25th Anniversary of

Gliwice Scientific Meetings

November 18-20, 2021

Satellite Event for the jubilee ***XXV Gliwice Scientific Meetings***

Joint online seminar of the *Polish Proteomics Society* and the *Finnish Proteomics Society:*

Proteomics of Extracellular Vesicles

November 18, 2021, 14:00 – 20:00 CET

Chairpersons: **Tuula Nyman** *(Oslo)* & **Piotr Widłak** *(Gliwice)*

14.00 – 16.00 Part 1

Maija Puhka *(Finnish Institute of Molecular Medicine, HiLIFE, University of Helsinki, Helsinki):* Considerations of factors affecting proteomes in extracellular vesicle isolates: preanalytics and beyond.

Harry Holthöfer *(Finnish Institute of Molecular Medicine, University of Helsinki, Helsinki and University Medical Center Hamburg-Eppendorf, Hamburg):* Urinary extracellular vesicles: from process optimization to applications.

Marit Inngjerdingen *(Institute of Clinical Medicine, Oslo University Hospital, Oslo):* Dissection of functionally distinct EV subset released from NK cells guided by proteomics.

Raghu Kalluri *(The University of Texas MD Anderson Cancer Center, Houston):* The biology and function of exosomes in cancer.

16.00 – 16.15 Break

16.15 – 18.15 Part 2

Mari Palviainen (*EV-group, University of Helsinki, Helsinki):* Ins and outs of extracellular vesicles.

Susana Garcia-Silva *(Microenvironment and Metastasis Group, Spanish National Cancer Research Center – CNIO, Madrid):* Extracellular vesicles cargo on melanoma's service and against it.

Beate Vestad *(Research Institute of Internal Medicine, Oslo University Hospital, Oslo):* Plasma extracellular vesicles in people living with HIV and type 2 diabetes are related to microbial translocation and cardiovascular risk.

Anna Wojakowska *(Institute of Bioorganic Chemistry, Poznań):* Small extracellular vesicles represent different proteomic pattern in response to neoadjuvant radiotherapy in rectal cancer patients.

18.15 – 18.30 Break

18.30 – 20:00 Part 3

Mayank Saraswat *(Mayo Clinic, Rochester):* Extracellular vesicle composition and biology: What can we learn from quantitative proteomics?

Dmitry Ter-Ovanesyan (*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston):* Separating EVs from free proteins in biofluids.

Monika Pietrowska *(MSC National Research Institute of Oncology, Gliwice):* Analysis of exosomeshuttled proteins using immunocapture approaches followed by high resolution mass spectrometry.

Registration.

The seminar participants should register by November 17, 2021. The request for registration should be sent by e-mail via Polish Proteomics Society <proteom@man.poznan.pl> or Finnish Proteomics Society <maciej.lalowski@helsinki.fi>.

The relevant link to the **ZOOM platform** meeting will be sent to registered participants only.

The seminar has no registration fee.

The seminar organizing committee:

Maciej Lalowski *(University of Helsinki, Helsinki, Finland)*

Magdalena Łuczak *(Institute of Bioorganic Chemistry, Poznań, Poland)*

Tuula Nyman *(Institute of Clinical Medicine, Oslo University Hospital, Oslo, Norway)*

Monika Pietrowska *(Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland)*

Theresa Whiteside *(Hillman Cancer Center, University of Pittsburgh, Pittsburgh, USA)*

Piotr Widlak *(Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland)*

XXV Gliwice Scientific Meetings

Scientific Program

Friday, 19th November 2021

9.00 – 9.15 Opening of the conference

Welcome address: **Krzysztof Składowski** *(MSC-NRIO, Gliwice)*

9.15 – 10.15 Special session:

"Professor Mieczysław Chorąży – 70 years of research in tumor biology"

Rememberance of Prof. Mieczysław Chorąży

Announcement of the Laureate of the Prof. Mieczysław Chorąży Scholarship

The Mieczysław Chorąży Lecture 2021 –

Kari Hemminki *(Charles University, Pilsen & DKFZ, Heidelberg)*: Science and society.

10.15 – 10.30 Coffee Break

10.30 – 13.00 Session: "**Cancer Moonshot – Early Detection of Lung Cancer**" Chairpersons – Piotr Widłak & Witold Rzyman

Witold Rzyman *(Medical University of Gdańsk, Gdańsk)*: Lung cancer screening - where we are?

Luis Montuenga *(University of Navarra, Pamplona)*: The role of molecular markers in the lung cancer screening route [online].

Fabrizio Bianchi *(Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo)*: The small world of microRNAs and their big role in lung cancer [online].

Piotr Widłak *(MSC-NRIO, Gliwice)*: Serum metabolome profiling in early detection of lung cancer.

Joanna Polańska *(Silesian University of Technology, Gliwice):* Radiomics of lung cancer.

Marek Kimmel *(Rice University, Houston)*: Modeling risk of lung cancer: Coordination of molecular events and growth characteristics of tumors.

13.00 – 14.00 Lunch Break / poster viewing

14.00 – 18.00 Session: "**Regulation of Gene Expression and Chromatin"** Chairpersons – Joanna Rzeszowska & Sergey Razin

Sergey V. Razin *(Lomonosov Moscow State University, Moscow)*: Order and stochasticity in the 3D organization of individual genomes [online].

Aleksandra Pękowska *(Nencki Institute of Experimental Biology, Warszawa)*: Interplay between chromatin architecture and gene expression.

Michal Gdula *(Adam Mickiewicz University, Poznań)*: How to shut down a whole chromosome? - SmcHD1, epigenetics and 4D genome in X inactivation.

Gracjan Michlewski *(International Institute of Molecular and Cell Biology, Warszawa)*: Regulation of miRNA biogenesis - mechanisms and future therapies.

Agnieszka Ciesielska *(10x Genomics, Stockholm)*: Scale and resolution. Single-cell and spatial transcriptomics - more than marriage from convenience.

16.00 – 16.15 Coffee Break

Martin Simard *(Université Laval, Quebec)*: Regulation and modulation of the microRNA silencing pathway [online].

Victor Ambros *(University of Massachusetts Medical School, Worcester)*: Argonaute mutations that broadly disrupt microRNA activity in worms and human disease [online].

