV, the major component of cell basement membrane, as well as inhibited the formation of colonies during the 3D growth in Geltrex both in U251MG and U87MG cell lines.

In summary, this *in vitro* study indicated that the *WWOX* gene expression is associated with the inhibition of biological processes that could affect the progression of glioblastoma multiforme.

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[IV-3] REGULATION OF PRODUCTION AND NEUTRALIZATION OF REACTIVE OXYGEN AND NITROGEN SPECIES

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Superoxide radical and nitric oxide are important signaling molecules which may influence many cellular processes. They are part of wide group of Reactive Oxygen and Nitrogen Species (ROS and RNS respectively), and are produced by cells in processes of cellular respiration or purposely by special enzymes like Nitric Oxide Synthase (NOS) or NADPH oxidase (NOX). NOS is specific enzyme which after increase of oxidative conditions can switch from production of nitric oxide to superoxide.

We compared the expression of genes that participate in ROS and RNS production and neutralization in cells of 400 cell lines. Comparison was performed on data obtained from Cancer Cell Line Encyclopedia. This analysis showed that neutralization of nitric oxide and superoxide radicals can be achieved differently in various cell lines what may influence overall changes in redox environment of cells. However some groups of redox regulating genes showed high expression in all studied cell types and these genes are part of old evolutionary pathways that regulate many cellular processes.

Maintaining proper ROS levels seems to be important factor of cell survival after exposure to ionizing radiation which increases the cellular level of ROS and induces oxidative stress. Using the time lapse fluorescent microscopy we observed changes of superoxide and nitric oxide levels and changes of their distributions in cells exposed to 4Gy of ionizing radiation. The same localization of nitric oxide and superoxide radical sources suggested that directly after irradiation and also before cell divisions, NOS switches between superoxide and NO production giving increase of both superoxide and nitric oxide in the same areas of the cell. Close localization of superoxide and nitric oxide could result in increased levels of cellular peroxynitrite and increased protein nitrosylation. Such regulation may be important for cellular proliferation and response to radiotherapy.

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[IV-4] DIFFICULTIES IN HEALING THE POSTOPERATIVE WOUND AFTER TREATMENT OF TETHERED CORD SYNDROME

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Introduction: The tethered cord syndrome (TCS) describes each low position of the spinal cord (below the L3 shank) caused by a thickened and/or end thread. It is a result of erroneously progressive embryogenesis during the process of retrograde differentiation of a set of cells in the caudal region. In the anchored spinal syndrome, symptoms such as back pain, a weakness of lower extremities, sphincter dysfunction, and sciatica-type pain may occur. In addition to neurological symptoms, these changes can be accompanied by skin stigmas, foot deformity, scoliosis of the spine, and muscular atrophy of the lower extremities.

Aim: The aim of this study was to describe two cases of neurosurgical treatment of the tethered cord syndrome and difficulties in wound healing after decompression, and also evaluation of the level of the transforming growth factor beta 1 and 2 (TGF1 and 2). Diagnosis was established based on the reported symptoms, neurological examination and Magnetic Resonance Imaging (MRI) diagnostics.

Material and methods: The first case in this study is a 28-year-old woman reporting pain in the sacro-lumbar region with radiation to the lower limbs and sphincter disorders with urine and feces incontinence. She also complained of weakness in the lower limbs that had been progressing for about a year. The second case is a 53-year-old man reporting urinary retention problems, cleavage sensory disorders, and lower back pain. MRI revealed a low-end spinal cone with a thickened terminal thread and a lesion corresponding to Intrathecal fat in both patients. Neurosurgical therapy consisted of laminectomy, opening of the spinal canal and the dura mater, followed by the cutting of the end thread. The procedure was carried out under the control of neuromonitoring. The level of transforming growth factor 1 (TGF1) in fluid fistula was determined by ELISA assay.

Results: The meninges sac and the tissues above it were closed using skin sutures. In the postoperative course, the expected improvement was observed neurologically. At the same time, the cerebrospinal fluid leaked from the wound after surgery which resulted in a fluid fistula. The microbiological culture of the fluid was negative. Treatment consisted of puncturing the fistula and then aspiration of the cerebrospinal fluid into the syringe. This procedure was repeated at regular intervals for several days until the pressure between the fluid collection and the meninges was equal and the wound was completely healed. It was not observed the significant differences in the genes expression between patients (TGF1 714.08 vs 710.03 pg/ml; TGF2 84.02 pg/ml vs 90.66 pg/ml).

Conclusion: Diagnosis of anchored spinal syndrome is an absolute indication for neurosurgical treatment. Due to the small number of tissues (muscles) in the lumbosacral region, the occurrence of difficulties in healing the postoperative wound should be considered, which should be reported to the patient.

[IV-5] ENCORAFENIB ALTERS MELANOMA CELL PROXIMITY TO THE APOPTOTIC THRESHOLD – IMPLICATIONS FOR COMPLEMENTARY THERAPEUTIC STRATEGY USING SELECTIVE BH3 MIMETICS

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Targeted therapies usually fail to achieve complete tumor regression despite targeting BRAFV600 or MEK1/2, which are the driver oncogenes in melanoma. One obstacle in attaining durable response is that targeted drugs predominantly affect fast-proliferating cells while not inducing robust cytotoxic response. The aim of the present study was to systematically investigate apoptosis-related response of patient-derived BRAFV600E melanoma cell lines to encorafenib (*Braftovi*), an inhbitor of BRAFV600 recently approved by FDA for patients with unresectable or metastatic melanoma.

We have shown that encorafenib induced apoptosis only in selected cell lines as demonstrated by real-time assessment of caspase-3/7 activation and PARP cleavage. The abundance of pro-survival proteins determined by immunoblotting was poorly associated with response of melanoma cells to encorafenib. In turn, encorafenib modulated the balance between apoptosis-regulating proteins as it significantly increased levels of BIM and BMF, and attenuated NOXA. Consequently, the extent of apoptosis was dependent on both i) cell-intrinsic proximity to the apoptotic threshold (inital mitochondrial priming) and ii) the abundance of encorafenib-induced BIM (iBIM) that has been collectively determined using standard and Dynamic BH3 Profiling, functional assays that measure capacities of peptides mimicking BH3 domains of specific pro-apoptotic proteins to depolarize mitochondrial membrane. In addition, we have shown that encorafenib modulated melanoma cell reliance on particular pro-survival proteins. While co-inhibition of MCL-1 and BCL-XL/BCL-2 was indispensable for apoptosis in drug-nave melanoma cells, encorafenib altered cell dependence to MCL-1, and reliance on either BCL-XL or BCL-2 was additionally found in cell lines that were highly primed to apoptosis by encorafenib. This translated into robust apoptosis when encorafenib was combined with selective BH3 mimetics targeting either MCL-1, BCL-XL or BCL-2.

These findings present preclinical evidence that a selective MCL-1 inhibitor potentiates encorafenib activity in BRAFV600E melanoma cell lines, whereas pharmacological inhibition of either BCL-XL or BCL-2 might be relevant but only for a narrow group of patients treated with encorafenib.

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[IV-6] HMGA1 EXPRESSION LEVEL IN CANCER TISSUE AND BLOOD SAMPLES OF NON-SMALL CELL LUNG CANCER PATIENTS – PRELIMINARY RESULTS

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Introduction: High mobility group protein HMG-I/HMG-Y, encoded by *HMGA1* is a non-histon protein that regulates inducible gene transcription. Levels of *HMGA1* products are often high in tumor-transformed cells; an increase in their concentration correlates with the advancement of the disease or the ability to metastasize. Prognostic value of the *HMGA1* expression level was demonstrated in some cancers, e.g. pancreatic, gastric, endometrial, hepatocellular cancer. However, knowledge about its significance in lung cancer is still limited. To date, no date is available concerning the meaning of the blood expression level of the HMGA1 in lung cancer.

Methods: Relative *HMGA1* expression level was assessed by means of real-time PCR in the postoperative cancer tissue samples and blood samples collected at the time of diagnosis, 100 days after and one year after surgery from 47 non-small cell lung cancer patients. Then, its association between clinico-pathological features and overall survival was evaluated.

Results: Mean *HMGA1* expression level in blood decreased systematically form time of diagnosis of cancer to one year after surgery. The blood *HMGA1* expression level measured 100 days after surgery was significantly correlated with blood platelet count, and the level measured one year after surgery was associated with tobacco smoking status of patients. Patient with high blood *HMGA1* expression level both 100 days after surgery and one year after surgery had worse overall survival than those of low expression level.

Conclusions/Novel aspect: Blood *HMGA1* mRNA level decreased in time after surgery resection of the tumor and it could be promising factor in prognostication of non-small cell lung cancer patients.

[IV-7] DIFFERENCES IN EXPRESSION PATTERN OF THE TUMOR NECROSIS FACTOR ALPHA AND ITS RECEPTORS IN THE KERATINOCYTES EXPOSED TO LIPOPOLYSACCHARIDE A

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Introduction: Tumor necrosis factor alpha and its receptors reveal different types of expression depending on the type of cells, and play a critical role in inflammation.

Aim: The goal of this study was to assessment the differences in expression profile of the mRNA *TNF-* and its receptors - *TNFR1* and *TNFR2*in the keratinocytes exposed to lipopolysaccharide A.

Material and methods: Human keratinocyte cells (HaCaT) were cultured according to the manufacture`s protocol. The first step of this research, the sulphorodamine B **c**ytotoxicity assay was performed. We used the three different concentrations of LPS: 1 g/ml, 2g/ml, and 10g/ml in comparison with untreated keratinocytes. Next, for keratinocytes exposed to 1 g/ml LPS for 2 (H2),8 (H8) and 24 hours (H24) compared to a control cell culture (C), to determinate the expression profile of mRNA *TNF-* and its receptor the microarray and RTqPCR analyses were performed. Statistical analysis comprised the ANOVA analysis and the post hoc Tukey test (*p* 0.05). Differences in genes expression were shown as a fold change (FC) of mRNA expression compared to a control.

Results: The cytotoxicity assay shown that LPS used in the range of concentration 1g/ml 10 g/ml did not any cytotoxicity effects. The microarray analysis showed changes in expression profile as follow: for TNF- H2 vs C FC=+2.78; H8 vs C FC= +4.01; H24 vs C FC=+5.17; for *TNFR1* H2 vs C FC=+3.45; H8 vs C FC=+4.01; H24 vs C FC=+4.47, and for *TNFR2:* H2 vs C FC= +3.01; H8 vs C FC=+2.11, and H24 vs C FC= +2.84. The data was validated by RTqPCR test. Statistical analysis showed the statistical significance for all comparisons (p0.05).

Conclusion: The study provided that TNF, TNFR1 and TNFR2 seem to be the useful of supplementary molecular related to inflammation in keratinocytes.

[IV-8] CYTOTOXICITY OF STYRYLQUINAZOLINE DERIVATIVES AND THEIR APPLICATION IN GLIOBLASTOMA THERAPY

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Glioblastoma multiforme (GBM) is the most common cancer among primary tumors of the brain and central nervous system accounting for as much as half of all glioblastomas. The median survival with GBM is 12-15 months and the 5-year survival rate remains below 5% despite intensive treatments. One of the possibilities to improve the therapeutic potential of and increase the arsenal of applied cancer therapies is to design and introduce novel tyrosine kinase inhibitors. Tyrosine kinases are enzymes that mediate the transfer of a cellular signal, thus participating in and controlling many important cellular processes. such as proliferation, growth, migration, metabolism and cell death. Precise regulation of these processes through kinases determines the proper functioning of cells throughout the body. It is worth emphasizing at this point that in the case of many of cancer their function is disturbed by mutations or excessive activity, so that they become promising targets for anticancer therapies. [1][2]

Recently, we have focused on searching of new derivatives that could become potential drugs in the fight against GBM. Our team has developed and synthesized a large library of styrylquinazoline (SQ) derivatives over the course of several years, from which promising inhibitors were selected for further research for glioblastomas.[3][4] The cytotoxicity of styryloquinozoline derivatives was performed using by MTS tests on human glioblastoma cancer cell lines: DKMG/EGFRvIII, U87 and human lung cancer A549 (as reference due to EGFR overexpression). In general, styrylquinazoline derivatives exhibited very high activity against DKMG and U87 cells (IC50 below 1.5 M). In the next step, we focused on a deeper investigation of the molecular mechanism of action of SQ. First, we investigated the influence of tested compounds on cell cycle progression and apoptosis induction in two cancer cell lines U87 and A549. The obtained results showed that tested compounds may inhibit the cell cycle in G0/G1 and G2/M phases. Finally, the possibility of using novel SQ derivatives in combination therapy with the known EGFR inhibitors Osimertinib and Afatinib was investigated. The analysis of the obtained results revealed several synergistic interactions between the tested inhibitors.

References: [1] 10.1016/j.ctrv.2019.101896; [2] 10.1186/s12943-018-0804-2; [3] 10.1016/j.bmc.2010.02.025; [4] 10.1016/j.ejmech.2018.12.012

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[IV-9] Evaluation of SLC14A1 gene expression in hemodialyzed patients with end-stage renal disease

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Introduction: Urea reduction ratio (URR) is one of the simplest methods to evaluate the effectiveness of haemodialysis. According to the *Health Care Financing Administration* (HCFA) achieving high URR values is associated with a reduction of the relative risk of mortality (RRM). The *SLC14A1* gene encodes a membrane glycoprotein called *UT-B1*, that functions as an urea transporter (*UT*) expressed in erythrocytes and kidney. Knowledge of *UTs* expression and function regulations is important to explain findings of abnormal water and nitrogen balance in certain clinical conditions. The aim of this study was to evaluate the expression level of *SLC14A1* gene in hemodialyzed patients with end-stage renal disease and determine the correlation between expression level of examined gene and clinical parameters of those patients.

Materials and methods: 40 samples of RNA were isolated from peripheral blood of hemodialyzed patients with end-stage renal disease from one Dialysis Station. Causes of end-stage renal disease in examined group included chronic glomerulonephritis (13 patients), diabetic nephropathy (13), polycystic kidney disease (2), hypertension (6) and unknown (6). Measured parameters were as follows: urea reduction ratio (URR), haemoglobin level, interdialytic weight gain (IDWG) in kg and %, duration of a single haemodialysis procedure and duration of renal replacement therapy. To evaluate expression level of examined gene, real-time polymerase chain reaction was performed. Statistical analysis of results was done using Statistica 13.1. p0.05 was considered as statistically significant.

Results: Expression of *SLC14A1* gene was found in each sample. There was no statistically significant difference in relative *SLC14A1* expression level between the groups according to sex ($p=0.252$) and cause of end-stage renal disease ($p=0.382$). There was no statistically significant correlation between relative level of expression of examined gene and age ($p=0.328$), URR ($p=0.675$), haemoglobin level ($p=0.344$), IDWG in kg ($p=0.421$) and % ($p=0.892$) and duration of a single haemodialysis procedure in minutes ($p=0.309$). A statistically significant correlation was demonstrated between the level of examined gene expression and duration of renal replacement therapy in months ($p=0.048$).

Conclusions: There is a statistically significant correlation between the level of *SLC14A1* gene expression and the duration of renal replacement therapy - along with a longer duration of therapy, the observed level of mRNA decreased. It is possible, that haemodialysis is a factor, that affects expression of *SLC14A1* gene. The study should be continued on a larger group of patients.

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[IV-10] MURINE BREAST CANCER: CELL MIGRATION, TUMOR GROWTH AND METASTASES IN MOUSE MODEL WITH IMAGING USING POWER DOPPLER ULTRASOUND AND BIOCHEMICAL ANALYSIS

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The ability of forming metastases is the basic survival mechanisms of cancers. Although many proteins are involved in the process, TGF-1 seems to play an important role by stimulating biochemical pathways responsible for controlling adhesion, proliferation and migration of cancer cells. One of the main processes, by which cancer cells gain migratory potential, is the epithelial-mesenchymal transition. Through this process cancer cells can detach from tumor mass and enter blood or lymphatic vessels.

The aim of this study was to investigate whether enhanced migratory potential of E0771 murine breast cancer cells, will influence the process of metastasis and associated tumor microenvironmental features (vascularization, redox state) in C57bl mice. Experiments were conducted on three experimental groups of mice with orthotropic murine breast cancer: mice inoculated with cells without treated (control), mice inoculated with cells treated with TGF-1 for 4 weeks (TGF) and mice inoculated with selected clones of cells characterized by high migratory potential (24x2). Ultrasound (Power Doppler and B-projection) were used for imaging of the tumors. Immunohistochemical staining of endothelium and for nitrotyrosine (marker for redox state) was also applied. Western Blot analysis was performed to determined expression of EMT-related proteins. Additionally, microvascularization and redox state changes in metastases were examined.

Tumors from both treatment groups grew slower and exhibited a different spatial organization of the vessels compared to the control group. In addition, the 24x2 group was characterized by higher ratio of vascularization to tumor volume. In general we observed higher density of vasculature in group treated with TGF-1 than in control. The TGF and 24x2 groups exhibited a higher nitrotyrosine level than the control group and were characterized by a higher microvessel density and size, respectively. Metastases were only seen in the treated groups, which is consistent with the examined expression of EMT-related proteins.

[IV-11] METABOLISM AS ONE OF THE WAYS TO FIGHT CANCER

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Cancer is currently one of the most frequent causes of human death in Poland and in the world. Despite numerous studies, effective cancer treatment has not yet been developed. Over the last few years, interest in compounds that can affect cell metabolism has emerged. That is why mitochondria is one of the promising objectives, and there are a number of reactions in this structure that enable the cell to function. Cancer cells usually need more glucose - an essential raw material from which energy is consumed, and therefore more glucoproteins for glucose transport (GLUTs) are present in their membranes. The differences in the metabolism of cancer cells and normal cells were described by German biochemist Otto Warburg, who proved that ATP produced by cancer cells, even in the high presence of oxygen, comes from anaerobic respiration (so-called aerobic glycolysis), in which hexoses (mainly glucose) are used as the only substrate. The first stage in aerobic glycolysis is phosphorylation of glucose (or other hexose) to glucose 6-phosphate (G6P), this reaction is catalyzed by the enzyme Hexokinase.