Grant McFadden *(Arizona State University, Phoenix)*: *Ex vivo* oncolytic virotherapy with myxoma virus [online].

19.30 Dinner& Social event (*Dworek u Hrabiego*, ul. *Świętokrzyska 6, 44-100 Gliwice)*

Saturday, 20th November 2021

9.00 – 10.00 **Virtual Poster Session** (part 1)/coffee

Short presentations of selected posters (5 minutes allocated to each presentation)

Poster Session 1

Ryszard Smolarczyk *Anticancer effect of vascular disrupting agent (CA4P) in combination with STING agonist (cGAMP) in murine breast cancer and melanoma therapy*
Marta Gawin *Intra-tumor heterogeneity revealed by mass spectrometry imaging is associated with the prognosis of breast cancer*
Kinga Pogoda-Mieszczak *The usefulness of murine mesenchymal cells as a myxoma virus carrier for the planned therapy of murine glioblastoma*
Agnieszka Potęga *Glutathione conjugation of the antitumor-active unsymmetrical bisacridine C-2028 in human liver subcellular fractions – the major role of glutathione S-transferase M1-1*
Adrianna Rutkowska *Analysis of the applicability of antibodies recognizing EGFRvIII in the case of primary glioblastoma cell lines*
Mateusz Smolarz *Analysis of miRNAs in serum and serum-derived exosomes – putative biomarkers for early detection of cancer in participants of lung cancer screening study*
Ali Maruf *Trehalose-Releasing Nanogels: A Potential Trehalose Delivery System for Autophagy Stimulation*
Joanna Pilch *Folic acid-quantum dots platforms for efficient delivery of unsymmetrical bisacridines to lung and prostate cancer cells*
Jolanta Kulesza *c-Myc protein level affected by unsymmetrical bisacridines plays a role in apoptosis induced by these compounds in HCT116 colorectal and H460 lung cancer cells*
Seweryn Gałecki *Variants in OPN and CD44 genes may influence recurrence and prognosis in non-small cell lung cancer treated with radiotherapy and chemoradiotherapy*
Marcelina Jureczko *How fast can be the vincristine removed by white-rot fungi?*
Marcela Krzempek *COVID-19 vaccinations decrease SARS-CoV-2 incidences: single medical Institute report*

10.00 – 11.45 Session: "**Radiation Biology and Medicine"**

Chairpersons – Dorota Słonina & Nicolas Foray

Nicolas Foray *(INSERM, Lyon)*: The radiation-induced nucleoshuttling of the ATM protein and its medical applications in radiotherapy and radiology.

Carmel Mothersill *(McMaster University, Hamilton)*: New non-targeted mechanisms identified after low dose radiation exposure.

Ludwik Dobrzyński *(National Centre for Nuclear Research, Świerk)*: Yonezawa effect and its modelling.

Marek Janiak *(Military Institute of Hygiene & Epidemiology, Warszawa)*: Antineoplastic effects of low-dose whole-body exposures to X-rays combined with inactivation of two immune checkpoints in mice.

11.45 – 12.00 Coffee Break

12.00 – 14.00 Session: "**Targeted Drug Delivery and Experimental Therapies**"

Chairpersons – Wiesław Szeja & Anna Kasprzycka

Grzegorz Grynkiewicz *(Pharmaceutical Research Institute, Warszawa)*: Sustainable chemistry for circular economy [online].

Andrzej Gamian/Beata Orzechowska (*Institute of Immunology and Experimental Therapy, Wrocław)*: Viral vaccine - new challenges [online].

Tomasz Sarnowski *(Institute of Biochemistry and Biophysics, Warszawa):* M13 bacteriophage and anti-SARS-CoV-2 VHH as a potential therapy against COVID-19 [online].

Ilona Wandzik *(Silesian University of Technology, Gliwice)*: Micro- and nanosized hydrogels for biomedical applications [online].

Katarzyna Jelonek *(Centre of Polymer and Carbon Materials, Zabrze)*: Biodegradable micelles for targeted delivery of anticancer drugs.

Wiesław Szeja *(Silesian University of Technology, Gliwice)*: Glut-targeting improves the properties of drug glycoconjugates in relations to neoplastic cells.

14.00 – 15.00 **Virtual Poster Session** (part 2)/coffee

Short presentations of selected posters (5 minutes allocated to each presentation)

Poster Session 2

Marta Kasprzyk *Functional dissection of IGH enhancers and enhancer RNAs in B-cell lymphomas*

Paulina Marona *The role of MCPIP1 protein in tumor initiation and stemness of normal epithelial cells*

Marta Podralska *Identification of irradiation-induced ATM-dependent lncRNAs*

Damian Sojka *p53-depletion enhances colon cancer cells' resistance to manumycin A*

Magdalena Surman *Ectosomal transfer of αvβ3 and αvβ5 integrins from melanoma cells to recipient vascular endothelial cells promotes their migration*

Agnieszka Gogler-Pigłowska *Deficiency of the HSPA2 gene promotes a more differentiated phenotype of human epidermal keratinocytes and induces alterations in the organization of the three-dimensional reconstructed epidermis*

Paweł Piłat *Identification of partners interacting with N4BP1 protein and determination of its cellular functions and localization*

Joanna Syrkis *Immunohistochemically detected LOX expression is higher in metastases than in primary tumors in ovarian cancer*
Wiktoria Płonka *The impact of DGCR8 inhibition on microRNA biogenesis in B-cell lymphoma*
Agnieszka Będzińska *The dual role of p53 in regulation of interferon signaling pathways*
Patryk Janus *The role of HSF1 in the regulation of the transcriptional response to estrogen at the level of chromatin organization*
Sybilla Matuszczak *Influence of the tumor microenvironment on the effectiveness of radiotherapy of head and neck squamous cell carcinomas in vitro*
Łukasz Skoczylas *HPV-positive head and neck squamous cell carcinoma cells release exosomes carrying HPV peptides*
Weronika Szukała *Mcpip1 in skin tumorigenesis*
Weronika Bagrowska *Modelling the cytoplasmic domain of the Toll-like receptor and initial studies of structure's dynamics*

15.30 – 15.45 Presentation of awarded posters and closing remarks
15.45 – Lunch

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Conference venue:
Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice Branch, Wybrzeże Armii Krajowej 15, 44-102 Gliwice, Jeremi Święcki Lecture Room.

Given the ongoing and expected effects of COVID-19, we plan to hold a hybrid type meeting with a limited number of participants in person and with online access to a wider audience.

More information at the conference web page: http://gsn.io.gliwice.pl

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LECTURE ABSTRACTS

SESSION: "**CANCER MOONSHOT – EARLY DETECTION OF LUNG CANCER**"

[L-1] LUNG CANCER SCREENING – WHERE ARE WE?

Witold Rzyman

Medical University of Gdańsk, Gdańsk

Lung cancer is the most common cancer in Poland and worldwide, and the leading cause of cancer-related deaths. Compared to the present day, the annual number of new cases of lung cancer will have increased by approximately 50%, by 2030. The overall ratio of mortality to incidence totals 0.87 and is among the highest. Despite the ongoing effort including implementation of multiple regimes in clinical oncology and thoracic surgery, the poor outcome of lung cancer treatment resulting, at best, in average 5-year survival of 11-16%, has remained virtually unchanged throughout the last 5 decades. Any preventive action, either primary or secondary, tends to be less expensive than treatment of advanced diseases, provided that it is appropriately designed and targets a population at risk. In contrast to basic sciences, in which the invested resources rarely are related to economic gain, medical prevention always entails the economic return. Effective primary and secondary prevention are the only ways to improve these fatal statistics.

In 2016, lung cancer screening using low-dose computed tomography (LDCT) was introduced to routine clinical practice in the United States following the publication of the largest randomized study, The National Lung Screening Trial that showed 20% mortality reduction in the screening group. The implementation of screening programs in Poland and the rest of Europe also seems unavoidable since the results NELSON trial showed 26% mortality reduction. In 2020 national demonstration lung cancer screening programs have been launched in Poland, Great Britain, Croatia and Hungary. Remaining European countries are either in the preparation phase to lung cancer screening implementation or awaiting the impulse to undertake such an action.

Although implementation is in the rapid phase of development there are several weaknesses of LDCT lung cancer screening that needs to be resolved. Nodule management, risk factors adoption to the screening process, application of AI tools for CT image interpretation, application of molecular signatures as diagnostic tests are among the most vivid issues in lung cancer screening efforts that will be discussed in the presentation.

[L-2] THE ROLE OF MOLECULAR MARKERS IN THE LUNG CANCER SCREENING ROUTE

Luis Montuenga

CIMA. University of Navarra and CIBERONC,Pamplona, Spain

In March this year, the US Preventive Services Task Force (USPTF) has updated its former recommendations regarding Low Dose CT (LDCT)-based lung cancer screening. The USPTF recommends annual screening for lung cancer with low-dose computed tomography (LDCT) in adults aged 50 to 80 years who have a 20 pack-year smoking history and currently smoke or have quit within the past 15 years. The initial observational results published by the I-ELCAP consortium and the NLST and NELSON randomized trials, together with other smaller trials, have clearly shown the efficacy of this lung cancer early detection strategy in high risk individuals. Nevertheless, there are still limited numbers of lung cancer screening programs worldwide, considering the at-risk population. In Europe only Poland, Croatia and some other few regions have implemented population-based LDCT lung cancer screening programs and other are currently considering this implementation. The relevant international societies have recommended for LDCT screening in high-risk individuals, together with smoking cessation programs. In the March 2021 JAMA publication of the USPSTF criteria, there is a final paragraph on research and current gaps, two of which are related to molecular biomarkers. My talk will focus on the potential role of molecular biomarkers in the different landmarks found in the route of LDCT-based lung cancer screening, with special interest in the management of nodules and diagnosed lung cancers. Biomarkers may optimize the effectiveness, including cost-effectiveness, of screening at least in three aspects. (a) Refining the selection of persons which should be advised to undergo LDCT screening, in order to complement the available epidemiological data-based risk models; (b) contributing to the characterization of the risk level associated to indeterminate nodules detected in the LDCT screening process; and (c) predicting outcome or response to therapy, once a lung cancer has been diagnosed. A fourth potential role of molecular markers being recently proposed is in the prediction of the response to chemo- or immuno-prevention strategies in patients with nodules. I will summarize some of examples of the available biomarkers, which are undergoing clinical validation in intended to use (screening) cohorts. None of the current biomarkers has reached the level of clinical utility validation. I will discuss potential designs to reach a rigorous validation for clinical utility. I will also present some examples of recently developed biomarkers or new technologies to identify novel molecular markers for early detection. I will focus on several recently published technologies, including those based on the deep sequencing of circulating DNA, some of which have been designed as tools for pan-cancer detection. I will comment of the pros and cons of these novel technical approaches from the different points of view (accuracy, affordability, cost-effectiveness, etc.). Finally, I will discuss the potential advances of biomarkers in this field in the future.

[L-3] THE SMALL WORLD OF MICRORNAS AND THEIR BIG ROLE IN LUNG CANCER

Fabrizio Bianchi, PhD

Head of Cancer Biomarkers Unit, IRCCS Casa Sollievo della Sofferenza Foundation, 71013 San Giovanni Rotondo - Italy

Biomarker-driven lung cancer early detection and prognostication nowadays represent two of the most promising approaches to reduce the burden of this deadly disease. In the past few years, we focused our research on intracellular and circulating microRNAs (small single stranded non-coding RNA molecules of 22nt in size) to tackle their potential as lung cancer biomarkers, and to study their function in the realm of non-small cell lung cancer (NSCLC). We identified modules of miRNAs intertwined with cancer molecular networks which allowed us to identify new aggressive lung cancer subtypes and mechanisms of chemoresistance. Furthermore, we identified extracellular miRNAs which hallmark the presence of asymptomatic lung tumors. Here, I will present most recent data which underline the critical role of miRNAs in lung cancer biology as well as their reliability as diagnostic and prognostic biomarkers.