The aim of the study was to investigate the influence of four new hexokinase II inhibitors selected from N-benzylidenebenzohydrazide (BBH) derivatives on human liver cancer cells HepG2 and Huh-7. Cytotoxicity of the tested compounds was determined by the MTT test. This test is based on the ability of the succinate dehydrogenase enzyme to reduce yellow, water-soluble tetrazolium salt to insoluble purple formazan and This reaction occurs only in living cells. Then the inhibitory effect of the tested compounds on cell proliferation was assessed using the clonogenic assay. As part of the experiment, 5000 cells were sown into plates and then the compounds in appropriate concentrations were added. The next stage was washing the plates with water, followed by drying, photographing and colony evaluation.

The cells were incubated for 72 hours with compounds in appropriate concentrations and then their cytotoxicity was tested. The cytotoxicity effect depended on both the concentration and cell line. All derivatives showed toxic properties for both cell lines. The derivatives KSZ-008 and KSZ-009 showed higher toxic properties for both cell types. Analogous results were obtained on the basis of a clonal test. All derivatives affected the formation of colonies.

Based on our results, the new hexokinase II inhibitors appear to be very promising anti-cancer compounds, but there is a need for further research into their mode of action.

[IV-12] USEFULNESS OF FULLY AUTOMATED IN VITRO COMET ASSAY FOR GENOTOXICITY DETECTION IN SAECS FROM UPPER RESPIRATORY AIRWAYS, AS ENDANGERED FOR EXPOSURE TO AIR POLLUTING NANOPARTICLES.

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Introduction: Micro- and nanomaterials are incorporated into a wide variety of consumer products across the globe. Unintentionally or not, some of these particles, or the results of their degradation, may be introduced into the environment or come into contact with humans or other organisms (through touching, swallowing or inhaling) resulting in unexpected biological effects, including DNA damage. So it is necessary to have rapid and robust methodology that can be used to critically assess and/or predict their potential genotoxicity (ISO 10993-1). A single cell gel electrophoresis (SCGE)/comet assay is a simple and effective way for evaluating DNA damage in single cells. The alkaline version of comet assay is very sensitive, and it is used to detect even small amounts of damage, including single and double-stranded DNA breaks. The *in vivo* comet assay to assess DNA damage is globally harmonized as OECD TG 489. However, a comet test guideline that evaluates DNA damage without sacrificing animals does not yet exist. Additionally the use of targeted tissues, instead of immortalized or cancerous cells lines, leads to results more corresponding to the reaction of a whole human organism.
The goal of this study was an optimization of the *in vitro* comet assay to assess DNA damage in human upper respiratory tract.

Methods: The experiment was conducted on selected cell line of SAEC (Human Small Airway Epithelial Cells)(Lonza, Switzerland). The line was thoroughly characterized prior the experiment, including viability, the rates of adhesion and proliferation as well as uniformity. In order to minimize variation one suppliers kit of chemicals (Trevigen Comet Assay, USA) and fully automated image analysis system (CometScan Metafer, MetaSystems, Germany) were used. Cells were grow four times in triplicates. The reproducibility of the *in vitro* comet assay was evaluated with appropriate statistics.

Results: There were no statistically significant differences between biological, nor technical replicates.

Conclusion: This study suggests that SAEC/automated comet assay is an optimal *in vitro* system to assess DNA damage caused in upper respiratory airways by inhaling of micro- or nanoparticles.

[IV-13] HOW CAN P53 INHIBIT THE WNT SIGNALING PATHWAY?

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Introduction: The p53 protein is a major transcription factor, which has an impact on many processes in cells. Studies carried out all over the world show the role of p53 as a key tumor suppressor in humans and other mammals, mediating cell-cycle arrest, senescence, the repair of genotoxic damage, cell death, angiogenesis, and even Wnt signaling pathway. The exact relationship between p53 with the Wnt signaling pathway is being intensively studied. Loss of the p53 tumor suppressor protein correlates with the activation of Wnt signaling in cancer. We have observed, that two substances, which stimulate p53 in different ways: actinomycin D and nutlin-3a, when acting simultaneously (A+N) induce synergistic activation of p53 in different cancer cell lines and in normal human fibroblasts. Probably, the synergy of these molecules results from the fact that actinomycin D stimulates phosphorylation of p53 by various kinases, whereas nutlin-3a helps the kinases in efficient phosphorylation of p53 by blocking the negative regulator of p53, the MDM2 protein. The analysis of transcriptome by RNA-Seq of cancer cell lines exposed to A+N revealed a significant increase in the expression of over 2000 genes, including expression of 500 genes up-regulated at least 10-fold. Based on our results and available databases of p53 binding sites, we have found several genes not yet associated with p53 regulation. Some of these genes are negative regulators of signalling through Wnt pathway: *DRAXIN* (Dorsal Inhibitory Axon Guidance Protein) and *FRMD8* (FERM Domain Containing 8).

Material and Methods: The cells in culture (A549 derived from lung cancer) were treated with: actinomycin D and nutlin-3a. In order to confirm the hypothesis, the gene regulatory regions of investigated genes with a potential p53 binding site were cloned into pGL3-Basic reporter vector. The results of RNA-seq have been validated by Real-Time PCR. *FRMD8* and *DRAXIN* validated by qRT-PCR, were also tested for their dependence on p53, namely, we compared their expression in control conditions or following A+N treatment in A549 cells with knocked-down expression of p53 and in controls for knock-down.

Results: We have confirmed our hypothesis, that p53 affects the induction of negative regulators of Wnt signaling pathway: *DRAXIN* and *FRMD8.* The cloned promoters of investigated genes contain *bona fide* p53 response element.

Discussion and conclusions: We identified new biological link between p53 and Wnt signaling pathway.

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[IV-14] EFFECT OF GENETIC VARIATION IN NHEJ PATHWAY ON COMBINATION TREATMENT OUTCOME IN HEAD AND NECK CANCER.

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One of the most conserved pathways to repair DNA double-strand breaks (DSBs) is non-homologous end joining (NHEJ) mechanism. NHEJ is active throughout the cell cycle, repairing such harmful DNA damage. Pathway initiation occurs when Ku70/Ku80 (XRCC6/XRCC5) heterodimer binds to a strand break and recruits and activates DNA-PKcs (XRCC7). This starts a signaling cascade involving XRCC4 and XLF proteins, which then allows DNA ligase IV (LIG4) to join DNA and rebuild the strand sequence. XRCC4 is a key protein that enables interaction of LIG4 with damaged DNA and thus final ligation. Defective NHEJ results in increased sensitivity to ionizing radiation or chemotherapeutic agents. Radiation therapy (RT) and cisplatin-based chemotherapy (CHT) are the cornerstone of treatment in head and neck cancer (HNC). They induce DNA damage, the most serious of which are DSBs. Genetic variation, such as common polymorphisms (SNPs), in NHEJ genes may modulate efficiency of the repair process and possibly affect individual sensitivity to damage-inducing anticancer treatment.

This work aimed to investigate the impact of ten polymorphic variants in five core NHEJ pathway genes on clinical outcome in HNC patients treated with RT combined with cisplatin-based CHT. Variants were identified using TaqMan probes. Kaplan-Meier method and Cox proportional hazards regression were used for data analysis. We found two SNPs *XRCC5* -1401 and *XRCC7* intron 31 to be significant risk factors for overall survival (OS) in multivariate models. Moreover, patients with unfavorable genotypes for *XRCC5* -1401 and *XRCC7* intron 31 demonstrated significant, nearly 3-fold increase in risk of death as compared with the reference group ($p = 0.0018$). The presence of both adverse genotypes for *XRCC6* 3UTR and *XRCC7* intron 31 SNPs was associated with over 3-fold higher risk of locoregional failure ($p = 0.004$). The analysis also revealed that the *XRCC5* -1401/*XRCC7* intron 31 combination was an independent risk factor for shorter OS, while the *XRCC6* 3UTR/*XRCC7* intron 31 combination was the only independent predictor of poor locoregional recurrence-free survival.

Our data show that some inherited variants in *XRCC5*, *XRCC7* and *XRCC6* genes may help predict the effects of therapy in patients receiving combination treatment for HNC. Presented results highlight the special role of these SNP combinations in modulating the risk of death and locoregional relapse in the cohort. Further studies and findings validation in independent groups are necessary.

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[IV-15] COMBINED BER GENE VARIANTS ARE ASSOCIATED WITH RADIOCHEMOTHERAPY RESULTS IN HEAD AND NECK CANCER PATIENTS.

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Head and neck cancer (HNC) is a serious clinical problem as most patients present with advanced disease. In locally advanced and inoperable HNC, radiotherapy (RT) and cisplatin-based chemotherapy (CHT) are the standard treatments. Ionizing radiation and platinum compounds generate reactive oxygen species that damage DNA. Base excision repair (BER) plays a key role in removing oxidative and alkylation damage including single-strand breaks. BER pathway requires interaction of several proteins such as APEX1, XRCC1, and PARP1. APEX1 is apurinic/apyrimidinic endonuclease which cuts phosphodiester bond at the site of lesion. APEX1 also monitors the correctness of nucleotides inserted by polymerase. Moreover, it regulates many transcription factors, e.g. p53 and HIF-1. APEX1 high expression has been linked to resistance to RT and CHT. It cooperates with XRCC1 protein, that coordinates and stabilizes the repair complex. XRCC1 central domain binds to PARP1, which activity is essential at many stages of BER. Its role is primarily detection of DNA strand damage and recruitment of other proteins to this site. It is assumed that single nucleotide polymorphism (SNP) in BER genes, by modulating the levels and activity of these proteins, may influence RT and CHT outcome.

Thus, the study aim was to examine whether functional SNPs in three genes encoding essential proteins of BER mechanism affect treatment results and prognosis in 203 HNC patients receiving cisplatin-based RTCHT. We found that combination of adverse genotypes for *APEX1* 148 and *XRCC1* -77 SNPs constituted significant risk factors for poor overall (OS; $p = 0.038$) and locoregional recurrence-free survival (LRRFS; $p = 0.029$). The presence of *APEX1* 148 and *XRCC1* -77 risk genotypes was identified as an independent indicator of unfavorable OS and locoregional relapse.

Our pilot study suggests that combinations of certain SNPs in BER genes may be genetic modifiers of treatment response contributing to poor clinical outcome in HNC patients receiving cisplatin-based RTCHT. Out of six SNPs investigated, the impact of combinations of two common variants *APEX1* 148 and *XRCC1* -77 - on OS and LRRFS was observed in our dataset. These results must be validated in larger populations.

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[IV-16] HSPA2 DEFICIENCY IN KERATINOCYTES MAY AUGMENT CYTOKINE-INDUCED PSORIASIS-LIKE INFLAMMATION

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HSPA2 is one of the chaperones of the HSPA (HSP70) family. Apart from the function in regulating male germ cell differentiation, its potential roles in extratesticular cells are poorly characterized. Recently, we showed that HSPA2 is produced in the basal layer of the human epidermis. In vitro functional study allowed us to reveal that HSPA2 plays a role in regulating keratinocytes differentiation. We found that its downregulation promoted the transition of keratinocytes from the undifferentiated state to the more mature phenotype. Also, we showed that HSPA2 expression in keratinocytes in under negative transcriptional control of Hypoxia-Inducible Factor 1 (HIF-1). Psoriasis, a chronic immune-inflammatory skin disease, is characterized by uncontrolled proliferation and abnormal differentiation of epidermal keratinocytes. Our preliminary immunohistochemical analysis shows that HSPA2 is present in human psoriasis lesions.

This study was aimed at examining whether modifications in the protein level of HSPA2 in keratinocytes may influence on keratinocytes response to psoriasis-related pro-inflammatory cytokines. To mimic psoriasis-like inflammation HaCaT cells, a spontaneously immortalized epithelial keratinocyte cell line, were exposed to a specific set of cytokines. We found that pro-inflammatory stimulation caused reduction in the endogenous *HSPA2* gene expression, both at the mRNA and protein levels. This response was paralleled by an increased stability of HIF-1 subunit and elevated expression of the HIF-1-target gene (*CA9*). Interestingly, both HIF-1 and HSPA2 shifted from cytoplasm into nucleus upon cytokines stimulation. We used cell differing by the HSPA2 level cells with stable RNAi-mediated downregulation or upregulation of the HSPA2 protein production. Our results show that HSPA2-deficient HaCaT cells expressed higher levels of mRNA encoding pro-inflammatory mediators, both under standard condition and after stimulation with cytokine cocktail. In turn HSPA2 overproduction had no effect on expression of pro-inflammatory mediators.

Altogether, we showed for the first time that inhibition of the HSPA2 gene expression may be a part of pro-inflammatory response induced by psoriasis-like microenvironment. Our results allow to speculate that HIF-1 may act as a repressor of the HSPA2 gene activity in psoriatic keratinocytes. Further research should resolve above-mentioned questions.

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[IV-17] ESTIMATION OF B4GALT1-7 GENE EXPRESSION LEVELS IN MELANOCYTES AND MELANOMA CELLS

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Introduction: Changes in the profile of protein glycosylation are a hallmark of ongoing neoplastic transformation. A unique set of tumor-associated carbohydrate antigens, expressed on the surface of malignant cells, may serve as powerful diagnostic and therapeutic targets. Cell-surface proteins with altered glycosylation affect growth, proliferation and survival of those cells, and contribute to their acquisition of the ability to migrate and invade. Recently we have found that glycosylation profile of glycoproteins in melanoma cell differs from melanocytes in higher amount of Gal1-3GlcNAc- units presented on N-oligosaccharides. We hypothesized that these structures are a consequence of downregulated expression of genes encoding known human 1,4-galactosyltransferases. Therefore, the aim of this study was the comparison of *B4GALT1-7* gene expression levels in melanocytes and melanoma cells from different stages of cancer progression.

Methods: Adult melanocytes, uveal primary melanoma cells and cutaneous primary and metastatic melanoma cells were cultured in monolayers. Total RNA was extracted from cell lysates and transcribed to cDNA. Housekeeping (*GUSB, HPRT1, PGK1, RPS23, SNRPA*) and target (*B4GALT1-7*) gene-specific mRNAs were amplified with real-time qPCR reactions. The GenExpA software was use to choose the best reference and calculate target genes expression levels.

Results: We found that in melanoma cells, in particular metastatic melanoma cell line WM266-4, the expression of all *B4GALTs* genes decreases relative to melanocytes. The exception is WM793 melanoma cell line from the vertical growth phase, in which only the expression of *B4GALT6* gene decreases markedly and the expression levels of the remaining *B4GALTs* genes show no statistically significant differences compared to their expression levels in melanocytes.

Conclusions: Downregulated expression levels of *B4GALTs* genes can potentially become molecular markers for melanoma diagnostic approaches.

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[IV-18] COMPARISON THE CHROMATOGRAPHIC AND THEORETICAL LIPOPHILICITY PARAMETERS OF DANAZOL

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Lipophilicity is one of the most studied physical properties which believes to influence drugs activity. Therefore, it is commonly used in drug discovery. Determination of lipophilic properties of various drug substances can be performed experimentally using different analytical methods including thin-layer chromatography (TLC) as well as theoretically by means of various calculation methods [1]. This study aims to compare the chromatographic parameters of danazol obtained by using TLC in different chromatographic systems consisted of various stationary and mobile phases with those obtained by means of calculation methods. Danazol is a synthetic steroid used in the treatment of endometriosis and breast disease. Four mobile phases consisted of methanol-water, 1,4-dioxane-water, acetonitrile-water and acetone-water in different volume compositions and chromatographic plates precoated with RP18, RP18W, RP8 and RP2 were applied for the purpose of experimental determination of lipophilicity parameter of examined compound i.e. RMW value. The chromatographic parameters of lipophilicity were compared with theoretically values of partition coefficient (logP) obtained by using calculation methods such as ALOGPS, AClogP, miLogP, ALOGP, MLOGP, XLOGP2, and XLOGP3 [2]. On the basis of similarity analysis was stated strong connections between the value of lipophilicity parameter (RMW) determined by means of silica gel RP2 and RP18W plates and among those determined with the use of mobile phases consisted of 1,4-dioxane-water and acetone-water, respectively. The average value of theoretically obtained partition coefficient (logP) of danazol equal 3.97 0.60 correlates well with all obtained chromatographic lipophilicity descriptors (RMW) except of those determined by using acetonitrile-water. The results confirm applicability of both, chromatographic and computational procedures for the estimation of lipophilic properties of examined steroid.

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[IV-19] HEAT SHOCK FACTOR 1 (HSF1) AS A NEW TETHERING FACTOR FOR ESR1 SUPPORTING ITS ACTION IN BREAST CANCER

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HSF1 is a well-known regulator of cellular stress response induced by various environmental stimuli. It mainly regulates the expression of Heat Shock Proteins (HSPs), which function as molecular chaperones. HSF1-regulated chaperones control, among others, the activity of estrogen receptors (e.g. ESR1=ER). In our previous work, we have found that the sex hormone 17-estradiol (E2) stimulates activation of Heat Shock Factor 1 (HSF1) in estrogen-dependent breast cancer cells via MAPK signaling.

Here, to study the cooperation of HSF1 and ESR1 in estrogen signaling and the influence of HSF1 on E2-stimulated chromatin organization, we created novel experimental models based on HSF1-deficient MCF7 (ER-positive breast cancer) cell line. HSF1 was stably deactivated using CRISPR/Cas9 editing system. All cell lines (*wild type, CRISPR/Cas9 control, i.e. HSF1-proficient, and HSF1-deficient*) were untreated or subjected to E2 for indicated time to induce an estrogen signaling pathway. Co-localization and cooperation of HSF1 and ESR1 was analyzed using Proximity Ligation Assay (PLA) and global profiling of binding sites (ChIP-seq).

First, we compared the chromatin binding patterns of ESR1 and HSF1 in *wild type* cells. Only a few common binding sites (with overlapping peaks) for both transcription factors were identified in untreated cells and more than 200 after estrogen treatment. When we combined ESR1 and HSF1 ChIP-seq peaks with data from chromatin interaction analysis by ChIA-PET (data from Fullwood et al. 2009), it was evident that the HSF1-bound regions could be ESR1 interacting loci even if ESR1 binding was not detected in the same anchor. Thus, we postulate that HSF1 binding may influence the organization of the chromatin loops created after estrogen stimulation. Putative HSF1/ESR1 interactions (dramatically increased after E2 treatment) have also been found using PLA. Global analyses of ESR1 binding to chromatin in *HSF1-proficient* and *HSF1-deficient* cells revealed that in unstimulated cells, ESR1 binding was more efficient in the absence of HSF1. E2 treatment generally enhanced ESR1 binding, however, in *HSF1-deficient* cells, it was weaker and primarily manifested by enhanced binding in already existing sites. These results were confirmed by ChIP-qPCR in the new model of HSF1-deficient MCF7 (obtained by DNA-free CRISPR/Cas9 approach).

Our results suggest that HSF1 may be a new tethering factor for ESR1. Although the chromatin co-binding of HSF1 and ESR1 might be not an essential factor in the regulation of ESR1 activity, HSF1 may influence the organization of the chromatin loops created during estrogen signaling, and ultimately affect the expression of ESR1-regulated genes.

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[IV-20] The impact of SNP C1236T in ABCB1 gene on the occurrence of non-small-cell lung cancer and the grade of the lung cancer malignancy.

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Introduction: Lung cancer is currently considered to be the second most common cancer in humans. The disease can be divided into small-cell-lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The ABC transporters (ATP-binding cassette) represent the superfamily of proteins responsible for transport of substances, such as drugs, across membranes. The human multidrug resistance gene 1 (MDR1) also known as ABCB1 gene encodes the P-glycoprotein which protects from multi-drug resistance and xenobiotics. It has been suggested that some SNPs of the ABCB1 gene could significantly influence the presence of lung cancer. In recent years significant attention has been given to C to T transition at nucleotide position 1236. Previous studies prove the correlation between the C1236T genotype and the prevalence of other cancers.

Material and methods: The study involved 49 patients diagnosed with NSCLC. All blood samples came from the Copernicus Regional Specialist Hospital in Lodz and were collected from surgically operated patients. Above-mentioned 49 specimens were genotyped for SNP in position C1236T of the ABCB1 gene. The genotyping process was performed using the automated sequencing method. The obtained data was compared to results of genotyping obtained for the control group (96 blood samples from healthy people). The specimens of healthy patients had been already studied in the Laboratory of Molecular Diagnostics and Pharmacogenomics. The presented study was based on the data concerning the TNM stage, grade of histological malignancy, histological type of tumor and other information like age, gender or tobacco smoking.

Results: The polymorphism distribution in both investigated and control groups of patients was in Hardy-Weinberg equilibrium. The percentage of TT genotype in the lung cancer patients was elevated in comparison to the control group as well as the CT genotype was more frequent in the subset of healthy individuals than in the test group ($p=0.0351$). According to further analyzes, at least one C allele was significantly more frequent in healthy subjects compared to lung cancer patients ($p= 0.01547$). In terms of the histological malignancy grade, at least one C allele is significantly more common in patients with grade G3 ($p = 0.0276$). In addition, the T allele and the TT genotype are more common in patients with the G1 or G2 grade ($p=0.0155$ and $p=0.0334$ respectively).

Conclusion: Obtained results suggested that SNP C1236T of ABCB1 gene may be useful in the assessment of the risk of NSCLC and the prognosis of its malignancy. In the future, large-scale studies are warranted to appropriately investigate this possibility.

[IV-21] THE IMPACT OF STING AGONIST INTARTUMORAL ADMINISTRATION ON NEUTROPHILS (LY6G+ CELLS) INFILTRATION

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Neutrophils seem to have diversified functions in tumors, depending on the microenvironment and stage of tumor development. There is a specific type of neutrophils in tumors called tumor associated neutrophils (TANs). Stimulator of interferon genes (STING) is one of the most important component of innate immune response. Apart from protecting host from pathogens infection, it has also emerged as promise target for anti-tumor therapeutic strategies. One of the first effects observed 24h after intratumoral administration of STING agonist (23-cGAMP) is massive neutrophils infiltration to the tumor site. The aim of the work was to assess neutrophils infiltration to the tumor site after intratumoral 23-cGAMP administration.

The experiments were conducted on B16-F10 murine melanoma model and 4T1 murine breast cancer model. 23-cGAMP was administered intratumorally in a dose 2,5g/mice in B16-F10 tumors, and in a dose 5g/mice in 4T1 tumors. To visualize the neutrophils in tumor tissue, frozen sections were stained with antibody against Ly6G (clone: 1A8) antigen. Microscopic observations were performed using LSM 710 Zeiss confocal microscope. To assess percentage of Ly6G+ cells, tumors were collected. The number and phenotype of neutrophils were determined using anti-CD11b, anti-Ly6G and anti-ICAM-1 antibodies. Analyses were performed with BD FACSCanto flow cytometer. To assess approximate time when neutrophils infiltration can be observed in 4T1 tumors, luminol sodium salt was administered intraperitoneally in a dose 6mg/mice. The images were taken using IVIS Lumina System in four different time points (0h, 6h, 24h, 48h) after PBS- or 23-cGAMP intratumoral administration.

A strong neutrophils (Ly6G+ cells) infiltration 24h after STING agonist administration in B16-F10 tumors was observed. However, there were no significant differences in neutrophils (Ly6G+ cells) infiltration 24h after STING agonist administration in 4T1 tumor model. After luminol administration, there was observed strong luminescence signal at the tumor site 6h after STING agonist - 23-cGAMP administration in 4T1 tumors. Additionally, there was observed luminescence signal at the site of lungs both in control and 23-cGAMP treated mice with 4T1 tumors.

4T1 and B16-F10 tumor models seem to have different abundance of Ly6G+ cells. Neutrophils (Ly6G+ cells) infiltration after STING agonist administration seems to occur in different time points depending on the tumor model.

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[IV-22] Lack of CD44 overexpression and application of concurrent chemoradiotherapy with cisplatin independently indicate excellent prognosis in patients with HPV-positive oropharyngeal cancer

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Bockground: HPV-16 positivity in patients with squamous cell carcinoma of oropharynx (OPSCC) is associated with have better prognosis. However in more than 40% of HPV infected patients progression of cancer disease is observed, which indicates the presence of cancer cells resistant to therapy. Some studies suggest that there may be a subpopulation of cancer stem cells (CSCs), which simultaneously exhibit unlimited ability to self-renew and differentiate towards neoplastic cells. However, the relation between HPV16 infection and biomarkers of CSCs is unclear. The aim of the study was to compare the expression of CD44, CD98, and ALDH1/2 in oropharyngeal cancer patients with or without HPV16 infection, as well as to analyze the prognostic potential of selected CSCs biomarkers in these two subgroups.

Materiał and Methods: The study was performed in a group of 63 patients. HPV16 infection status was analyzed by quantitative polymerase chain reaction, while CD44, CD98, and ALDH1/2 expression by immunohistochemistry. In survival analysis, two endpoints were applied: overall survival (OS) and disease-free survival (DFS).

Results: Among 63 cancers, HPV16 infection was found in 25 tumors (39.7%), overexpression of CD44, CD98, and ALDH1/2 in 43 (68.2%), 30 (47.6%), and 33 (52.4%) cancers, respectively. In the HPV16-positive subgroup, DFS rate of 100% was observed in patients with tumors characterized by lack of CD44 overexpression and those treated with concurrent chemoradiotherapy with cisplatin (CisPt-CRT). In the HPV16-negative subgroup, none of the CSCs biomarkers evaluated in the study had any impact on OS or DFS.

Conclusion: In patients with HPV16-positive oropharyngeal cancer, lack of CD44 overexpression and application of CisPt-CRT were found to be positive prognostic factors.

[IV-23] DESIGN, SYNTHESIS AND POTENTIAL BIOLOGICAL ACTIVITIES OF 1,2,3-TRIAZOLE-DIAZAPHENOTHIAZINE HYBRIDS CONTAINING DIQUINOTHIAZINE UNITS.

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Bioactive heterocyclic compounds of natural and synthetic origin contain various heterocyclic ring systems play an important role for the development of novel scaffolds in medicinal chemistry. One of the most bioactive heterocyclic rings is a 1,4-thiazine ring. Phenothiazines, the oldest synthetic antipsychotic drug, are used in the treatment of schizophrenia and anxiety. Recent reports describe very promising anticancer and antibacterial activities, also reversal of multidrug resistance and possibility of treatment of Creutzfeld-Jakobs, Alzheimers and AIDS diseases for classical and newly synthetised phenothiazines.

The modification of the structures of phenothiazine is mostly carried out by changing the substituent at the thiazine nitrogen atom, introduction of substituents into the benzene ring, and replacing one or two benzene rings by the heteroaromatic system (pyridine, pyridazine, pyrimidine, pyrazine, 1,2,4-triazine, quinoline, quinoxaline) or bicyclic homoaromatic system (naphthalene).The most perspective modifications of the phenothiazine structure can be achieved by substitution of the benzene ring with a quinoline ring to form pentacyclic diazaphenothiazines . We have modified the phenothiazine structure with a quinoline moiety to obtain linearly and angularly condensed diquinothiazines (diquino[3,2-b;2,3-e][1,4]thiazines and diquino[3,2-b;3,4-e][1,4]thiazines). In the reactions of pentacyclic diquinodithiin or 2,2-dichloro-3,3-diquinolinyl sulfide with amines and ammonia we obtained pentacyclic linear condensed diquinothiazines. For the synthesis of angular condensed diquinothiazines, 2,2-dichloro-3,3-diquinolinyl disulfide and 3-aminoquinoline were used as starting materials. We have obtained a large group of pentacyclic diazaphenothiazines of significant anticancer, immunosuppressive and antioxidant activities.

As part of ongoing research we present an effective method for the three-step synthesis of new diquinothiazines with different phenyltriazolylmethyl substituents. The title diquinothiazines were obtained in the Huisgen cycloaddition reaction with the use of propargyldiquinothiazines, substituted phenyl azides and copper (I) iodide as the reaction catalyst. This reaction allowed to obtain these compounds with high yields. 1H NMR, 2D NMR and HR MS spectroscopic analysis was used for identify of the chemical structure of the products.

Hybridization of diquinothiazines motifs together with triazoles motifs can lead to substances with good biological properties. Based on the results obtained with the PASS program, 1,2,3-triazole-diquinothiazine hybrids can show activity as glycosylphosphatidylinositol phospholipase D inhibitor, MAP kinase inhibitor, angiogenesis inhibitor, cytokine production inhibitor. In addition, analgesic, anticancer, and antineoplastic activities are prognosis.

[IV-24] CANCER-ASSOCIATED FIBROBLASTS IN MURINE MODELS OF BREAST CANCER (4T1) AND COLON CARCINOMA (CT26)

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Introduction: Tumor microenvironment (TME), besides cancer cells, comprises cellular components, such as cancer-associated stromal cells and their extracellular products. Cancer-associated fibroblasts (CAF) may constitute over 50% of the tumor mass. Among cellular components of TME, CAF have recently gained more interest as a relevant for tumor progression. At tumor site, CAF are able to modulate tumor progression, angiogenesis, response to treatment (chemo- and radioresistance), invasion and metastasis.

Methods: BALB/c mice were inoculated with 2x105 4T1 (murine breast cancer) or 2x105 CT26 (murine colon carcinoma) cells. Two weeks after inoculation, mice were sacrificed and tumors were excised. The obtained tumors were stained to determine CAF abundancy with the following antibodies: SMA, FAP, CD90.2. Additionally, CAF were isolated from freshly removed tumors and separated using immunomagnetic sorting. CAF were then cultured and immunophenotyped.

Results: 4T1 and CT26 murine models had different CAF composition. The model of 4T1 breast cancer was rich in CAF cells, determined either as: SMA or FAP or CD90.2 positive cells. Additionally, SMA positive cells were not pericytes. The model of CT26 colon carcinoma was poor in CAF cells. SMA positive cells were closely associated with endothelial cells (CD31). SMA positive cells were rather pericytes than CAF in that tumor model. FAP positive CAF in CT26 tumor occupied far less area of tumor tissue. The level of fibroblast marker CD90.2 was also smaller. The separation efficacy of CAF (CD45-CD90.2+) from primary 4T1 tumors using immunomagnetic sorting exceeded 98%. The number of obtained CAF in a single separation ranged from 510x105. CAF in standard culture condition exhibited expression of SMA, PDGFR and vimentin and did not expressed pan cytokeratin (marker of epithelial cells).

Conclusions: Our preliminary results indicate that the distribution of cancer associated fibroblasts in 4T1 and CT26 tumors is different. Hence, this two models may response differentially on treatments directed against stromal fraction of TME. They can provide a useful tool for studies of the effect of CAF on the applied therapies and investigation of CAF-targeted anticancer strategies.

[IV-25] In ferroptosis-sensitive A549 cells, oxidative stress results in a specific lipid peroxidation death pathway

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Introduction: To date, many types and subtypes of cellular death have been discovered and described in literature [1]. One of which, ferroptosis, is characterized by lipid cell membrane oxidation, which leads to its eventual damage [2]. Each cell line reacts differently to this type of death induction some show resistive phenotype, whereas some are very susceptible to ferroptosis [3].

Materials and methods: The research aimed to determine the sensitivity of adenocarcinoma human alveolar basal epithelial cells (A549), and normal human dermal fibroblasts (NHDF) to ferroptosis induction, followed by Erastin (Sigma) 24h exposure at doses of 5 M and 10 M. A specific molecular marker mRNA level was examined by qRT-PCR (BioRAD): ACSL4 for ferroptosis and Nrf2 for antioxidative pathways, respectively. A 24h long-term spectrophotometric determination using BODIPY Lipid peroxidation sensor (ThermoFisher) was made every hour. Finally, the lipid peroxidation of the cell membranes and confluence were confirmed by bright-field microscopy analysis using JuLi BR (NanoEnTek) system and PHANTAST plugin [4] for FiJj ImageJ 2.1.0 / 1.53c [5]. Cell viability was estimated with a 24h MTT assay.

Results and conclusions: ACSL4 gene expression in ferroptosis pathway was elevated in sensitive A549 cancer cells. NRF2 gene expression in the antioxidant pathway was elevated in sensitive A459 cancer cells, which corresponds to the lipid peroxidation. Lipid peroxidation occurred in A549 cells treated with Erastin (24 h at 5M and 10M). Microscopic images of A549 showed cellular death resulted from cell membrane damage. These findings were also confirmed by cell viability measurements with MTT assay and lowered cell confluence calculated from live imaging. A549 cells are sensitive to ferroptosis induction.

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[IV-26] MICELLAR CARRIERS AND CONJUGATES BASED ON GRAFTED OR HETEROGRAFTED COPOLYMERS - PRELIMINARY IN VITRO EVALUATION FOR COSMETIC APPLICATIONS

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Introduction: Novel polymeric carriers based on the graft polymers with variety of architecture have been designed. Their main chain was based on a copolymer of alkyne modified 2-hydroxyethyl methacrylate (AlHEMA) and methyl methacrylate (MMA) or methacrylate macromonomer of poly(ethylene glycol) ethyl ether (MPEGMA). Then click reactions with an azide functionalized poly(ethylene glycol) (PEG) were carried out resulting in homografted P((HEMA-*graft*-PEG)-*co*-MMA) or with poly(-caprolactone) (PCL) or polydimethylsiloxane (PDMS) to form heterografted P((HEMA-*graft*-PCL)-*co*-MPEGMA) and P((HEMA-*graft*-PDMS)-*co*-MPEGMA). The amphiphilicity of polymers provided self-assemblies with ability to encapsulate active substances (arbutin, vitamin C). Additionally, the antioxidants ferulic acid (FA) and lipoic acid (LA) were conjugated yielding P((HEMA-*click*-FA)-*co*-MPEGMA) and P((HEMA-*click*-LA)-*co*-MPEGMA). The micellar carriers and the conjugates with the best physicochemical parameters for application in delivery of active substances in cosmetic products (i.e. hydrodynamic diameter (Dh 300 nm), active substance loading content (77%) or amount of conjugated active substance (30-40%), the percentage of substance released from the carrier (90%)) were selected for the biological research.

Methods: The permeability through artificial skin was tested in Franz diffusion cells. The cytotoxicity of new carriers and released substances were evaluated across cell lines including, human dermal fibroblasts (NHDF), human epidermal keratinocyte (HaCaT), malignant melanoma cells (Me45) and human metastatic melanoma variant of WM164 cell line (451-Lu) using MTT test. Cell cycle and cell apoptosis were evaluated by flow cytometry.

Results: The study of the permeability through artificial skin showed that the active substance was released by the carrier with a high efficiency (Rmax 68%). The active substance released from the carrier penetrated through the artificial skin only in a small amount (3-8%), while the rest with a high probability penetrates into the membrane and during the experiment (1-4 h) does not diffuse into the solution. The tested micellar carriers and conjugates did not affect the viability and proliferation capacity of healthy skin cell lines (NHDF, HaCaT). Furthermore, there no impact of carriers containing active substances on cell apoptosis or the cell cycle interruption.