[L-4] SERUM METABOLOME PROFILING IN EARLY DETECTION OF LUNG CANCER

Widlak Piotr[1], Jelonek Karol[1], Smolarz Mateusz[1], Kurczyk Agata[1], Żyła Joanna[2], Sitkiewicz Magdalena[3], Veronesi Giulia[4], Polańska Joanna[2], Rzyman Witold[3]

[1]*MSC National Research Institute of Oncology, Gliwice, Poland*
[2]*Silesian University of Technologies, Gliwice, Poland*
[3]*Medical University of Gdańsk, Gdańsk, Poland*
[4]*IRCCS San Raffaele Scientific Institute, Milan, Italy*

Serum metabolome and components of extracellular vesicles (EVs) present in the blood are promising sources of molecular biomarkers that could support the early detection of lung cancer in screening programs based on low-dose computed tomography. Several panels of metabolites present in serum end serum-derived small EVs (exosomes) that differentiate lung cancer patients and healthy individuals were reported, yet none of them were validated in the population at high risk of developing cancer. Here we analyzed metabolome profiles in participants of two lung cancer screening studies: MOLTEST-BIS (Gdańsk, n=369), and SMAC (Milan, n=93). Three groups of screening participants were included from each cohort: patients with screen-detected lung cancer, individuals with benign pulmonary nodules, and those without any lung alterations. Concentrations of about 400 metabolites (lipids, amino acids, and biogenic amines) were measured by a mass spectrometry-based approach. We observed a reduced level of lipids, in particular cholesteryl esters, in sera of cancer patients. However, despite several compounds showing significant differences between cancer patients and healthy controls were detected within each study, only a few cancer-related features were common when both studies were compared, including reduced levels of LPC(18:0). A large heterogeneity of serum metabolomes was observed, both within and between studies, which impaired the accuracy of classifiers based on specific metabolites. The average AUC values of three-state classifiers were 0.60 and 0.51 for the test (MOLTEST) and validation (SMAC) cohorts, respectively. Therefore, a hypothetical metabolite-based biomarker for early detection of lung cancer would require adjustment to lifestyle-related confounding factors that putatively affect the composition of serum metabolome. Moreover, the lipid profiles of the total fraction of small EVs obtained from sera of participants of the MOLTEST study were analyzed. A few lipids whose levels were different between compared groups were detected, including ceramide Cer(42:1) upregulated in vesicles from cancer patients. On the other hand, the contribution of phosphatidylcholines with poly-unsaturated acyl chains was reduced in vesicles from lung cancer patients. However, high heterogeneity of lipid profiles of small EVs was observed, which impaired the performance of classification models based on specific compounds (the average AUC value of the three-state classifier was 0.58 in the test subset). Hence, the data obtained do not support the concept of using the lipidome components of the total mixture of extracellular vesicles present in serum as biomarkers for early lung cancer detection.

[L-5] RADIOMICS OF LUNG CANCER

Joanna Polańska

Silesian University of Technology, Gliwice

[L-6] MODELING RISK OF LUNG CANCER: COORDINATION OF MOLECULAR EVENTS AND GROWTH CHARACTERISTICS OF TUMORS

Marek Kimmel

Departments of Statistics and Bioengineering, Rice University, Houston TX

Lung cancer is a deadly disease still claiming around 200,000 lives per year in the United States. This talk summarizes research on quantitative understanding of progression of lung cancer which was carried out for past 30 years by a group mainly at Rice University, MD Anderson Cancer Center and Baylor College of Medicine. I will first review statistical concepts of screening for early detection of cancer. They culminated in a major clinical trial that demonstrated, in 2011, the ca. 20% mortality reduction potentially achievable by screening for lung cancer using computed tomography. However, earlier than this, mathematical modeling predicted a reduction of similar magnitude. This was accomplished by a careful studies of incidence and mortality, by researchers from all over the world, which then informed mathematical models capable of predicting the long-term effects of public health interventions.

Building such models involves splitting the natural course of disease into an early phase at which the tumor is growing slowly and remains localized, so that removal may lead to cure, and later phases with faster growth and local and distant aggression, which might be targeted by systemic therapies, but with less certainty of cure. Due to recent progress in DNA sequencing, including single-cell sequencing, insights can be gained into the timing of the waves of mutations and other genome transformations, which leave trace in the cancer cell genomes. Analysis based on probabilistic models of genetics can help estimate the relative rates of evolution of different clones and hence the relative durations of phases corresponding to small vs. large and slowly vs. fast growing tumors. I will review some of these estimates and discuss their potential importance. This work is still in progress.

References:

Flehinger BJ, Kimmel M, Melamed MR. The effect of surgical treatment on survival from early lung cancer: Implications for screening. Chest. 1992 Apr 1;101(4):1013-8.

Foy M, Yip R, Chen X, Kimmel M, Gorlova OY, Henschke CI. Modeling the mortality reduction due to computed tomography screening for lung cancer. Cancer. 2011 Jun 15;117(12):2703-8.

Dinh KN, Jaksik R, Kimmel M, Lambert A, Tavaré S. Statistical inference for the evolutionary history of cancer genomes. Statistical Science. 2020 Feb;35(1):129-44.

Credits to:

Xing Chen, Shenyan Medical University
Khanh Ngoc Dinh, Columbia University
Roman Jaksik, Silesian University of Technology
Ivan Gorlov, Baylor College of Medicine
Olga Gorlova, Baylor College of Medicine
Andrew Koval, Rice University
Amaury Lambert, Sorbonne Universites
Simon Tavaré, Columbia University

LECTURE ABSTRACTS

SESSION: **“CHROMATIN AND REGULATION OF GENE EXPRESSION”**

[L-7] ORDER AND STOCHASTICITY IN THE 3D ORGANIZATION OF INDIVIDUAL GENOMES

Sergey V. Razin

Institute of Gene Biology RAS and Lomonosov Moscow State University, Moscow Russia

It was demonstrated that the eukaryotic chromosomes are separated into the contact chromatin domains. These contact domains are believed to be structural-functional blocks of genome, which restrict the areas of enhancers action. Indeed, it was shown on several model systems that the removal of contact domain boundaries or the creation of new boundaries leads to a change in the spectrum of genes activated by an enhancer. At the same time, no similar effects were observed in other genome regions. These contradictory results can be caused by a plasticity of the 3D genome. Most of the observations concerning the role of 3D genome organization in the regulation of transcription were made in experiments performed on cell populations. However, it is clear that population Hi-C maps show an average picture of the spatial organization of the genome. The spatial organization of the genome in individual cells can differ significantly from this average picture. Indeed, using the FISH technique it has been demonstrated that the physical distances between the same genomic loci can vary significantly in individual cells. From a fundamental and applied point of view, it is extremely important to assess the degree of variability in the spatial organization of individual genomes. To this end, we have developed experimental approaches for Hi-C analysis of individual cells. Using these approaches, the profiles of genome partitioning into contact domains in individual mouse and Drosophila cells were analyzed. It turned out that in mouse cells only about 30% of the boundaries of the contact domains coincide between cells, whereas in Drosophila cells more than 40% of the boundaries are constant. Constant boundaries are characterized by a high level of transcriptionally active chromatin marks. Polymer simulations demonstrate that chromatin folding is best described by the random walk model within contact domains and is most suitably approximated by a crumpled globule build of Gaussian blobs at longer distances. The shape of contact domains and the path of DNA within a chromosomal territory vary significantly between individual cells. We observe prominent cell-to-cell variability in the long-range contacts between either active genome loci or between Polycomb-bound regions, suggesting an important contribution of stochastic processes to the formation of the Drosophila 3D genome.

This work was supported by Russian Science Foundation (RSF) grant #19-14-00016

[L-8] INTERPLAY BETWEEN CHROMATIN ARCHITECTURE AND GENE EXPRESSION

Aleksandra Pękowska

Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur Street, 02-093 Warsaw, Poland

Topologically Associating Domains (TADs) are megabase-sized genomic intervals of preferential genomic contacts. TADs are implicated in the functional interplay between cis-regulatory elements. The formation of TADs critically relies on the concert action of cohesins and CTCF, an eleven zinc finger insulator protein. Cohesins tether sequences to each other while CTCF underlies the formation of TAD boundaries by limiting the interactions to regions within TADs. The two CTCF-enriched TAD boundaries frequently form a strong interaction reminiscent of a loop.
I will show our previous and current work where we combined high-throughput sequencing approaches, including in-situ HiC, and computational tools to address the question of how cohesins and CTCF participate in the regulation of genome topology and in the control of gene expression. I will present data connecting cellular energy consumption and cohesin action on chromatin. In the second part of the talk, I will show the timing of the maturation of chromatin structure during the earliest stages of mammalian development.

[L-9] HOW TO SHUT DOWN A WHOLE CHROMOSOME? - SMCHD1, EPIGENETICS AND 4D GENOME IN X INACTIVATION

Michal Gdula

Adam Mickiewicz University, Poznań

X chromosome inactivation is the mechanism that evolved in mammals to equalize levels of X-linked gene expression in XX females relative to XY males. Cells of early female embryos selectively inactivate a single X chromosome, usually at random, resulting in the formation of a stable heterochromatic structure, the Barr body. The inactive X chromosome (Xi), once established, is highly stable, and is maintained in somatic cells throughout the lifetime of the animal. The inactive X chromosome (Xi) in female mammals adopts an atypical higher-order chromatin structure, manifested as a global loss of local topologically associated domains (TADs), A/B compartments and formation of two mega-domains. The non-canonical SMC family protein, SmcHD1, which is important for gene silencing on Xi, contributes to this unique chromosome architecture. Specifically, allelic mapping of the transcriptome and epigenome in SmcHD1 mutant cells reveals the appearance of sub-megabase domains defined by gene activation, CpG hypermethylation and depletion of Polycomb-mediated H3K27me3. These domains, which correlate with sites of SmcHD1 enrichment on Xi in wild-type cells, additionally adopt features of active X chromosome higher-order chromosome architecture, including A/B compartments and partial restoration of TAD boundaries. Xi chromosome architecture changes also occurred following SmcHD1 knockout in a somatic cell model, but in this case, independent of Xi gene derepression. SmcHD1 is a key factor in defining the unique chromosome architecture of Xi.

[L-10] REGULATION OF MIRNA BIOGENESIS - MECHANISMS AND FUTURE THERAPIES

Gracjan Michlewski

International Institute of Molecular and Cell Biology, Warszawa

MicroRNAs (miRs) regulate gene expression by binding to complementary target mRNAs and repressing their translation and/or triggering RNA degradation. Primary miR molecules (pri-miRs) undergo nuclear and cytoplasmic processing events into precursor (pre-miR) and mature miR, respectively. The regulation of microRNA levels is crucial in development, differentiation, and numerous diseases. We and others have demonstrated that RNA-binding proteins (RBPs) that bind to the pri- and pre-miRs can influence their processing.

Therapeutic approaches targeting RNA-protein interactions have shown promising effects on some diseases that were previously regarded as incurable. Here, I will demonstrate a novel fluorescent on-bead screening platform, RNA Pull-Down COnfocal NAnoscanning (RP-CONA), used to identify RNA-protein interaction modulators in eukaryotic cell extracts. Using RP-CONA, we identified several small molecules that disrupt the interaction between HuR, an RBP inhibitor of brain-enriched miR-7 maturation, and the conserved terminal loop of pri-miR-7-1. Importantly, miR-7's primary target is an mRNA of α-synuclein, which contributes to the aetiology of Parkinson's disease (PD). Thus, upregulation of miR-7 and downregulation α-synuclein should contribute towards alleviating PD.

In summary, RP-CONA identified a small molecule able to upregulate cellular miR-7 levels and downregulate the expression of α-synuclein. This opens up new therapeutic avenues towards treatment of PD as well as provides a novel methodology to search for modulators of RNA-protein interaction.

[L-11] SCALE AND RESOLUTION. SINGLE-CELL AND SPATIAL TRANSCRIPTOMICS - MORE THAN MARRIAGE FROM CONVENIENCE

Agnieszka Ciesielska

10x Genomics, Stockholm

[L-12] REGULATION AND MODULATION OF THE MICRORNA SILENCING PATHWAY

Martin Simard

Université Laval, Quebec, Canada

The Argonaute proteins are at the core of the gene silencing pathways mediated by microRNAs and are essential for development across the animal kingdom. In *C. elegans,* the microRNA-specific Argonautes ALG-1 and ALG-2 regulate multiple processes required for the animals' proper developmental timing and viability. These developmental cues can be monitored at various stages of their lifespan and represent a unique opportunity to uncover new regulatory mechanisms with genetic and molecular approaches. During this talk, I will discuss our recent progress in identifying new ways of controlling Argonaute proteins for modulating the microRNA-mediated gene silencing in animals.