Conclusions: The presented polymers after preliminary biological studies seems to be good candidates for application as carriers of cosmetic substances in products, such as mask, eye pads. Despite the satisfactory characteristics of the obtained carriers, further detailed biological tests are required for the final evaluation to apply them in cosmetology.

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[IV-27] NOVEL OLEANOLIC ACID OXIMES CONJUGATED WITH IBUPROFEN AND KETOPROFEN DOWNREGULATE THE NRF2-ARE SIGNALING PATHWAY IN HUMAN HEPATOCELLULAR CANCER CELLS IN CONTRAST TO NORMAL HEPATOCYTES

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Chronic inflammation is a key factor in the etiology of neoplastic diseases, including hepatocellular carcinoma (HCC). Nonsteroidal anti-inflammatory drugs (NSAIDs) were suggested for liver and pancreas cancers prophylaxis. That supports the idea of testing nonsteroidal anti-inflammatory drugs (NSAIDs) as potential candidates for drugs with chemopreventive and/or chemotherapeutic properties. Plant-derived oleanolic acid (OA) possesses anti-inflammatory activity and the ability to inhibit the proliferation of human hepatocellular carcinoma cells (HepG2). Its low bioavailability justifies alternating OA structure into more active derivatives. Coupling of triterpenoid analogs with NSAIDs may enhance this effect and prevent the unfavorable side effects related to NSAIDs long term use. Moreover, the pharmacological activity of OA derivatives can be increased by conjugating with conventional NSAIDs. Nrf2-ARE pathway plays key role in regulation of cellular homeostasis and is overexpressed in HCC cells.

Therefore, this study aimed to evaluate the effect of novel oleanolic acid oximes (OAO) conjugated with ibuprofen (IBU) and ketoprofen (KET) on Nrf2-ARE signaling pathway in HepG2 hepatoma cells and THLE-2 normal human hepatocytes.

HepG2 and THLE-2 cells were incubated for 24h with tested compounds in the concentrations selected based on MTT assay. The activation of Nrf2 was assessed by the evaluation of its translocation into the nucleus and binding to ARE sequence by the ELISA assay. Expression of Nrf2 and SOD-1 was evaluated by RT-PCR and Western blot method.

Cell viability was affected mostly by 3-ibuprofenoxyiminoolean-12-en-28-oic acid morpholide and 3-ketoprofenoxyiminoolean-12-en-28-oic acid morpholide in both cell lines. The level of Nrf2 in the nucleus and binding to ARE sequence was decreased in HepG2 cells, in contrast to the effect exerted by those compounds in the THLE-2 normal hepatocytes. In HepG2 cells, a decreased expression level of SOD-1, both on mRNA and protein levels, was observed.

These results indicate that conjugation of IBU and KET with novel OAO may protect cancer cells against chemoresistance through inhibition of the Nrf2-ARE pathway and, at the same exert a chemopreventive effect in normal hepatocytes. Those novel compounds might be considered the potential modulators of hepatocellular carcinoma therapy and chemopreventive agents.

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[IV-28] COMBINATION OF VASCULAR DISRUPTING AGENT (CA4P) AND STING AGONIST (CGAMP) IN MURINE BREAST CANCER THERAPY

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Tumor blood vessel formation is a key process for tumor expansion. Hence, targeting tumor blood vessels seems to be an effective solution in anti-cancer therapy. One of the strategies targeting tumor blood vessels is the use of vascular disrupting agents, such as combretastatin A4 phosphate (CA4P). CA4P by depolymerization of tubulin in endothelial cells induces cell apoptosis which leads to blood vessels destruction, and following tumor core necrosis. However, after a short period of time, tumor re-growth from remaining viable tumor cells is observed. Therefore, in order to reduce tumor volume and prevent its re-growth, it seems to be necessary to combine vascular disruptive agents with other anti-cancer agents. Thus the combination of CA4P with one of the most prominent immunostimulatory agent STING agonist (23-cGAMP) seems to be rationale. Binding of the 23-cGAMP molecule to the STING protein results in type I IFN production and strong antitumor immune response. The aim of the work was to check the effectiveness of the CA4P and 23-cGAMP combination therapy and to study the role of immune cells in achieving the therapeutic effect.

Experiments were conducted on 4T1 murine breast cancer model. In the study, CA4P (vascular disrupting agents) was combined with 23-cGAMP (STING agonist) in two therapeutic schemes. In the first one, 23-cGAMP was administered one day prior to CA4P administration. In the second scheme, contrariwise. CA4P was administered intraperitoneally in a dose 50 mg/kg mice body weight. 23-cGAMP was administered intratumorally in a dose 2,5 g/mice.

In both combination tumor growth inhibition was observed. The highest number of CD8 cytotoxic T lymphocytes was observed in tumors after 23-cGAMP monotherapy. In tumors after combination (both schemes) there was not observed significant increase in CD8 T cells influx. The highest number of natural killer cells (NK) was observed in tumors after combination therapy where 23-cGAMP was administered prior to CA4P. Additionally, there was observed decrease in tumor blood vessel after CA4P monotherapy and increase after 23-cGAMP monotherapy and combination therapy where CA4P was administered prior to 23-cGAMP. We have also observed the changes in M1 and M2 macrophages populations after conducted therapies.

The proposed therapeutic strategy where 23-cGAMP was administered prior to CA4P seems to be beneficial compared to both monotherapies.

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[IV-29] LYMPHOCYTE SUBPOPULATIONS AND CYTOKINE PROFILE ANALYSIS OF TRANSPLANTATION MATERIAL COLLECTED FROM HEALTHY BONE MARROW DONORS.

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Introduction: Hematopoietic stem cells (HSC) transplantations are an important part of the many hematological malignancies therapies, often as a last resort for patients suffering from leukemias or lymphomas. HSCs reside in bone marrow but are capable of migrating to peripheral blood and later coming back to the bone marrow cavities. HSCs can be collected by aspiration directly from bone marrow or by the peripheral blood leukapheresis. There is evidence that the method of collection can affect the transplantation procedure and the following course of treatment. Higher percentages of Graft vs Host Disease occurrence was described in patients undergoing peripheral blood stem cell transplantation. It can be explained with both the different composition of the transplantation material and functional state of transplanted cells themselves. The number of cells required for transplantation is determined by various factors and it can only be established after the end of the collection. Sometimes the collected number of cells is insufficient. It would be greatly beneficial to develop a method to predict the number of collected HSCs before the collection occurs.

Methods: Material consists of routinely collected peripheral blood and bone marrow samples obtained during the bone marrow collection for following transplantation. Samples were obtained from 49 healthy bone marrow donors undergoing collection procedure. Samples were analyzed for total blood count, cytokine levels by ELISA assay and lymphocyte subpopulations by flow cytometry. ELISA panel consisted of IL-1, IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-, TGF- and TNF- assays. Flow cytometry panel evaluated total lymphocytes, total T-cells, T-helper cells, Cytotoxic T-cells, T-regulatory cells, B-cells, NK-cells and CD34+ HSCs.

Results: The results obtained after comparing peripheral blood and bone marrow of healthy bone marrow donors provided the following. Statistical analysis revealed significant differences in all of the white blood cells parameters and almost all of the red blood cells and platelets parameters. Cytokine levels were significantly different for IFN-, IL-1, IL-2, IL-4, IL-6, TGF- and TNF-. No significant differences for IL-2, IL-10 and IL-17A were observed. Materials exhibit a different percentages of total lymphocytes, T-cells, T-helper cells, NK-cells and B-cells in bone marrow and blood samples. Statistical analysis revealed a number of correlations between lymphocyte subpopulations, cytokine levels and blood count parameters. Statistically significant correlations between lymphocytes subpopulations and number of HSCs in transplantation material were observed.

Conclusions: Based on the above findings it is possible, within limits, to predict the number of efficiently collected CD34+ HSCs deriving from bone marrow by analysing donors blood lymphocyte subpopulations. This hypothesis needs further verification with prospective studies.

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[IV-30] NEW MELPHALAN ANALOGS AS COMPOUNDS WITH IMPROVED ANTIPROLIFERATIVE PROPERTIES

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Multiple myeloma (MM) constitute 10% of all haematological malignancies, being the second most common hematological neoplasm for which chemotherapy is one of the most important pharmacological treatments. High dose melphalan followed by autologous stem cell transplantation remains the standard of treatment for transplant-eligible patients with multiple myeloma. Due to high systemic toxicity and the occurrence of drug resistance, there is a strong need to develop new therapies.

Several melphalan derivatives showing improved cytotoxic activity in human tumor cells - THP-1, HL-60 and RPMI-8226 have been synthesized. Chemical modifications included both the carboxyl and the amine groups. The resulting compounds were methyl and ethyl esters (EE/EM-MEL) and methyl ester of melphalan additionally modified with the morpholine group (EM-MOR-MEL). The cell cycle distribution analysis was performed using the flow cytometry method, measuring the fluorescence at excitation =488 nm. Cell populations at particular phases of the cell cycle were quantified from DNA histograms using Flowing Software.

The antiproliferative properties of antitumor compounds result primarily from their ability to inhibit the cell cycle. All tested derivatives lead to the accumulation of cells in the G2/M phase at the expense of the G1 and S phases. A significant increase in the number of cells was also observed in the subG1 phase. The strong increase in subG1 fraction in the tested cell lines after incubation with the tested analogues confirms the pro-apoptotic properties of the compounds. At the same time, all tested derivatives caused the accumulation of cells in the G2/M phase. It is noteworthy that the number of cells arrested in G2/M increased several-fold, while the population of G1 cells decreased, thereby causing a reversal of the cell cycle profile in preparations treated with melphalan derivatives. Cell cycle arrest in this phase is related to the mitotic catastrophe process. In previous experiments we showed that the tested melphalan derivatives have strong pro-apoptotic properties. The assessment was made on the basis of morphological and biochemical changes as well as the analysis of caspase-3/7, -8 and -9 activity. Strong increase of subG1 fraction in the tested cell lines after incubation with the tested analogues confirms the pro-apoptotic properties of the compounds, the results of the cell cycle analysis correlated with the results of the analysis of apoptosis induction.

In conclusion, our study showed that the tested melphalan analogues exhibit enhanced antiproliferative activity. Significant changes in the distribution of the cell cycle following treatment with the new analogues compared to cells treated with melphalan were observed. This indicates that the proposed modifications can serve as an effective therapeutic drug system and be a response to the problems associated with the currently used melphalan.

[IV-31] GENOME-WIDE ASSOCIATION STUDY IDENTIFIES NEW GENETIC VARIANTS ASSOCIATED WITH COLORECTAL CANCER IN A POLISH POPULATION

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Introduction/Rationale: Colorectal cancer (CRC) is one of the most common cancers in the world. Approximately 90% of CRC cases are sporadic without family history or genetic predisposition, while in less than 10% a causative genetic event has been identified. Susceptibility genes and the underlying mechanisms for the majority of risk loci identified by genome-wide association studies (GWAS) for CRC risk remain largely unknown. We have conducted GWAS to identify new genetic variants associated with CRC in a Polish population.

Methods: Pooled-DNA samples from 432 patients with CRC (18 pools) and 672 controls (28 pools) were analyzed individually on Illumina Human Omni2.5-Exome BeadChip microarrays. TaqMan SNP Genotyping Assays (Thermo Fisher, USA), a SensiMix II Probe Kit (Bioline Ltd., UK) and a 7900HT Real-Time PCR system (Thermo Fisher, USA) in a 384-well format was used for validation of GWAS findings with individual genotyping. The loci were chosen that were represented by blocks of SNPs associated with CRC at the *p* 5 103 for which the interval between all pairs of adjacent SNPs was 30kb. From each of the independent loci, the most strongly associated SNP (at *p* 104) was selected as an index SNP for further verification with individual CRC (*N* = 465) and control (*N* = 1548) DNA samples and stepwise forward logistic regression analysis. All computations were performed according to R environment statistics.

Results: Based on GWAS findings, seven susceptibility loci were selected for further verification. Five of the analyzed SNPs exhibited differences in allele and genotype frequencies between the CRC and control groups, significant after the Benjamini-Hochberg algorithm adjustment for multiple testing (*padj* 0.05). All these associations have not previously been reported for CRC. The strongest association was observed for rs10935945 in long non-coding RNA variant *LINC02006* at 3q25.2 (*padj* = 1.26 105 and 1.29 105 for allele and genotype frequencies, respectively). The next three SNPs revealed allelic associations at *p* 3.92 104. Apart from rs10935945, three other SNPs were located within gene regions (rs17575184 in *NEGR1* at 1p31.1, rs11060839 in *PIWIL1* at 12q24.33 and rs12935896 in *BCAS3* at17q23.2) and one was at intergenic location (rs9927668 at 16p13.2). The minor allele of two SNPs was associated with an increased risk of CRC development and three SNPs showed a protective effect. The effect size of all five susceptibility loci was relatively moderate (OR 1.45 or 0.77).

Conclusions/Novel aspect: Our study revealed five new variants associated with CRC susceptibility. Three variants were protective and two predisposed to the CRC development.

[IV-32] CLINICOPATHOLOGICAL AND PROGNOSTIC SIGNIFICANCE OF SPDL1 EXPRESSION IN PANCREATIC DUCTAL ADENOCARCINOMA

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer death worldwide and simultaneously one of the most lethal malignancy as its incidence rate is almost equivalent to mortality. According to the above, PDAC remains a serious both diagnostic and therapeutic challenge. Therefore, based on the knowledge that tumorigenesis may be accompanied by genetic alterations such as defects in chromosome segregation process leading to genome instability (GIN), we selected spindle apparatus coiled-coil protein 1 (SPDL1) involved in mitotic spindle formation and the segregation of chromosomes as a potential biomarker of PDAC the impaired expression of which may be associated with PDAC pathogenesis and the shorter survival time of PDAC patients.

The aim of the study: To evaluate the immunohistochemical expression of SPDL1 and *in silico* analysis of *SPDL1* gene expression in PDACs and normal peritumoral pancreatic tissues in relation to clinicopathological features, including patients survival.

Materials and Methods: SPDL1 expression level was examined by immunohistochemistry on tissue microarrays containing 68 PDAC specimens and 65 non-tumor adjacent tissues. In turn, *in silico* analysis of *SPDL1* gene expression was performed with mRNA-seq data of PDAC tumor and healthy samples from the TCGA and GTEx, respectively, which were obtained from public sources. All data were analyzed using suitable statistical methods.

Results: Both spindly protein and mRNA levels were markedly up-regulated in PDAC tissues relative to non-cancer normal tissues (p *0.0001*). Moreover, Kaplan-Meier analysis of our cohort showed that high expression of SPDL1 was significantly associated with better overall survival (OS; *p = 0.01;* 294 days *vs.* 647 days). In the multivariate Cox analysis, overexpression of SPDL1 was found to be an independent prognostic marker for better OS (HR = 0.42, 95% CI 0.18-0.99; p = 0.046). Contradictive to protein expression, Kaplan-Meier survival analysis of the TCGA cohort revealed that the high *SPDL1* expression group showed a significantly shorter median OS than the low expression group (695 days *vs.* 334 days; p *0.0001*). In the multivariate Cox analysis, *SPDL1* remained an independent poor prognostic factor for OS (HR = 2.40, 95% CI 1.53-3.75; p *0.0001*). Furthermore, there was no association between both spindly protein and mRNA levels and analyzed clinicopathological traits (p *0.05)*.

Conclusions: The above data suggest that SPDL1 is prognostically valuable for PDAC. However, further studies including *in vitro* experiments are required not only to understand the mechanism of action of the presented marker but also to explain discrepancies between mRNA and protein levels and their impact on PDAC patients survival.

[IV-33] COMBINATION OF OLAPARIB AND METFORMIN IN BRCA WILD TYPE OVARIAN CANCER - CYTOTOXICITY ASSESSMENT

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The initial, standard-of-care, adjuvant chemotherapy in epithelial ovarian cancer is usually a platinum drug, such as cisplatin or carboplatin, combined with a taxane. However, despite surgical removal of tumor and initially, high response rates to first-line chemotherapy, around 80% of women will develop cancer recurrence. Effective strategies are necessary to improve prognosis. Olaparib (AZD2281) is the first Food and Drug Administration (FDA) approved poly (ADP-ribose) polymerase inhibitor (PARPi). It may increase the level of replication stress and in consequence, lead to genome instability and cell death. However, its effectiveness may be limited to patients with BRCA mutation and unfortunately, PARPi resistance is common. Metformin is a biguanide, oral hypoglycemic drug and is widely used in the treatment of type 2 diabetes but may also be used in metabolic and polycystic ovarian syndrome. Metformin inhibits cell proliferation in several human cancers like pancreatic cancer, thyroid cancer, gastric carcinoma, endometrial carcinoma and ovarian cancer. Therefore, combining olaparib with metformin may be a new treatment option for ovarian carcinomas without BRCA mutation.

The aim of the study was to sensitize ovarian cancer cells to olaparib by using the combined action of olaparib and metformin. We determined cytotoxic effect of drugs in two ovarian cancer cell lines the human OV-90 (human malignant papillary serous carcinoma, ATCC CRL-11732) and SKOV-3 (human ovarian adenocarcinoma, ATCC HTB-77). OV-90 carries a mutation in the TP53 gene. The cytotoxicity was determined with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and clonogenic assay.

Olaparib at increasing concentrations did not cause high decreases in survival rate. Metformin was more cytotoxic than olaparib for ovarian cancer cells and caused a dose-dependent decrease in cell viability. Combined treatment significantly decreased viability after 24 h, starting with a concentration of 20 M + 20 mM (O+M) for both cell lines. The clonogenic assay confirmed the results of the MTT assay. The drug synergism in most of the tested combinations was observed.