[L-13] ARGONAUTE MUTATIONS THAT BROADLY DISRUPT MICRORNA ACTIVITY IN WORMS AND HUMAN DISEASE

Ye Duan[1], Yi Yi[2], Ganesh Prabhakar[2],Anna Zinovyeva[2], Amelie Piton[3], Victor Ambros[1]

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MicroRNAs (miRNA) are small regulatory RNAs that exist in all multicellular eukaryotes. Mature miRNAs are bound by Argonaute proteins to form the miRNA induced silencing complex (miRISC), which post-transcriptionally regulates target gene expression through microRNA-guided base pairing to target mRNAs. Recently, multiple *de novo* coding variants in a human Argonaute gene *AGO1* were reported to cause neurodevelopmental disorders with intellectual disability (Schalk et al, Piton, Gerard 2020 *BioRxiv*). Interestingly, most of the amino acids altered in the in human *AGO-1* mutations are identical between *H. Sapiens* and *C. elegans* microRNA Argonautes, suggesting that they are associated with evolutionarily conserved miRISC functions. To better understand how these amino acid changes in human *AGO1* could affect miRISC function, we used CRISPR/Cas9 to generate the identical mutations in the *C. elegans AGO1* homolog, *ALG-1*. We found that several of these *ALG-1* mutations resulted in developmental defects in *C. elegans* that are characteristic of *ALG-1* loss-of-function, but with an intriguing twist: Aspects of the phenotypes are expressed with a severity greater than that of the *ALG-1 null* mutation, indicating that these particular amino acid changes have *antimorphic* effects on *ALG-1*. We interpret the antimorphic properties of these mutant *ALG-1* proteins to indicate that they bind to microRNAs, but are impaired in one or more step in miRISC assembly or function, thus sequestering microRNAs in non-functional complexes – and hence deleteriously competing with paralogous argonaut proteins (the worm's *ALG-2*). To characterize how the *ALG-1* mutations impact post-transcriptional gene regulation by microRNAs, we used immunoprecipitation of *ALG-1* from worm lysates, followed by small RNA sequencing, and found that that each mutant *ALG-1* protein displays a characteristic profile of sequestered microRNAs. Similarly, ribosome profiling of the worm mutants reveals allele-specific disruptions in expression of hundreds of worm genes. We hypothesize that the human AGO-1 mutations corresponding to those that we modelled in *C. elegans ALG-1* could likewise disrupt miRISC function globally in human cells through antimorphic deleterious competition with the human paralogs of *AGO-1*, *AGO-2*, *-3*, and *AGO-4*. We are currently testing these ideas using mammalian models. Further, we anticipate that the precise locations and nature of the amino acid changes leading to antimorphic Argonaute activity provide a powerful framework for guiding structure-function studies of evolutionarily conserved miRISC activities.

[L-14] *EX VIVO* ONCOLYTIC VIROTHERAPY WITH MYXOMA VIRUS

Grant McFadden

Arizona State University, Phoenix

LECTURE ABSTRACTS

SESSION: "**RADIATION BIOLOGY AND MEDICINE"**

[L-15] THE RADIATION-INDUCED NUCLEOSHUTTLING OF THE ATM PROTEIN AND ITS MEDICAL APPLICATIONS IN RADIOTHERAPY AND RADIOLOGY

Nicolas Foray

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The evaluation of the radiation-induced (RI) risks is of medical, scientific and societal interest. However, despite considerable efforts, there is still neither consensual and unified mechanistic models nor predictive assays for describing the three major RI effects, namely radiosensitivity, radiosusceptibility and radiodegeneration. The ATM protein is a major stress response factor involved in the DNA repair and signaling that appears upstream most of pathways involved in the three precited RI effects. The rate of the RI ATM nucleoshuttling (RIANS) has been shown to be a good predictor of radiosensitivity at high doses and notably after radiotherapy. In the frame of the RIANS model, irradiation triggers the monomerization of the cytoplasmic ATM dimers, which allows ATM monomers to diffuse in nucleus. The resulting nuclear ATM monomers phosphorylate the H2AX histone, which triggers the recognition of DNA double-strand breaks and their repair. The RIANS model has permitted to define three subgroups of radiosensitivity and provided relevant explanation for the radiosensitivity of syndromes caused by mutated cytoplasmic proteins, especially neurodegenerative diseases. At low doses, the RIANS model has provided molecular explanation of the hyper-radiosensitivity to low dose, the hormesis and the adaptive response phenomena which leads to a new reading of epidemiological data from individuals exposed to ionizing radiation.

[L-16] NEW NON-TARGETED MECHANISMS IDENTIFIED AFTER LOW DOSE RADIATION EXPOSURE

Carmel Mothersill

McMaster University, Hamilton

This presentation will review our latest understanding of the radiation induced bystander effect. There will be a special emphasis on the new UV and acoustic signals whic appear to trigger the ownstream events. The production, transmission and repsonse to these signals will be discussed with special emphasis on likely uses in radiotherapy and mitigation of effects in radiation protection.

[L-17] YONEZAWA EFFECT AND ITS MODELLING

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The Yonezawa Effect (YE) is a good example of the adaptive response in the organisms subjected to low priming dose of ionizing radiation followed after some time by the challenging dose which is or is close to be fatal. As one shows the priming dose results in prolongation of lifetime of mice, it is also seen in the number of e.g. micronuclei appearing in human and mice cells. Another side of this effect is a possibility of performing radiation training which would allow patients to better endure high radiotherapeutic doses. The aim of the presentation is to show examples of this effect and how it may be modelled.

[L-18] ANTINEOPLASTIC EFFECTS OF LOW-DOSE WHOLE-BODY EXPOSURES TO X-RAYS COMBINED WITH INACTIVATION OF TWO IMMUNE CHECKPOINTS IN MICE

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As evidenced by the results of preclinical and clinical investigations, whole-body irradiations (WBI) with X-rays at less than 0.1-0.2 Gy per fraction induce remissions of various neoplasms without inciting adverse side effects. In the present study a murine model of human non-small-cell lung cancer was employed to evaluate for the first time the anti-cancer efficacy of WBI combined with blockade of two major immune checkpoints. The results indicate that WBI used alone and in conjunction with the inhibition of the function of CTLA-4 and PD-1 markedly reduced tumourigenesis in mice implanted by three different routes with syngeneic Lewis lung cancer cells and suppressed clonogenic potential of these cells in vitro. Sole application of the tested inhibitors did not appear to affect the growth of cancer cells in vivo and in vitro, but when both blockers were used concurrently with WBI the anti-neoplastic effect of the latter treatment was noticeably enhanced. These results were associated with the relevant changes in the profile of immune cells recruited to the growing tumours and the circulating anti- and pro-inflammatory cytokines. The obtained results supplemented with mechanistic explanations provided by future investigations will help design the effective treatment of lung and other cancers based on inactivation of immune checkpoints combined with low-dose radiotherapy.

SESSION: "**TARGETED DRUG DELIVERY AND EXPERIMENTAL THERAPIES**"

[L-19] SUSTAINABLE CHEMISTRY FOR CIRCULAR ECONOMY

Grynkiewicz Grzegorz

Warsaw Scientific Society

European Green Deal (EU Climate Target Plan 2030), which is focused on radical reduction of the atmospheric content of greenhouse effect gases (GHG; primarily CO2), requires a prompt switch of industrial sectors to sustainable development mode and adapting it to the circular economy (CE) principles. Consequently, a pharmaceutical industry that is essential for supporting the healthcare system is facing radical changes in the entire supply chain from raw materials and intermediates, through active pharmaceutical ingredients (API) and auxiliary formulation materials, to packaging. Currently used substances, manufactured from petrochemicals based on non-renewable fossils will need to be replaced by new materials manufactured with the use of clean energy and feedstock based on renewable carbon resources coming from biomass. In order to illustrate the magnitude of dramatic changes which the entire chemical sector will face in immediate future to comply with the treaties striving for reduction of atmospheric GHG level, we will shortly discuss the concept of biorefineries as industrial facilities designed to supply energy and chemicals from renewable feedstock. Lignocellulose biomass (LCB), produced by plants through photocatalytic CO2 fixation in ca. 1011 tons/y is composed of three organic polymers: cellulose, hemicellulose, and lignin, which are rich in carbon (ca 50%) but need quite substantial energy to get deconstructed and converted into low molecular weight compounds, which can be used as platform chemicals, fuels, and solvents. Chemical and biotechnology processes designed for the transformation of biomass sugar polymers into value-added products will be discussed in some detail to illustrate trends in the current and future pharmaceutical industry.

[L-20] VIRAL VACCINES - NEW CHALLENGES

Andrzej Gamian, Beata Orzechowska

Institute of Immunology and Experimental Therapy, Wrocław

[L-21] M13 BACTERIOPHAGE AND ANTI-SARS-COV-2 VHH AS A POTENTIAL THERAPY AGAINST COVID-19

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In December 2019, a previously unknown coronavirus that causes atypical pneumonia, emerged in the city of Wuhan in China. This virus, named SARS-CoV-2, has reached virtually all corners of the world within a few months. According to official information, in November 2021, SARS-CoV-2 had caused the deaths of more than 5,000,000. More than 250,000,000 people worldwide were confirmed to suffer of SARS-CoV-2 caused COVID-19 disease. Since the pandemic's beginning, the whole world has been working on developing vaccines and an effective COVID-19 treatment. As the mutation rate of the virus is very high the risk that newly emerging variants may become resistant to currently developed vaccines and proposed therapies is considerable.

We synthetized *de* novo using template –free Gibson method, overexpressed and purified fragments of SPIKE protein of SARS-CoV-2 virus. Such fragments were used for the identification of specific VHH. The VHHs alone and fused with the M13 bacteriophage exhibited their functionality in the blocking of coronavirus entry in *in vitro* tests. Given their properties and safety they represent interesting and valuable candidates for establishment of new antiviral therapy against SARS-CoV-2 coronavirus and may serve as the basement for therapies against other factors with pandemic potential.

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[L-22] MICRO- AND NANOSIZED HYDROGELS FOR BIOMEDICAL APPLICATIONS

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Hydrogels are polymeric materials with high water content formed by crosslinking polymer chains. They exhibit a wide range of beneficial properties such as biocompatibility, high loading capacity, and sensitivity to environmental stimuli, what makes them especially relevant for biomedical engineering applications. The present study is focused on the development and the potential use of micro- and nanogels in 3D cell culture and drug delivery systems.

Keywords: hydrogels, trehalose, 3D cell culture, drug delivery, autophagy, biocompatibility

[L-23] BIODEGRADABLE MICELLES FOR TARGETED DELIVERY OF ANTICANCER DRUGS

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Polymeric micelles have been widely studied and developed as drug carrier for targeting solid tumors. The micelles have a core-shell structure formed by self-assembly of amphiphilic block copolymers, so they can incorporate drugs of poor water solubility in the hydrophobic core, while the outer hydrophilic corona provides biocompatibility and prolonged circulation in blood by avoiding rapid clearance by the reticuloendothelial system. Compared to surfactant micelles, block copolymer-based micelles are characterized by higher stability and larger versatility for controlling micellar structure and functionality by choices of polymer composition, architecture, molecular weight and monomer chemistry. The most popular polymer forming the outer shell is poly(ethylene glycol) (PEG) due to its hydrophilicity and biocompatibility. Poly(lactide) (PLA) is one of the most commonly used hydrophobic blocks of the amphiphilic polymers because it is biocompatible and biodegradable.

Among the various aggregates, flexible filomicelles in analogy to filoviruses possess a long circulation time and display almost twice higher drug loading capacity as compared to spherical micelles due to larger core volume per carrier. Filomicelles were developed from poly(lactide)/poly(ethylene glycol) (PLA/PEG). Factors controlling morphology of PLA/PEG micelles, paclitaxel encapsulation efficiency and *in vitro* release process were identified. It was shown that the release of paclitaxel is strongly dependent on the degradation of polymer. Also, PLA/PEG filomicelles for multidrug delivery were developed. Simultaneous administration of two or more pharmacologically active agents with different mechanisms of action is recognized as more efficient compared to conventional therapy based on a single therapeutic agent. Crucial importance of intermolecular interactions for drug loading and release properties was revealed.