Combining PARP inhibitor with metformin enhances its anti-proliferative properties in ovarian cancer cells. Our results confirm that the combination regimen may be a promising option in treating BRCA-wild type EOC.

[IV-34] SYNTHESIS AND CHARACTERIZATION OF PIL GRAFT COPOLYMERS BEARING CLAVUNATE ANIONS: EFFECT OF POLYMER STRUCTURE ON RELEASE AND PHYSICOCHEMICAL PARAMETERS.

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Polymer carriers are mainly used in medicine to improve therapeutic efficacy of drug delivery. Nowadays these systems attract a lot of attention due to their ability of controlled drug administration. Among carriers there are conjugates, that can attach the drug by chemical bond. It is well known that solubility of the conjugated molecules is enhanced. One of the strategy is introduction of ionic pharmaceutics by the ion exchange reaction in a poly(ionic liquid) (PIL), including that with a graft topology. Advantageous for polymer carriers is possibility of regulating the release kinetics profiles and physicochemical parameters through the polymer structure, i.e. the grafting degree, the length of backbone and grafts [1][2].

The presented work is focused on anion exchange reaction with the use of potassium clavunate (KCLV). The polymer matrix was designed by combination of [2-(hydroxyethyl) methacrylate and methyl methacrylate copolymer as the main chain, and (methacryloyloxy)ethyl]trimethylammonium chloride and methyl methacrylate copolymer as the grafted chains (P(MMA-*co*-(HEMA-graft-(TMAMA/Cl-*co*-MMA)))). The *grafting from* strategy was carried out in the presence of multifunctional macroinitiators with two different contents of initiating groups to receive copolymers with diverse grafting degrees. Then the anion exchange reaction with pharmaceutical ion, i.e. clavulanate (CLV-) was accomplished.

The polymer self-assembling was determined by Critical Micelle Concentration (CMC). It was noticed that after anion exchange the CMC increased (0.005-0.026 mg/mL for Cl-; 0.012-0.045 mg/mL for CLV-). Furthermore, the hydrophilicity level of graft copolymers, evaluated by the water contact angle (WCA), decreased with the increase of trimethylammonium units and after exchange (44-68° for Cl-; 29-64° for CLV^{-}). These nanocarriers with Cl- and CLV- reached sizes ranged in 18-368 nm and 21-357 nm, respectively. Effective release of anionic drug was observed up to 3h, whereas after 48h the carriers were able to remove 26%-73% of CLV, which corresponded to 11-31 g/mL.

These studies proved that the grafted systems used as conjugates with pharmaceutical anions seems to be a good candidates for drug delivery. It has been shown that a relatively high amount of the drug can be released in experiment *in vitro*. The rapid release should be beneficial in the antibacterial treatment.

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[IV-35] CIRCULATING INNATE LYMPHOID CELLS (ILCS) IN PERIPHERAL BLOOD OF DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) PATIENTS.

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Adaptive immunity has been shown to play a critical role in the elimination of tumor cells, but still little is known about the role of innate lymphocytes in this process. The understanding of the cancer development and tumor microenvironment have been recently expanded with the recognition of the important role of innate lymphoid cells (ILCs). ILCs are functionally resemble T lymphocytes, both in cytotoxicity and cytokine production. But, in recent studies ILCs have emerged as novel important immune effector cells, playing a critical role also in tumor immunosurveillance, immune responses and tissue homeostasis.

Our project relates to important, but weakly explored issues in immunology, concerning the ILCs role in tumor immunity in diffuse large lymphoma B cell (DLBCL) patients. ILCs subpopulations (ILC1, ILC2, ILC3) have been already studied in some cancer settings, yielding ILC-specific or disease specific results, their significance in lymphoproliferative disease still awaits answer. This study examined frequency, subset distribution and function in newly diagnosed DLBCL patient (nodal and extra-nodal) and compare it with blood specimens from healthy donors.

Peripheral blood collected from 33 adult patients positively verified for DLBCLs diagnosis in Department of Bone Marrow Transplantation and Oncohematology, National Cancer Institute of Oncology in Gliwice was used. Control group consisted of 34 healthy bone marrow donors. Total circulating ILCs and subtypes was identified as percentage of total Lin CD127+, ILC1 Lin- CD127+ CD117- CRTH2-, ILC2 - Lin- CD127+ CD117+ CRTH2+, ILC3 - Lin- CD127+ CD117+ CRTH2-. Lineage cocktail for depletion T cells, B cells, NK cells, monocytes, granulocytes, basophiles, DC and hematopoietic progenitor cells included: anti-CD3, anti-CD4, anti-CD16, anti-CD19, anti-CD8, anti-CD20, anti-CD34, anti-CD15, anti-CD20, anti-CD33, anti-CD203c and anti-TCR. A minimum of 106 MNCs was acquired on a FACSCanto II flow cytometer (Becton Dickinson). Data was analyzed using Diva software (Becton Dickinson).

In the group of patients we observed significantly lower level of total circulating ILCs ($p = 0{,}004$) as well as ILC1 ($p=0{,}01$) and ILC3 ($p=0{,}009$) compared to the control group. Analysis of the ILC2 subpopulation showed no significant differences.

The final aim of the following project is to determine the immunological role of each ILCs subpopulations individually in response to lymphoma cells influence.

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[IV-36] INTRAMUSCULAR ADMINISTRATION OF HUMAN ADIPOSE DERIVED MESENCHYMAL STROMAL CELLS ACCELERATE MUSCLE REGENERATION IN A MURINE MODEL OF HINDLIMB ISCHEMIA

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For several decades, adipose derived mesenchymal stromal cells (ADSCs) have been extensively studied for their therapeutic potential across a wide range of diseases. In the preclinical setting, ADSCs demonstrate consistent ability to promote tissue healing, down-regulate excessive inflammation and improve outcomes in animal models. The critical limb ischemia is an advanced stage of a peripheral artery disease in which stenosis and occlusion of the arteries occur. One of the treatment method for this disease is therapeutic angiogenesis. The therapy utilizes growth factors or cells that secrete factors which stimulate the formation of new blood vessels and regenerate damage muscle fibers. There are still many inaccuracies regarding the site, timing, or appropriate number of cells administered in the treatment for critical limb ischemia.

The aim of present work was to compare three methods of human ADSCs administration on the muscle regeneration in murine model of hindlimb ischemia.

Unilateral femoral artery ligation was performed on males of the C57BL/6NCrl strain (8-10 weeks of age). hADSCs were administered into the gastrocnemius muscles: a) intramuscular into hindlimb where unilateral femoral artery ligation was performed, b) intravenous into tail vein, c) contralateral - into contralateral limb, where unilateral femoral artery ligation was absent, d) control mice - subjected only to femoral ligation procedure (ischemic). The analyzes were carried out on muscles (quadriceps and gastrocnemius) from the hindlimb where unilateral femoral artery ligation was performed. 7 and 14 days post injury (dpi), the muscles were obtained, fixed in liquid nitrogen and stained using immunohistochemistry. Microscopic observations were performed using a Nikon Eclipse 80i microscope.

Intramuscular administration (into hindlimb where unilateral femoral artery ligation was performed) of human adipose derived mesenchymal stromal cells accelerate quadriceps and gastrocnemius muscles regeneration in a murine model of hindlimb ischemia. The greatest number of muscle fibers and the greatest amount of regenerative muscle fibers was observed in gastrocnemius muscles 7 days after injury.

This observation might potentially lead in the future to an attractive novel strategy of administration of the mesenchymal stromal cells.

This study was financed by Grant UMO-2018/29/N/NZ4/01689.

[IV-37] NEW MELANOMA PROGNOSTIC MARKERS AND THERAPY MONITORING BY MEANS OF LABEL-FREE METHODS

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Malignant melanoma is one of the most common type of skin cancer originating from melanocytes. Its diagnosis is difficult and generally relies on subjective assessments. In particular, there is a lack of quantitative methods allowing melanoma diagnosis as well as monitoring melanoma therapies [1].

Changes in the glycosylation pattern of cells are often associated with various diseases including cancer. Lectins are proteins recognizing various carbohydrate structures with specific binding affinities [2]. Lectin-carbohydrate interactions can be found in a wide variety of regular biological processes (adhesion and migration) or can be applied for detecting glycans present on cancerous cells [3].

Label-free methods like the quartz crystal microbalance with the dissipation monitoring (QCM-D) may be applied for the differentiation of tumor cells in different progression stages based on lectin-glycan interaction as well as viscoelastic properties of cells [4, 5]. On the other hand, the only hope for patients with advanced melanoma is the inhibition of metastasis. Anandamide is an endocannabinoid, that has a potential anti-tumor effect [6].

In our studies, the real-time binding between lectin Concanavalin A and glycans present on the surface of melanoma cells from different stages of cancer progression was examined by label-free methods and compared with that observed on melanocyte surface. Two procedures have been developed to detect the differences in the cellular glycosylation profile using cell-based sensors. The observed changes in lectin-glycan interactions among the studied cell types (melanoma cells and melanocytes commercial and self-isolated) enabled an early cancer detection as well as the distinction of melanoma cell types. Two prognostic markers were identified based on the lectin-glycan interaction measurements the affinity and the viscoelastic index. The treatment of metastatic melanoma cells with anandamide results in a change in the glycosylation profile towards the less aggressive forms of melanoma. The established methodology can be applied as an additional diagnostic/prognostic procedure for patients with melanoma skin cancer.

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[IV-38] ANALYSIS OF THE CYTOTOXIC EFFECT INDUCED BY TEMOZOLOMIDE AND AFATINIB ON PRIMARY GLIOBLASTOMA CELL CULTURES

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Primary glioblastoma (GB) cell cultures provide a valuable model for *in vitro* studies of GB cell sensitivity to compounds of interest to scientists and physicians. GB is the most aggressive malignant primary brain tumor and is therefore classified as WHO grade IV. To date, there is a list of compounds that have been tested for the treatment of glioblastoma, but none of them is considered fully effective, and the average survival time of GB patients has not lengthened much over the years. Afatinib is an irreversible tyrosine kinase inhibitor, that blocks activation of EGFR, HER2, HER4 and EGFRvIII by binding to their ATP binding site. Afatinib showed limited activity in clinical trials in patients with GB. Temozolomide is the standard chemotherapeutic agent used in GB patients and it works through DNA alkylation. The aim of the studies was to determine the impact of afatinib and temozolomide on cell viability and to analyze EGFR and EGFRvIII expression in the GB cells.

The experiments were carried out on primary GB cell cultures that were derived from GB samples obtained after neurosurgical tumor resection. EGFR and EGFRvIII expression was assessed by real-time RT-PCR. Moreover, immunocytochemical analysis was also performed to confirm the origin of the cells. Cells were incubated for 48 hours with temozolomide or afatinib, then cell viability was assessed by MTS and IC50 values were calculated.

The analyzed cells were characterized by different status of mRNA for EGFR and EGFRvIII. In the case of cells incubated with temozolomide, it was possible to determine the IC50 value for the tested compound in all primary cell lines. Contrary to the effects of temozolomide, only some primary GB cell lines showed sensitivity to afatinib. The results of afatinib treatment are interesting in connection with the results of EGFR and EGFRvIII expression in GB cells. There is no strict correlation between the sensitivity of cells to afatinib and the state of the analyzed genes.

Since primary GB cell lines showed different sensitivity to treatment with temozolomide and the mechanism of action of this compound is well known, it would be interesting to compare the results of patients treated with temozolomide with the *in vitro* efficacy of this compound on primary cell cultures derived from patients tumor. The same approach could potentially be used for afatinib treatment, but is not approved for the treatment of GB.

Such an approach could potentially allow a predictive assessment of the effectiveness of chemotherapy in patients with GB. Based on the performed studies, the analysis of the cytotoxicity of simultaneous incubation of cells with afatinib and temozolomide and the subsequent evaluation of the cytotoxicity caused by these compounds seems also to be interesting.

[IV-39] PHYTOCHEMICALS AND THEIR COMBINATIONS AFFECT THE CELL CYCLE, APOPTOSIS AND PROLIFERATION OF HEPG2 CELLS WITH DIFFERENT EFFICIENCY

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Hepatocellular carcinoma (HCC) is the fourth most common cause of cancer-related death worldwide. Phytochemicals such as xanthohumol (XAN), phenethyl isothiocyanate (PEITC), indolo-3-carbinol (I3C), and resveratrol (RES) possess anti-tumorigenic activities and the ability to inhibit the proliferation of human hepatocellular carcinoma cells. Recent studies indicate that using combinations of phytochemicals may enhance this effect.

The aim of this study was to evaluate the effect of the above-mentioned phytochemicals and their combinations on the cell cycle distribution, induction of apoptosis, and proliferation in HCC derived HepG2 cells.

The cells were treated with these compounds, alone or in combination, at the concentration of 10 M or 20 M, selected based on MTT assay. Muse Cell Analyzer was used to flow cytometric assessment of cell cycle progression by propidium iodide staining, induction of apoptosis by Annexin V binding to cells externalizing phosphatidylserine, and proliferation based on Ki67 expression.

XAN and PEITC and their combination similarly as Topotecan, used as a positive control, reduced the percentage of the cells in G0/G1 and increased in G2/M. This combination also decreased the total number of apoptotic cells and showed the most pronounced antiproliferative effect.

These results indicate that XAN, PEITC, and their combination are more effective inhibitors of cell proliferation, and inducers of cell cycle arrest, than the other tested phytochemicals. Thus, the mixture of XAN and PEITC might be considered as a potential HCC chemopreventive and/or therapeutic agent.

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[IV-40] IDENTIFICATORY OF GENOME WIDE DISCORDANT METHYLATION PATTERNS OF THE ADJACENT CPG SITES

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INTRODUCTION: With the advances of the genome wide methylation screening technologies including Illumina BeadChip technique and next generation sequencing (NGS), increasingly more detailed maps of the genome wide methylation patterns of both healthy and pathologically changed tissues are available. At the same time, the increasing number of the disease case-control studies based on mining of the genome wide methylation profiling data identify a significant number of the methylation changes at single CpG sites. Those changes do not seem to involve regions of high density CpG sites such as CpG island (CGI) for which a general principle of concordant co-methylation is observed (Lovkvist, Nucleic Acids Res, 2016; Affinito, Genomics, 2020). According to that phenomenon the probability of the methylation level of the CpG site is not random and is strongly influenced by distance to a next CpG site, with CpG sites in proximity of less than 50bp displaying identical methylation status (Affinito, Genomics, 2020). In general terms studies of the genomes at the single CpG site resolution are in agreement with the co-methylation phenomenon and all the CpG sites in the regulatory elements such as CGIs are either methylated or non-methylated.

METHODS: We have developed a bioinformatics approach allowing to identify single CpG sites with more than 30% methylation level difference between adjacent CpG sites (in the proximity of up to 50bp) and used Illumina MethylationEPIC BeadChip data from 65 healthy blood samples to investigate co-methylation patterns of the consecutive CpG sites in the proximity of less than 50bp.

RESULTS: Overall, we identified 2024 CGs with methylation levels different more than 30% from the methylation of the nearby CpG sites (up to 50bp distance). We then mapped those CpG sites to the genome and found that majority of them (1815 CpGs) are located outside of CGIs.

CONCLUSIONS: Our preliminary results indicate that a substantial number of the CpG sites in human genome does not follow the general principle of the co-methylation and those CpG sites are located mainly outside of CGIs. The functional significance of this phenomenon is not known and needs to be investigated.

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[IV-41] Regeneration of rare T lymphocytes in patients after allogeneic haematopoietic stem cell transplantation (alloHSCT) from fully vs half matched donor

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Introduction/Rationale: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is an effective and often the only therapeutic option in haematopoietic malignancies. The alloHSCT procedure is preceded by the high-dose radio- or chemotherapy, which ultimately damages the patients defective hematopoiesis. CD34 + cells from donors material provide the restoration of hematopoiesis and very slow regeneration of patients immunity. During the first months after alloHSCT immune recovery relies on the peripheral expansion of donor T cells present in the transplanted cell material. These are mainly memory T cells and naive T cells that become alloreactive in contact with the host cells. The most affected and the last to restore after alloHSCT are CD4+ T cells. Especially valuable, but also hardly renewable are so-called recent thymic emigrants (RTE) which are produced in the thymus and are the earliest T lymphocytes detected in the blood. While detailed T-cell reconstitution has been fairly well described for alloHSCT from a donors fully matched in HLA (*human leucocyte antigens*), this aspect is still poorly understood for alloHSCT from donor matched in only half of the HLA (so called haploidentical or **haploHSCT**). Although rarely performed, haploHSCT is becoming increasingly important for patients who do not have HLA-matched donor. Taking into account the above premises, we undertook a comprehensive evaluation of rare T lymphocytes reconstitution after HSCT from haploidentical donor (haploHSCT) vs fully matched unrelated donor (**MUD-HSCT**).

Methods: The study was performed on 40 patients who underwent alloHSCT (haploHSCT or MUD-HSCT) as a therapy for high-risk hematopoietic malignancy. Immunophenotypic assessment was aimed to detect the following T cell subpopulations: Th (CD4+), Tc (CD8+), naive/memory T lymphocytes, double negative T cells (CD3+ CD4- CD8-), Treg regulatory lymphocytes (CD4+ CD25 high CD127-), RTE (recent thymic emigrants, CD4+ CD45RA+ CD62L+ CD31+).

Results: Our preliminary results indicate a more efficient early post-transplant naive T lymphocyte reconstitution, including recent thymic emigrants (RTE) and naive regulatory T cells (Treg) in patients after haploHSCT compared with patients after MUD-HSCT. On the contrary, double negative T cells (DN) were restored less efficiently in patients after haplo-HSCT. There were no substantial differences between studied groups in restoration of T cell populations, like total CD3+ T cells or CD8+ T cells. It means that nave T cell and RTE cells are restored post haploHSCT regardless of other lymphocyte populations, that shed a light on the whole process of T cell regeneration.

Conclusions/Novel aspect: Although haploidentical HSCT is performed as a last resort, our results shows that it is promising in terms of efficient de novo T cell regeneration. This research will allow a deeper understanding of the fundaments of immune restoration after alternative forms of alloHSCT, related to the emergence of individual T-cell subpopulations (especially valuable RTE cells). In perspective it may provide the possibility for a detailed prognosis of individual T-cell subpopulations renewal based on the degree of donor-recipient compatibility.

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[IV-42] NOVEL MECHANISM OF TUMOR RESISTANCE TO SUNITINIB IN CLEAR CELL RENAL CELL CARCINOMA.

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Introduction: Due to high level of tumor vasculature, therapy of more advanced stages of clear cell renal cell carcinoma (ccRCC) is based on new anti-angiogenic drugs, which inhibit multiple tyrosine kinase receptors. The most widely used tyrosine kinase inhibitor (TKI) is sunitinib. This anti-angiogenic drug that primarily target VEGF receptors (VEGFRs), despite its clinical efficacy, fails due to adaptation of tumor cells to treatment. The mechanism responsible for acquiring resistance is still not fully understood. The understanding of exact mechanisms and factors involved in developing resistance to TKIs may help in improvement of therapeutic strategies. The main aim of our study was to determine the mechanism of developing tumor resistance after treatment with sunitinib.

Materials and Methods: ccRCC cell line Caki-1 was constantly stimulated with sunitinib or DMSO (control) for 7-21 days to obtain resistance. Resistant cells were then analyzed with Western blot, IF staining, qPCR, and conditioned media were collected. Conditioned media and mice plasma were used for ELISA assays, and for stimulation of HUVEC endothelial cells. For animal studies GFP+ Caki-1 cells were treated for 5 weeks with the drug. Next, cells were mixed with wild type Caki-1 in 1:1 ratio and injected *sc*. into Nod-Scid mice. After 6 weeks tumors were harvested and analyzed with Western blot and IHC staining.

Results: Our results indicate that mechanism of resistance to sunitinib is due to the acquisition of senescent state. The viability of sunitinib resistant cells decreases compared to control cells. Resistant cells are more circular, and form clone-like structures. Moreover, after sunitinib stimulation cells express high levels of e-cadherin, b-catenin, Oct4 and c-Met receptor. Sunitinib treatment results in cell senescence and an increased secretion of pro-angiogenic factors like IL8 and IL6. Stimulation of ECs with conditioned media from resistant Caki-1 cells resulted in VEGFR2 and Src phosphorylation, and leads to the disruption of ECs monolayer integrity due to phosphorylation and internalization of VE-cadherin. Sunitinib resistant tumors, despite lower mass were characterized by an increased lung metastasis due to upregulated phosphorylation of c-Met receptor. Furthermore, we observed better developed vascularity with a high level of blood vessel marker CD31 and raised secretion of IL8 to plasma compared to control.

Conclusions: Our results suggest that despite of multi-target inhibition, tumor cells are able to compensate inactivation of pathways involved in angiogenesis. In consequence, the activation of another proteins and signaling pathways leads to increased aggressiveness and survival of tumor cells. Furthermore, resistant cells are able to stimulate ECs via secretion of pro-angiogenic factors and in result develop more complex vasculature. The obtained results may contribute to development of new treatment strategies to counteract the emergence of resistance.

[IV-43] Association of c.470T>C CHEK2 variant with papillary thyroid cancer – original study and meta-analysis

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Introduction. Predisposition to papillary thyroid cancer (PTC) is multigenic, with interactions among genes and environmental factors determining individual susceptibility. Various genes associated with PTC have been identified, among them is also CHEK2 gene. The present study investigated the frequency of c.470TC CHEK2 variant among PTC cases and controls in the own study. Next, a meta-analysis was performed for c.470TC CHEK2 variant with the available Polish data.

Material and Methods. The frequency of the c.470TC (known as I157T) CHEK2 variant in DNA derived from blood samples of 2248 PTC cases and 1158 controls was investigated. PTC cases were referred to the Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch for treatment. Control subjects were recruited from consenting volunteers. The c.470TC variant was analyzed with the allelic discrimination assay (TaqMan probes). In the meta-analysis of the c.470TC variant data from five Polish studies were included with the total number of 5124 PTC cases and 8511 controls.

Results. A significant association with PTC was observed for C alle of c.470TC CHEK2 variant (OR = 2.03; 95% CI: 1.53-2.70) These results were confirmed by the meta-analysis C allele of c.470TC variant was significantly associated with PTC (OR = 2.09, 95% CI: 1.79-2.44).

Conclusion. Our results from the largest number of the PTC cases analyzed up to date confirmed that c.470TC CHEK2 variant is significantly associated with PTC. The results were confirmed in meta-analysis performer in all available Polish data.

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[IV-44] DETECTION OF G-QUADRUPLEXES AND HEME OXYGENASE 1 INTERACTIONS BY PROXIMITY LIGATION ASSAY

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G-quadruplexes (G4) are stacked nucleic acid structures, reach in guanine and interacting with heme. In cells, they possibly affect DNA replication and gene transcription. Several helicases can unwind G-quadruplexes. However, their role in cells and putative sensitivity to heme is unclear, because of difficulties in the visualization of G4-binding proteins. We found that hematopoietic stem cells (HSCs) from heme oxygenase-1 deficient (*Hmox1-/-*) mice have upregulated expression of G4-unwinding helicases, and show weaker staining for G-quadruplexes. This can result from a direct role of HMOX1 or enhanced cell cycle in *Hmox1-/-* HSCs. Therefore, we investigated the potential interaction between HMOX1 protein and G-quadruplexes in HEK293 and iPSC model cells. First, we demonstrated that the proximity ligation assay (PLA) can detect cellular co-localization of G-quadruplexes with helicases, as well as with HMOX1, suggesting a potential role of HMOX1 in G4 modifications. Using cells engineered to express only nuclear or only cytoplasmic form of HMOX1 we found that nuclear localisation correlated with reduced levels of G-quadruplexes. Finally, treatment of cells with exogenous heme enhanced G4-staining, especially in HMOX1-deficient cells. To sum up, heme seems to stabilize G-quadruplexes in cells, which could be prevented by HMOX1. Cellular G4-protein colocalizations can be quantitatively analysed using PLA.

[IV-45] GLOBAL DNA METHYLATION IN PATIENTS WITH HEAD AND NECK CANCER AND PATIENTS WITH CHRONIC TONSILLITIS

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Introduction. DNA methylation is a pivotal mechanism to contribute to gene expression and associated with carcinogenesis, such as head and neck squamous cell carcinoma (HNSCC) - the six most common cancer in the world. Aberrant methylation has also been reported in inflammatory diseases. Chronic tonsillitis is a chronic inflammation and is a serious health problem.

The aim of the current study was to evaluate the global DNA methylation in HNSCC patients and for the first time in patients with chronic tonsillitis.

Methods. The study population comprised 30 patients with HNSCC (including 24 men and 6 women), 56 patients with chronic tonsillitis (26 men and 31 women) and 29 healthy individuals (13 men and 16 women). Global DNA methylation was analysed in tumour sample and margin sample collected from HNSCC patients, in tonsillar tissues taken during tonsillectomy from chronic tonsillitis patients, and in epithelial cells of the oral mucosa collected from healthy individuals. The methylation level was examined by a 5-mC DNA ELISA Kit (Zymo Research, Germany). The data were analysed using T Student test.

Results. We observed a significantly higher degree of global DNA methylation in TC patients compared to tumour samples, margin samples and control study (5.32% vs. 2.58%; 5.32% vs. 1.52%, 5.32% vs. 1.99% respectively, p-value ≤0,05). Moreover, we detected a significantly higher level of DNA global methylation in the tumour samples compared with margin samples (2.58% vs 1.52%, 1,99%, p-value <0,001).

Conclusions. The higher level of DNA global methylation among patients with chronic tonsillitis compared to other groups may suggest that hypermethylation is associated with chronic inflammation. Moreover, the higher degree of global methylation in the tumour sample compared to the surgical margin in HNSCC patients may be associated with the carcinogenesis process.

Key words: head and neck squamous cell carcinoma (HNSCC), chronic tonsillitis, global methylation, hypermethylation

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[IV-46] ASSESSMENT OF LYMPHOCYTE SUBPOPULATIONS AND IMMUNE ACTIVATION STATUS AFTER INFUSION OF SELECTED LYMPHOCYTES FROM ALLOGENIC, HLA-MISMATCHED UNRELATED DONORS IN PATIENTS WITH OROPHARYNGEAL CANCER

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Cancer cells can become a target of immune system. Cellular response and elimination of cancer depend on the presence of tumor-specific antigens presented in the context of HLA molecules. Effector cells consist mainly of CD8+ cells (Tc), requiring preceding activation from CD4+ cells (Th). This mechanism is sufficient during homeostasis and protects from development of the malignant tumors. In case that the malignant growth occurs and reaches critical mass, it can lead to establishing immune tolerance towards the cancer cells. The presence of lymphocytes infiltrating solid tumors (mainly cytotoxic T-cells) has been previously described, however they remain dormant and inactive. It is presumed that CD8+ cells require proper signaling from T-helper cells, which is absent due to the immune tolerance of the tumor. One of the proposed methods of inducing immune response towards cancer cells is an infusion of CD4+ (Th) cells derived from HLA mismatched donor. Th infusion could provide stimulus for CD8+ activation. That kind of approach was previously described in leukemia studies. Our research is the first to incorporate those findings in solid tumor therapy. The aim of our pilot study is to test this hypothesis and assess efficacy of the therapy.

The pilot study included three patients diagnosed with HPV-positive oropharyngeal cancer. Cells were collected from HLA-mismatched unrelated donors during leukapheresis procedure. The cytotoxic T lymphocytes were removed from the donor lymphocyte infusion (DLI) by negative immunomagnetic selection (CD8+ depletion) to minimize the risk of graft-versus-host disease (GvHD). Samples of peripheral blood were collected at various time points for three weeks after CD8-depleted DLI infusion and were analyzed for immune activation and lymphocyte subpopulations (B, Tc, Th, NK) by flow cytometry. MNC and lymphocyte subpopulations were sorted and the percentage of the donor lymphocyte chimerism was evaluated using STR analysis method.

The number of Th cells infused was (0,41-0,74) x108/kg b.w. Negative immunoselection was an efficient method for Tc depletion (1,3-71,5) x103/kg b.w. -GvHD prevention. There was no detectable level of donor Tc in blood of recipient. The donor NK and B-cells were detectable only to +3 day after DLI infusion. The percentage of the donor Th cells was about 2-6% and fell to the undetectable level between +5 and +10 day after infusion. Flow cytometry analysis has shown that the number of cells in all of the recipient lymphocyte subpopulations reconstituted in the first 2 weeks after DLI infusion. Analysis of both early and late markers of the lymphocyte activation has shown strong (up to 50%) activation of the recipient Tc. Other analysis: tumor volumetric changes, cytokines level and histopathology are pending.

Preliminary results shown that CD8-depleted DLI from HLA-mismatched unrelated donors seems to be an effective method for stimulation of immune system of patients with oropharyngeal cancer.

[IV-47] 3D IMAGING AND CHARACTERIZATION OF THE RB-LIKE PROTEIN ACTIVITY CHANGES IN ROOT MERISTEM CELLS OF *VICIA FABA* SUBJECTED TO REPLICATION STRESS

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Vicia faba is one of the earliest domesticated plants, still widely used in modern world (culinary, agricultural and medical). Its well-known for its resistance to unfavorable growth conditions (e.g. insufficient irrigation, increased salinity or chemically harmful soil factors). In the context of increasing climate changes and pollution on our planet, the capacity of a particular plant to grow and provide value (either nutritional or medical) in an adverse environment is one of the crucial factors that may determine our well-being. Root meristem cells of *V. faba* subjected to chemically-induced replication stress caused by hydroxyurea (HU) and co-treatment with caffeine (CF) show strikingly high survival rates, rarely observed in other organisms. In our opinion, this ability results from highly efficient stress-response mechanisms during DNA replication.

The preliminary investigation involved immunocytochemical detection and quantitative analysis of activity of the Rb-like regulatory protein which coordinates the transition from G1 to S phase of the cell cycle, acting as a suppressor of excessive cell growth. The specific form of Rb-like protein analyzed was phosphorylated at Ser807/811 (pRb) this form allows the progression from G1 to S and the start of replication. Additionally, we performed a detailed 3D modeling using Blender 2.9.1. software this method allows to visualize the data using high-resolution images that greatly support the ones originally obtained from microscope analysis. Weve executed 3D modeling that was strictly built upon the original data obtained from photographs as well as the general modeling, based on the conclusions after the statistical analysis.

In this project we present five distinctive patterns of phosphorylated Rb-like protein activity during S-phase. The patterns are related to particular chromatin regions and thus to the specific events during the replication. We were able to show that pRb activity is visibly diminished in HU-treated cells but the labeling patterns are still clearly recognizable even though they appear to be altered in response to replication stress. Our findings suggests that *V. faba* has a highly effective stress-response system that allows it to survive in harsh environment. The 3D modeling presented here is an effective and aesthetical way of making visual aids that support conclusions and experimental analysis. The modeling based on original data allows us to prepare a high-res images of the exact same object observed under the microscope, but with the option to add various modifications (e.g. different coloring for specific areas) this way enabling us to make a clearly understandable model that is very specific for the discussed subject. On the other hand, the general modelling was conducted on the basis of the analyzed statistical data that led to the hypothesis verification this approach is very useful for the visualization of a broader ideas and data summarizing.

[IV-48] SYNTHESIS AND BIOLOGICAL PROPERTIES IN VITRO OF NOVEL TETRACYCLIC PYRIDOQUINOTHIAZINES DERIVATIVES

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A series of novel tetracyclic pyridoquinothiazines derivatives was obtained. The effect of electron-acceptor and electro-donor substituents on cyclization mechanism was analyzed along with direction of substitution in the pyridine ring.

Antiproliferative activity of the obtained compounds was tested using chosen neoplastic cell lines: MDA-MB-231 invasive breast cancer (ATCC), SNB-19 glioma multiforme (DSMZ), amelanotic melanoma C-32 (ATCC) as well as normal fibroblast cell line HFF-1 (ATCC). The mechanism of antiproliferative action of the novel derivatives was studied. Strong binding to DNA isolated from examined cell cultures has been was found reaching levels higher than those for cisplatin, a widely used chemotherapeutic. Gene transcriptional activity was examined for histone H3 (*H3*), a proliferation marker, as well as for BCL-2 (*BCL-2*) and BAX (*BAX*), two mitochondrial apoptosis markers and cell cycle regulators.

Antimicrobial activity of the obtained compounds was investigated using six Gram-positive and six Gram-negative bacterial strains, as well as Candida albicans, an opportunistic pathogenic yeast. Greater activity was demonstrated towards Gram-positive strains.

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[IV-49] Effect of EGFR and c-Met inhibitors on motile and proteolytic activities of highly invasive vemurafenib-resistant melanoma cells

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Melanoma is one of the most aggressive cancers with an increasing number of diagnosed cases every year. About 50% of melanoma patients harbor a mutation in gene coding *BRAF* kinase (*BRAF* V600E), a part of the signal transducing pathway activated by receptor kinases a.o. EGFR (Epidermal Growth Factor Receptor) and MET (Hepatocyte Growth Factor Receptor). Vemurafenib, a specific inhibitor of BRAF V600E, efficiently blocks the action of constitutively active mutated BRAF, however, cancer patients quickly develop resistance to this treatment. To evaluate the effectiveness of previously tested combination therapy using inhibitors of EGFR (lapatinib) and MET (foretinib) we generated human melanoma cell lines resistant to vemurafenib. Established cells exhibited a slower proliferation rate, and increased invasive and proteolytic abilities compared to the parental lines. Administration of tested drugs decreased cell viability and spontaneous migration to the same extent in resistant and parental lines, however, generated lines demonstrated lower sensitivity to EGFR/MET inhibitors in terms of collective migration measured with scratch wound assay. Additionally, resistant cells showed an altered profile of secreted metalloproteases which protein levels and activity were also decreased upon inhibitors treatment. In summary, vemurafenib-resistant melanoma cells demonstrate elevated migratory, invasive, and proteolytic capabilities, which can be partially blocked by the administration of combination therapy composed of EGFR and MET inhibitors. However further research is required to completely abolish the motile abilities of cancer cells which are responsible for metastasis formation.

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[IV-50] 3D LYMPHOMA AGGREGATES FOR THE MAGNETIC NANOPARTICLES-MEDIATED HYPERTHERMIA TREATMENT.

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INTRODUCTION: Hyperthermic treatment involves heating tumor tissues to a moderate temperature of 4045 C to destroy tumor cells by starting a series of thermally induced apoptotic events. Magnetic-nanoparticle-mediated hyperthermia has the potential to enable localized tumor heating with minimal side effects. In this technique we targeting magnetic nanoparticles to tumor tissue and then we apply an electric field that induces localized heating. In order to thoroughly investigate the potential of nanomaterials in hyperthermia, we developed 3D mutlicellular aggregates, which consist of both: lymphoma and normal stromal cells. Proposed here biological model has more benefits than single cell studiem because tumour spheroids provide an accurate in vitro representation of the three-dimensional organization of cells in tissues.

MATERIAL AND METHODS: Two human cancer cell lines Ri-1 (Human Caucasian B‑ cell lymphoma), and HS-5 (human bone marrow stromal cell) were acquired from the American Type Culture Collection. Ri-1 cells were cultured in RPMI 1640 and HS-5 in Dulbeccos Modified Eagles Medium. Both medium containing 2mM Glutamine and supplemented with 10% Foetal Bovine Serum. For spheroids formation, agarose structures with spherical microwells were obtained by using a micro-mold made according to the manufacturers protocol (3D Petri Dish, Microtissues Inc., Providence RI, US). The Total number of cells seeded was 3,840 cells/190l with the ratio of Ri-1 cells to HS-5 cells, as followed: 1:1; 2:1; 3:1; 4:1; and 10:1.

RESULTS: Separately, both cell lines did not form mutlicellular spheroids. Ri-1 cells aggregated poorly or not at all, while HS-5 cells loosely grouped together. In co-cultures with ratio 4:1 and 10:1 Ri-1 cells to HS-5 cells first spheroids are formed after 48 hours. The best aggregation was obtained for a Ri-1 cells to HS-5 cells ratio of 4:1.

CONCLUSIONS: Numerous examples of 3D spheroids from epithelial neoplasms are given in the literature, but no data on lymphomas are available. Lymphoma cells grow in suspension and aggregate heavily. Here, we have shown that it is possible to obtain large-cell aggregates resembling 3D spheroids thanks to co-culture with stromal cells. This new model can be succesfully used in the magnetic- nanoparticles mediated hyperthermia studies.

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[IV-51] GLUCOSE-CONJUGATION ENHANCES THE ANTICANCER EFFECT OF METHOTREXATE VIA INCREASE OF APOPTOSIS IN MCF-7 BREAST CANCER CELLS IN HYPOXIC AND AGLYCEMIC CONDITIONS

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Introduction: Despite the progress in developing novel cancer therapies, many patients with breast cancer remain incurable. While different cytotoxic therapies are employed in breast carcinoma management, response rates are low, and acquired resistance is common. Moreover, the solid tumor microenvironment is characterized by inadequate oxygen and glucose supply, which promotes the rapid proliferation of cancer cells in oxygen and glucose-deprived conditions. Those hallmarks of the cancer niche can profoundly affect the cancer cell response in the presence of different chemotherapeutics by increased adaptation to apoptosis and autophagy. Methotrexate has been widely used in the clinic and incorporated in the treatment of several malignancies, such as colorectal, gastric, breast, or head and neck cancer. However, drug resistance of malignant cells and systemic toxicity in the course of treatment are alarming causes of chemotherapy failure. Therefore, it is desirable to explore novel therapeutic approaches. One approach could be the conjugation of glucose to drugs to target cancer cells. Recent evidence points to glucose transporter 1 and sugar metabolism as targets in cancer treatment. The aim of the present work was to design, synthesize, and biologically evaluate a novel glucose-methotrexate conjugate (Glu-Met) for the first time.

Methods: The cytotoxicity of Glu-MTX and free methotrexate after 72 hours of incubation on breast cancer cell line MCF-7 was measured by MTT assay. Cell migration was evaluated by wound-healing assay. Apoptosis detection was performed by flow cytometry using Annexin V Apoptosis Detection Kit.

Results: Glucose-conjugated methotrexate exerted an increased cytotoxic effect on MCF-7 breast cancer cells in comparison to free methotrexate in hypoxia (1% O2) and glucose starvation conditions. Furthermore, Glu-MTX attenuated cells proliferation and migration ability more effectively than free methotrexate after 48 hours. The evaluation of apoptosis level in hypoxic conditions by flow cytometry revealed that Glu-MTX has a greater potential in inducing apoptotic cell death compared to the free drug.

Conclusions: Our study reveals that the conjugation of methotrexate with glucose can overcome tumor microenvironment resistance to the chemotherapeutic drug in breast cancer *in vitro*. The improved cytotoxic effect of Glu-MTX may be linked with elevated glucose uptake and GLUTs overexpression in cancer cells in a tumor-like microenvironment. It is expected that the glycoconjugated methotrexate may become a scientific foundation for a novel therapeutic approach in the treatment of breast cancer.

[IV-52] OVERCOMING CHEMORESISTANCE IN SW480 COLON CANCER CELLS CANCER USING NOVEL GLYCOCONJUGATE OF METHOTREXATE IN HYPOXIC/AGLYCEMIC CONDITIONS

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Introduction: Colorectal cancer (CRC) is one of the most frequent malignancies globally, and chemotherapy is the leading treatment method commonly applied in combination with surgery or radiotherapy, depending on tumor advancement. During neoplastic cell proliferation, the tumor increases in size, resulting in low oxygen levels (less than 2%) and minimal glucose availability in cancer microenvironment. The mentioned cancer features deeply affect the cell response to different chemotherapeutics by increased adaptation to apoptosis. Thus, breaking hypoxia-induced drug resistance is necessary to elevate the efficacy of cancer chemotherapy and increase the patients lifespan. Glucose is a fundamental energy source that is absorbed by cells through the cell membrane. The glucose receptors (GLUTs) have been found to be overexpressed in a large percentage of cancers to increase glucose uptake and the flux of metabolites through glycolysis. This phenomenon, named the Warburg effect, arises from mitochondrial metabolic changes and is one of the cancers most common traits. In this study, we synthesized and biologically evaluated a novel glucose-methotrexate conjugate (Glu-MTX). Methotrexate (MTX) has been successfully used for many years in the treatment of patients with cancer. In our study, we investigated whether Glu-MTX could overcome MTX chemoresistance on oxygen and glucose-deprived SW480 colon cancer cells.

Methods: The experiments were carried out at hypoxic oxygen concentrations (1%) and in culture medium without glucose. Cytotoxicity was verified by MTT viability test and cell motility was examined using a wound-healing assay. Moreover, to establish the expression of crucial proteins related to programmed cell death (Bax, Bcl-2, caspase 3) following cell treatment, immunocytochemical staining was performed.

Results: In vitro cytotoxicity study demonstrated that Glu-Mtx had IC50 (concentration required to reduce 50% cell viability) values in the M range against several different solid tumor lines. The IC50 with free methotrexate was lower than the conjugate in SW480. The cytotoxicity of Glu-Met shows a rather broad antitumor spectrum and is greater than that of free methotrexate in hypoxia/aglycemia conditions. Moreover, Glu-MTX-treated cells had significantly slower migration than MTX-treated cells. High levels of proapoptotic proteins in cells treated with Glu-MTX and MTX compared to control. The intensity of staining and percentage positivity was more elevated in Glu-MTX- treated cells than in MTX- treated cells.

Conclusions: Our study reveals that conjugation of methotrexate with glucose overcome tumor microenvironment resistance to the chemoterapeutic drug in colon cancer cells. The increased cell death is linked with the elevated glucose-sonjugated methotrexate intake. Although the finding has been confined to in vitro studies, our observations shed light on a potential therapeutic approach to overcome chemoresistance in cancer.

[IV-53] SYNTHESIS AND CHARACTERIZATION OF PARTIALLY DEGRADABLE POLYESTER-POLYMETHACRYLATE POLYMERS WITH VARIOUS TOPOLOGY

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By combining various polymerization methods, such as - atom transfer radical polymerization (ATRP) and ring-opening coordination-insertion polymerization (ROP), the linear block polymers and miktoarm star polymers based on *N,N*-dimethylaminoethyl methacrylate (DMAEMA) and various cyclic esters, i.e., ε-caprolactone (CL), lactide (LA), glycolide (GA) were obtained. In the synthesis of linear block copolymers, a heterofunctional initiator having a hydroxyl group capable of initiating ROP and a bromoester group initiating ATRP was used. Star-shaped copolymers were synthesized by using a gluconolactonamide-based miktoinitiator with five bromoester groups and one azide group. The latter one was exploited in the *click* chemistry reaction between azide-functionalized star polymers and polyesters having alkyne end group. [1]

The structures of the obtained macromolecules were confirmed by spectroscopic analyzes, i.e., 1H NMR and ATR-IR. Because of the presence of polyDMAEMA blocks with thermo-/pH-sensitive properties, the cloud point temperatures of polymer solutions in PBS and H2O (TCP,H2O = 35-48C, TCP,PBS = 59-92C) were determined using UV-Vis spectroscopy. In addition, the critical aggregation concentrations of the polymers in H2O (CAC = 0.0030-0.0797 mg/mL) were measured by fluorescence spectroscopy. The important aspect of the studies was focused on the degradation of polyester segment by chemical and enzymatic methods. Chemical degradation was carried out in physiological saline solution at pH 5.0 and 7.4, while enzymatic degradation was performed with the use of the Novozyme 435. It was observed that enzymatic degradation occurs faster (within 3 weeks) than chemical degradation (within 6 weeks).

The obtained amphiphilic polymers can be used in medicine as drug delivery systems

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[IV-54] EFFECTIVENESS OF LIGHT-ENCODING ONCOLYTIC MYXOMA VIRUS-LOADED ADSCS TO DESTROY MURINE PANCREATIC CANCER CELLS

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Introduction: Modification of oncolytic viral constructs with specific biological activity profile could lead to novel approaches to anti-cancer therapy. A novel myxoma virus construct (MYXV, vMyx-Light-tdTomato-FLuc) encoding mouse tumor necrosis factor ligand superfamily member 14 (Tnfsf14, synonym Light) was designed to increase the influx of T-cells and intensify the immune response directed against the tumor. Light gene is under the control of a poxvirus synthetic Early/Late (E/L) promoter. The recombinant construct contains tdTomato reporter gene under poxvirus late p11 promoter and firefly luciferase under poxvirus synthetic E/L promoter. Effective administration and in vivo delivery of novel MYXV requires a protective carrier. Mesenchymal stem cells derived from adipose tissue (ADSCs) have been used for this purpose. ADSCs can be easily procured and possess natural tropism to inflammatory sites like tumor beds.

Aim: Assessment of novel myxoma virus construct usefulness for therapy of experimental murine pancreatic cancer making use of ADSCs as a viral cargo carrier.

Methods: Immunophenotype of MYXV-infected and uninfected ADSC, together with ability to differentiate into fat, bone and cartilage cells were confirmed using commercial kits (BD Biosciences). Permissiveness of ADSCs and murine Pan02 for MYXV was examined using flow cytometry at 24-72h post infection. Cytotoxicity of MYXV for the tested cells (ADSCs, Pan02, Panc-1, RK13) was determined using MTS viability test. Production of MYXV progeny was assessed under one-step multiplication conditions for ADSCs, Pan02 and Panc-1, treated or not treated with IFN to verify whether it does inhibit replication of the virus which would be detrimental for therapy. Early and late gene expression was tested using MYXV for ADSCs and Pan02 24h post infection.

Results: Presence of CD73, CD90 and CD105 surface markers was confirmed in both MYXV-infected and uninfected ADSC along with absence of blood cell lineage-specific markers: CD11b, CD19, CD34, CD45 and HLA-DR. Both infected and uninfected ADSCs were confirmed as capable of differentiating into fat, bone and cartilage cells. ADSCs and Pan02 were permissive to MYXV infection. While ADSCs remained viable after MYXV infection and their proliferation was not remarkably reduced, viability of pancreatic carcinoma cell lines diminished significantly. ADSCs, Pan02 and Panc-1 all produce infectious MYXV progeny. Presence of IFN did not affect MYXV ability to replicate. ADSC and Pan02 cells produce early and late genes at 24h post infection.

Conclusion: MYXV construct effectively infected ADSCs carrier cells which support replication of virus. On the contrary, infected pancreatic cancer cell cultures were effectively destroyed. The infected ADSC might therefore be used as Trojan horse for experimental therapy of murine pancreatic cancer.

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[IV-55] Pain, Poor Physical, Emotional, and Financial Functioning of Head and Neck Patients (HNC) Precede Worse Treatment Outcome

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Introduction: Global quality of life proved to be an important predictive factor for HNC patients survival. The question is whether a certain pattern or number of subjectively expressed symptoms may be related to future HNC treatment failure.

Methods: Seventy-nine patients (women - 28%, men - 72%, stage I/II - 17%, III - 15%, IV - 68%) were treated with by Accelerated Radiotherapy Alone (ARA - 60%) or Concurrent Chemo-Radiation (CCR 40%), 38% received induction chemotherapy. The patients filled quality of life questionnaires before and after ARA or CCR QLQ-C30 plus head and neck cancer-specific HN35 (35 sub-scales). Endpoints of the treatment such as loco-regional failure (LRF) at 180 days after ARA/CCR, loco-regional control (LRC), disease-free survival (DFS) and overall survival (OS) after 3 years were obtained. Kaplan-Meyer analysis and log-rank test were performed to differentiate which of QoL sub-scales are related to the treatment outcomes.

Results: LRF was related to worse physical functioning (PF median= 86.7/100, PF 86.7 LRF=13%, PF86,7 LRF=37%, p0,011) before ARA/CCR. LRC was unrelated to specific results of QoL. DFS was related to pain level (median PA=16.7/100, PA 16.7 DFS=31%, PA16.7 DFS - 59%, p0.03) and emotional functioning (median EF=66,7, EF66,7 - DFS=65%, EF66,7 DFS = 31%, p0.011) before ARA/CCR. OS was related to PF (PF 86.7 OS=49%, PF86.7- OS= 77%,p0.013) and financial problems (FI=33,3/100) before the treatment (FI33,3 OS=88%, FI33,3- OS=51%, p0,002).

Conclusions: In-depth QoL should be routinely done at oncology ward. A cluster of pain, poor physical and emotional functioning and financial problems impair survival and progression rates in HNC patients treated with radical intent. HNC patients having social and physical complaints need more psychological and medical care with more than others watchful observation during follow-up.

[IV-56] CAFFEINE-INDUCED CHROMOSOME SHATTERING IN ROOT MERISTEM CELLS OF VICIA FABA SUBJECTED TO PREMATURE CHROMOSOME CONDENSATION

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Introduction/Rationale: Parachute-like chromatin configuration (PALCC) and general chromosome shattering (GCS) was described by Hbner and co-workers for the first time in 2009, in the cells of Chinese hamster irradiated with UV light and followed by caffeine treatment. Strikingly similar formations (named parachute-like, PAL) were also observed in the PCC-induced root meristem cells of *Vicia faba* recently. The fact, that such similar results are obtained in different species (and using different methods) points us to the conclusion that there may be some core mechanisms underlying the formation of PALCC and PAL affected.

Results/Conclusions: As both methods used caffeine, we decided to take a deeper look at the mechanisms that may be impacted by its activity and tried to find a theoretical answer to the pathways of formation of such aberrant, yet interesting formations. In this project, we discuss the current state of knowledge regarding the events of CF-induced PCC, chromatin condensation and the disturbances in mitotic division and propose the conflict of interest model, an alternative and/or additional to the factor depletion model that was proposed by Hbner in 2009.

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[IV-57] CYTOTOXICITY OF DRUG-LOADED EXOSOME CARRIERS FOR MELANOMA CELL CULTURES

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Introduction: Exosomes used as drug carriers might have many advantages e.g. ease of acquisition, stability, target ability and safety of use. Exosomes have been tried for transporting drugs (e.g. paclitaxel or doxorubicin), enzymes, DNA, or even superparamagnetic iron oxide nanoparticles. Such kind of cargoscan be loaded by simple incubation or methods like sonication, extrusion or electroporation. Doxorubicin (Dox) is a well-known anthracycline chemotherapeutic often used for the treatment of various cancers. Dox extravasation following iv. administration can result in tissue ulceration, necrosis and is associated with cardiac toxicity thus drug shielding is beneficial. We compared possible anthracycline loading into exosomes using a simple approach. We tried Dox or WP760, a bis-anthracycline10-100 times more toxic to various types of melanoma cells as compared to other cell types using the Compare Program algorithm (National Cancer Institute). WP760 has a unique mode of action, which distinguishes this compound from 80 000 other anti-cancer agents screened. We strived to answer the question whether exosomes isolated from melanoma cell culture media would be able to incorporate WP760, or Dox as control. Such exosomes could be used todeliver WP760 to tumor foci *in vivo*.

Methods: The process of possible exosomal loading with WP760 was studied using *in vitro* cultures of two melanoma cell lines: human 1205Lu and murine B16-F10. At ca. 70% culture confluency, the regular medium was replaced with medium containing serum devoid of exosomes. 24 hours after the medium change, Dox or WP760 were added. Two concentrations of the chemotherapeutic agents were used in the experiments, both below cytotoxicity thresholds. After additional 48h, cell culture media were collected, pre-cleared by a series of centrifugations (200-10 000 g) and the supernatant filtered using a 0.22 m syringe filter unit. Exosome enrichment was achieved with Vivaspin centrifugal concentrator device. Exosome aliquots were loaded onto size exclusion chromatography columns (SEC) and 1 mL fractions were eluted with PBS. Size distribution profile of exosomes was determined using dynamic light scattering (Zetasizer). The presumably loaded drugs were extracted from exosomes using detergent and sonication and quantified by absorbance. Exosomal preparations were used to check their impact on nave cultures of melanoma cells.

Results: Exosomes possibly containing chemotherapeutic agents were eluted form SEC columns in fraction 5.Exosomal preparations were tentatively found to convey the cytotoxic effect to previously drug-untreated cell cultures.

Conclusions: Drug transfer *in vivo* using WP760-loaded exosomes remains feasible. Studies defining exosomal drug capacity, stability and retention kinetics are underway.

This study has been supported by an internal grant to AS from the National Institute of Oncology, Gliwice Branch, No. GW/CBT/18/2019.

[IV-58] INDUCTION OF CELLULAR SENESCENCE IN DK-MGHIGH EGFRVIII-POSITIVE CELLS WITH EPIDERMAL GROWTH FACTOR

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Introduction/Rationale: Glioblastoma (GB) is a highly aggressive type of brain tumor. One of the possible approaches used to treat glioblastoma patients is to induce senescence in glioblastoma cells instead of killing them. Glioblastoma cells proliferation in DK-MGhigh cell line is associated with a higher percentage of epidermal growth factor receptor variant III (EGFRvIII)-positive cells. EGFRvIII is characterized by a lack of binding site for an epidermal growth factor (EGF). In this study, we examined the EGF as a factor inducing senescence in DK-MG glioblastoma cells.

Methods: DK-MGhigh cell line was cultured in RPMI medium enriched with 10% fetal bovine serum (FBS). The medium was exchanged on RPMI serum-free (SF) medium for 24 hours before treatment with EGF. Cells in a test sample were treated and incubated with epidermal growth factor for 72 hours in 37C, while in a control sample they were not. Cellular senescence was analyzed by means of senescence-associated galactosidase (SA--Gal) activity assay. Immunocytochemistry (ICC) analysis was conducted to detect and evaluate the percentage of glioblastoma cells expressing EGFRvIII. Bromodeoxyuridine (BrdU) staining was performed to evaluate the proliferation of glioblastoma cells after treatment with and w/o EGF. Statistical data analysis was performed using 2way ANOVA with Bonferroni post hock test in GraphPad Prism Software.

Results: The percentage of proliferating cells treated with EGF was higher than in the control sample. Interestingly, at the same time, a slightly higher percentage of SA--Gal-positive cells was verified in the test sample with EGF treatment than in the control. Moreover, a slightly higher percentage of EGFRvIII-positive cells was observed for cells treated with EGF and the results are statistically significant (*p*0,05). In addition, the signal for EGFRvIII in DK-MGhigh cells exposed to EGF was more intense than in the control sample.

Conclusions/Novel aspect: EGF may induce an increased proliferation level of glioblastoma cells. Interestingly, at the same time, EGF seems to fractionally promote the induction of senescence in DK-MGhigh cells. The obtained results suggest also that EGF may increase the percentage of EGFRvIII-positive cells and the intensity of the signal for the EGFRvIII in comparison with cells not treated with EGF. The described phenomenon is contrary to the prevailing beliefs. Further study of the senescence induction in GB cells is needed to verify the mechanism responsible for this dualistic role of EGF and to find out how it depends on the genetic context of the glioblastoma cells.

This study was sponsored by the National Science Centre grant no. 2019/35/B/NZ3/03577.

[IV-59] NEW CINNAMANILIDES AND THEIR BIOLOGICAL ACTIVITY

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A series of novel ring-substitutedN-arylcinnamanilides was synthesized andcharacterized. All investigated compounds were tested against Staphylococcus aureusas the referencestrain, two clinical isolates of methicillin-resistantS. Aureus (MRSA), and Mycobacterium tuberculosis.(2E)-N-[3-Fluoro-4-(trifluoromethyl)phenyl]-3-phenylprop-2-enamide showed even better activity(minimum inhibitory concentration (MIC) 25.9 and 12.9M) against MRSA isolates than the commonlyused ampicillin (MIC 45.8M). The screening of the cell viability was performed using THP1-BlueNF-B cells and, except for (2E)-N-(4-bromo-3-chlorophenyl)-3-phenylprop-2-enamide (IC506.5M),none of the discussed compounds showed any significant cytotoxic effect up to 20M. Moreover, all compounds were tested for their anti-inflammatory potential; several compounds attenuated thelipopolysaccharide-induced NF-B activation and were more potent than the parental cinnamic acid. The lipophilicity values were specified experimentally as well. In addition, in silico approximationof the lipophilicity values was performed employing a set of free/commercial clogP estimators,corrected afterwards by the corresponding pKa calculated at physiological pH and subsequentlycross-compared with the experimental parameters. The similarity-driven property space evaluationof structural analogs was carried out using the principal component analysis, Tanimoto metrics and Kohonen mapping.

[IV-60] STRESS-INDUCED CHANGES IN GLOBAL TRANSCRIPTION. IS LAMIN PHOSPHORYLATION INVOLVED?

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The process of transcription is one of the key adaptive mechanisms and needs to be strictly controlled in response to environmental factors and stimuli (as heat shock). Recently, interest in this topic has been growing among scientists, because it is still not known much about the mechanisms controlling it. Heat shock is an invaluable model for studying mechanisms regulating gene expression and is well known and easy to control. Many papers report that during stress, transcription is shut down globally while only a few loci are highly activated. These active loci are connected with heat shock proteins (Hsp) family which functions as intra-cellular chaperones. Lamins are evolutionarily conserved proteins classified as type V intermediate filaments, which are involved in the regulation of gene expression, chromatin organization, DNA replication and repair, signaling, developmental regulation, and nuclear positioning. In order to play such a variety of functions, lamins interact with many different nuclear proteins, which are directly or indirectly responsible for a particular function. Lamins (the main component of the nuclear envelope) together with associated proteins built a complicated platform for the regulation of nuclear processes. It has been proved that the chromatin regions located near the nuclear envelope consist mainly of heterochromatin transcriptionally inactive regions. Our research suggests that lamins and associated/interacting proteins are significantly connected with transcription regulation. In this work, we focus on changes in gene expression profile in stress response. Our results also suggest that the phosphorylation status of HSF and lamins changes. Does the phosphorylation cause the transcription shut down or is it the result of it?

All experiments were performed on *D. melanogaster* embryonic cell line Kc. Cells were maintained at 23C as normal conditions. To induce the heat shock cells were incubated at 37C for 1 h before further experiments. To study the heat-induced changes in transcriptome we use RNA sequencing. For studying proteins and their phosphorylation status we used Western blotting followed by densitometric analysis and as the second technique - immunofluorescence staining with confocal microscopy.

As a result of our study, we have developed a protocol that allows us to study the stress response in cell cultures. We have found and functionally described changes in global transcription in response to stress. Our data show that the level of some transcripts, widely considered as stable reference genes, alter after stress exposure. RNA-seq analysis allowed us to select the set of genes that remain stable in heat shock response in Kc cells. We have shown that under stress the phosphorylation of HSF and lamin Dm occur. We also have shown one of a stress-dependent phosphorylation site in lamin Dm Ser25. These data indicate that lamins may play a role in heat-induced regulation of transcription, although further experiments need to be performed.

[IV-61] THE CLINICAL RELEVANCE OF THE BODY-WEIGHT SIGNALING PLAYERS: GDF15, GFRAL AND RET IN GASTRIC CANCER

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Introduction: Stomach cancer is one of the most common causes of death from tumour worldwide. Unfavourable prognosis of gastric cancer is associated with the lack of both strictly defined risk factors and the characteristic symptoms of the disease, which in turn leads to the diagnosis of cancer in advanced stages and limited treatments. Gastric cancer is heterogeneous, characterized by the different genetic and molecular model, which distinguishes two basic histopathological forms of this cancer, i.e. the intestinal and diffuse form. This is evidenced by different gene signatures for the above-mentioned forms: high expression of the HER2 protein in the intestinal form, and high expression of C-MET in the diffuse type. Due to the proven strong relationship between obesity and gastric cancer development, a new model of GDF15 signalling via the GFRAL-RET complex may prove helpful in the faster diagnosis of this cancer. Therefore, we aimed to analyze the expression levels of GDF15, GFRAL and RET in GC tissues in relation to each other and clinicopathological features, including patient survival, in order to establish a potential implication of the body-weight signalling pathway in the pathology and clinical outcome of GC.

Methods: Researches allowing to determine the level of GDF15, GFRAL and RET expression were carried out on archival tissue material containing 104 gastric cancer patients samples and normal gastric mucosa samples. Whereas gene expression data for The Cancer Genome Atlas cohort of 413 gastric cancer patients were obtained from public sources. The fixed material in the form of paraffin blocks was stained by immunohistochemistry on tissue microarrays. In order to determine the significance of the obtained results, a statistical analysis was performed.

Results: We found that the protein expression of GDF15, GFRAL and RET was significantly elevated and positively correlated in our set of GC tissues, which was reflected in their tendency to be overexpressed in low-grade and intermediate-grade tumours rather than high-grade ones. No other relationships between the expression status of the examined proteins and clinicopathological characteristics of GC patients were found. Through in silico data analysis, we showed that high GDF15 expression was associated with better overall survival of GC patients, whereas high levels of RET or GFRAL were associated with poorer overall survival. These results together with further in silico analysis suggested that overexpression of the GDF15-dependent GRFAL-RET complex correlated with poorer overall survival of GC patients, which could be related to cancer cachexia.

Conclusions: We conclude that GDF15-GFRAL-RET axis appears to be not only diagnostically and prognostically valuable for GC but also a promising target for its treatment and/or prevention of cancer-related syndromes, like anorexia and cachexia.

[IV-62] PATHOLOGICAL CHANGES OF THE LIVER CAUSED BY A HIGH-FAT DIET

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Non-alcoholic fatty liver disease (NAFLD) is currently considered the most common chronic liver disease in developed countries. NAFLD includes a spectrum of diseases ranging from isolated hepatic steatosis to nonalcoholic steatohepatitis (NASH), the progressive form of the disease characterized by inflammation, cellular injury, and fibrosis. Fibrosis may be a result of the epithelial-mesenchymal transition (EMT) process that leads to de-differentiation of epithelial cells to a mesenchymal-like phenotype. During EMT epithelial cells with high expression of E-cadherin change their morphology with the expression of i.e. vimentin, fibronectin, N-cadherin. An inducer of EMT and in consequence, fibrosis development, is Transforming Growth Factor beta (TGF).

The aim of our study was to check if high-fat diet induces EMT in vitro and in vivo. We found that prolonged treatment with fatty acids increased levels of TGF, MMP9 and -catenin important inducers of EMT. We also found that livers of mice fed high fat diet have features of fibrotic liver with increased levels of -smooth muscle actin (-sma) and increased expression of TGF. Our study showed that high fed diet induced EMT in vivo by increasing the levels of EMT-activating transcription factors including zeb-1, zeb-2 and snail and changed the protein profile to characteristic for TGF. Our results show a link between excess fat accumulation and the change in hepatocytes phenotype to mesenchymal.

[IV-63] Influence of the shield placed on the way of ionizing radiation on the type of cell death

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About 4% of patients undergoing radiation therapy have metal implants in the body. The literature shows that the error in the dose obtained due to the presence of metal implants close to the therapeutic target is outside the clinically acceptable range. Materials such as titanium implants, amalgam fillings or other implants scatter ionizing radiation and may change the effectiveness of radiotherapy.

The aim of the study is to investigate the influence of the shield placed on the way of ionizing radiation on the type of cell death on a cellular model.

We examined the effect of lead shield on the effectiveness of radiotherapy. The shield was placed under and over the irradiated cells. We examined two human cell lines: FaDu (hypopharyngeal carcinoma cells) and BEAS-2B (normal bronchial epithelial cells). Irradiation of cells in T25 culture flasks was performed with single-dose: 10Gy. The bottles with cells were placed in a phantom, at a depth of 10 cm. Photon radiation X 6 MV, with a beam power of 400 MU/min, was used. Two days later we analyzed the number of cells and phases of the cell cycle. Next, we analyzed density of lysosomes (autophagy) and the presence of caspase-3+ cells (apoptosis) in the treated cells.

Ionizing radiation increased the percent of cells in SubG1 phase and Out G2/M phase in both cell lines. Ionizing radiation increased the number of apoptotic cells and aneuploid cells which may indicate two types of cell death: apoptosis and mitotic catastrophe. The autophagy process was observed only in BEAS-2B cells. Only BEAS-2B cells increased density of lysosomes in treated cells. Lead shield especially placed under irradiated cells increased the percent of caspase-3+ cells in both cell lines. Also, we observed decrease of cell viability after radiotherapy for FaDu cells placed over shield.

The lead shield especially placed under irradiated cells decreased the number of live cells, increased the number of apoptotic cells and induce G2/M arrest of cell cycle in FaDu cell line.

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[IV-64] ADIPOCYTOKINES STIMULATE ADSCs TO ROS PRODUCTION, AND IMPROVE CANCER CELLS VIABILITY IN PRESENCE OF AgNPs

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Introduction: Adipose-derived stem cells (ADSCs) produce adipokines that influence the bodys metabolism, their level is correlated with the course of neoplastic diseases and the immune response in viral infection. Cancer stem cells (CSCs) can lead to tumor growth as well as promote viral infections. Adipocytokines are known as pro-oxidative and antioxidative factors a dose regulates redox equilibrium. AgNP as photosensitizers can disrupt cellular functions and cause toxic effects, including DNA damage and apoptosis, also with normal cells and elevate the level of reactive oxygen species (ROS) produced during treatment.

Aim: The research aimed at oxidative stress followed by adipocytokines cocktail treatment directly on the ADSCs, and observations of an adaptative potential in cancer SCC-25 cells to AgNPs treatments

Materials and Methods: Human Adipose-Derived Stem Cells (ADSCs Sigma) and human tongue squamous carcinoma SCC-25 (ATCC CRL-1628), cultivated with the mixture of Dulbeccos modified Eagles medium, were exposed to adipocytokines (adipoMIX: vaspin, chemerin, omentin, visfatin; concentrations from 0.125 to 1 ng/mL) or silver nanoparticles alone (AgNPs, concentrations from 0.068 to 0.5 g/mL), and a combination of AgNPs with adipocytokines, respectively. ROS evaluation was followed by *dihydrofluorescein* diacetate fluorescence (Sigma) staining for flow cytometry assay (Aria III, Becton Dickinson). Live microscopic observation was performed using JuliBr (NanoEntek), transit channel, magnification 100 x. The cytotoxic activity of AgNPs with diameters of 10 nm 4 nm (Sigma) was measured by 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Promega) assay.

Results and Conclusions: ADSCs produce adipocytokines for self-stimulation and stem colony-forming units, then differentiation into the adipocytes (adipose tissue enlargement). ADSCs responded with ROS production to cytokines cocktail what elevates oxidative stress in tissue. Delivering of pro-oxidative regulators from surrounding tissue (adipose tissue) can temporarily improve the resistance of cancer cells, eg. SCC-25 cells to AgNPs treatment.

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[IV-65] POLYSOME ASSOCIATION OF PTEN MRNA IN CONTROL AND IRRADIATED HCT 116 CELL LINE

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Translated mRNA describes the state of the cell over time. A thorough understanding of the translation process may contribute to a better understanding of the cells response to stress or explain the difference in mRNA expression levels with protein abundance observed in the cell.

In this work, the translation of PTEN mRNA in HCT 116 cell line was analyzed. Because PTEN expression level is associated with regulation of the cell cycle progression and genetic stability, the IR-induced alteration of polysome association of PTEN mRNA was studied too.

Polysomes were fractionated by centrifugation in sucrose gradients (20%-50%). The gradient was divided into four fractions: non-translated (free mRNA + small subunit) and translated mRNA (large subunit + monosome, light polysomes, and heavy polysomes). Total RNA was extracted from each fraction by using Universal RNA/miRNA Purification Kit (EurX). The level of PTEN transcript was determined by RT-qPCR. To determine the protein level, a part of cells from cell culture has been taken for the Western Blot analysis.

The obtained results showed that ionizing radiation does not induce a change in the polysome profile of PTEN mRNA. The slight alteration in PTEN mRNA polysome profile was observed only 12h after irradiation.

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XXIV Gliwice Scientific Meetings
virtual conference
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AWARDED POSTER PRESENTATIONS

Prize of the *Association for the Support of Cancer Research* for the best poster presentation:

Judyta Górka, Paulina Marona, Oliwia Kwapisz, Agnieszka Waligórska, Ewelina Pośpiech, Jerzy Dobrucki, Janusz Ryś, Jolanta Jura, Katarzyna Miękus: RNAse activity of the MCPIP1 protein inhibits tumor progression by regulating Wnt/β-catenin signaling pathways and Epithelial-Mesenchymal Transition in Clear Cell Renal Cell Carcinoma [poster II-1]

Distinctions in the contest for the best poster presentation:

Marta Pałka, Aleksandra Tomczak, Ryszard Rzepecki: Lamin-associated proteome after heat shock induction – is lamin the novel element in stress granules aggregates? [poster I-4]

Marta Podralska, Marcin Sajek, Antonina Bielicka, Magdalena Żurawek, Iwona Ziółkowska-Suchanek, Tomasz Kolenda, Marta Kazimierska, Marta Kasprzyk, Weronika Sura, Barbara Pietrucha, Bożena Cukrowska, Natalia Rozwadowska, Agnieszka Dzikiewicz-Krawczyk: Identification of irradiation-induced ATM-dependent lncRNAs [poster II-3]

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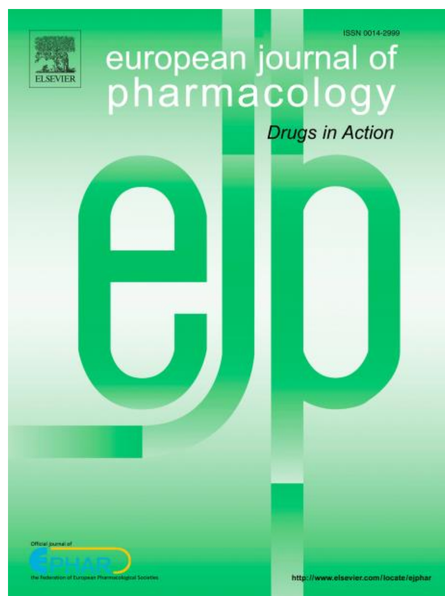

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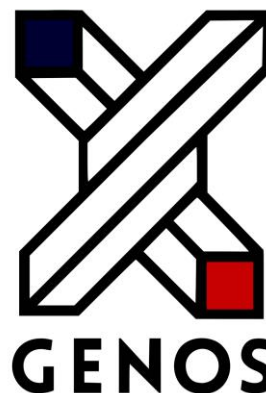

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