In the past decade, several strategies, which include specific ligand-decoration of micelles for receptor mediated endocytosis (active targeting) have been developed to enhance tumor cell uptake of drug-loaded biodegradable micelles. A variety of small molecules such as folic acid and biotin have been used as targeting ligands for cancer chemotherapy, because they are readily available, inexpensive, nontoxic, nonimmunogenic and easy to modify. Folate targeted filomicelles for delivery of phosphate derivative of betulin were developed and characterized. However, due to heterogeneity of tumor cells, targeting efficiency may be enhanced by using different types of targeting agents on the same drug delivery system. Therefore, the current study is focused on development and characterization of PLA/PEG micelles dual-targeted with folic acid and biotin for co-delivery of docetaxel and resveratrol.

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[L-24] GLUT-TARGETING IMPROVES THE PROPERTIES OF DRUG GLYCOCONJUGATES IN RELATION TO NEOPLASTIC CELLS

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The development of targeted approaches is the ultimate goal to achieve improvements in disease diagnostics and treatment. Facilitative carbohydrate transporters – GLUTs – have received wide attention over decades due to their essential role in nutrient uptake and links with various metabolic disorders including cancer [1]. GLUT1 has been found to be overexpressed in a variety of both solid and hematological malignancies, and became attractive targets for cancer research and medicinal chemistry, leading to the development of new approaches to cancer diagnostics and for cancer-targeting therapeutics [2,3]. The overall differences in GLUT composition between cells in conjunction with higher sugar consumption in cancer cells have provided a strong basis to view GLUTs as important therapeutic targets. Accelerated metabolism of glucose and high glucose transport in a significant percentage of cancers provided a strategy to improve the selectivity of chemotherapy drugs *via* glycoconjugation [4].

The project represents the first attempt to systematically evaluate the anticancer activities of novel methotrexate and gemcitabine conjugates *in vitro* and *in vivo*. We employed specific targeting of GLUT1 by glucose derivatives and investigated methotrexate or gemcitabine drug conjugates for their ability to induce cancer-specific cytotoxicity. Several linkages, those included amide, triazine, ester, and glycosidic linkages for conjugating glucose and drugs were assessed. The cytotoxicity of conjugates was assessed over 24h, 48h, 72h, and significant dependence between cancer-selectivity and conjugate structure was observed. The differences were found to depend on the type of linkers and functionalization of sugar hydroxyl groups. The results indicate that the glycoconjugates exhibit potent anticancer activity against a range of solid tumor cell lines with IC50 values significantly lower compared to free drugs.

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POSTER ABSTRACTS

[1] HYPOXIA-INDUCIBLE FACTOR 1α SUBUNIT IS A CLIENT OF HEAT SHOCK PROTEIN A2 IN HUMAN EPIDERMAL KERATINOCYTES

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The epidermis is a stratified squamous epithelium that is composed primarily of keratinocytes. As a dynamic and perpetually renewing tissue, the epidermis is divided into four layers according to keratinocyte morphology and position. Keratinocytes migrate from the basal layer to the surface of the skin to produce the horny layer during terminal differentiation process. This tightly regulated action is controlled in part by factors related to the underlying dermis, but also by intrinsic mechanisms that are specific for keratinocytes and can be modulated by microenvironment conditions. In this context it is noteworthy that the epidermis represents a type of hypoxic tissue. The oxygen concentration around the basal keratinocytes can naturally be as low as 0.1%. In such a condition the activation of a transcriptional program involving Hypoxia Inducible Factor 1 (HIF-1) occurs. It is believed that HIF-1 through impact on keratinocyte differentiation can regulate the formation of a fully stratified epidermis. HIF-1 is also considered a key factor in the keratinocyte response to UVB exposure.

In this study we found that HIF-1α, an oxygen-sensitive subunit of HIF-1, is a binding partner of HSPA2 molecular chaperone. HSPA2 is a poorly characterized and differentiation-related member of the HSPA (HSP70) family. HSPA2 is encoded by the HIF-1-dependent gene, which is expressed in a cell type specific manner in the testis, brain as well as in various multilayered epithelia. Previously, we showed that HSPA2 in the epidermis is produced primarily in keratinocytes located in the basal layer and that it can play a role in regulating keratinocyte differentiation. In this study, using PLA method we found that HIF-1α directly interacts with HSPA2 in human epidermis ex vivo, as well as in reconstituted 3D epidermal equivalent in vitro. HIF-1α-HSPA2 interactions were more numerous under hypoxic than normoxic conditions in keratinocytes grown in standard 2D culture. Co-immunoprecipitation experiments confirmed that HIF-1 can bind to HSPA2 in keratinocytes exposed to hypoxia in vitro. Using reporter gene assay we also observed that HSPA2 can effect on transactivation activity of HIF-1. We found that under hypoxia HIF-1 showed reduced capability to induce the reporter gene expression in keratinocytes deficient in HSPA2 due to CRISPR/Cas9-mediated gene knockout. We also observed that HSPA2-defcient keratinocytes were more sensitive to UVB, however potential functional link between this effect and aberrant activity of HIF-1 remains to be evaluated in subsequent experiments.

Our results point to the important novel aspects of HSPA2 activity in epidermis as a modulator of HIF-1 activity in epidermal keratinocytes. Our study suggests that HSPA2-HIF1α interactions might have a significant impact on keratinocyte differentiation program and epidermal homeostasis.

This work was supported by the National Science Centre, Poland, grant no. 2017/25/B/NZ4/01550.

[2] MODELLING THE CYTOPLASMIC DOMAIN OF THE TOLL-LIKE RECEPTOR AND INITIAL STUDIES OF STRUCTURE'S DYNAMICS

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Toll-like receptors (TLRs) are transmembrane proteins that are key regulators of the innate immune response. TLR receptors trigger a pro-inflammatory immune response by recognizing a variety of ligands, e.g. external pathogen-associated (PAMP) and internal damage-associated (DAMP) molecular patterns. In humans, ten TLRs have been identified. Each TLR contains three structural domains: a leucine-rich repeats (LRRs) motif, transmembrane helix, and cytoplasmic Toll/IL-1 receptor (TIR) domain. The LRRs part is responsible for pathogen recognition, whereas the TIR domain interacts with the signal transduction adaptor.

Characteristics of the structure of TLRs and studying their behaviour with the use of *in silico* methods is not trivial. Crystallographic structures of the individual components of TLRs are available only for selected members of the TLR family. Moreover, for most TLRs, the cytoplasmic domain has not been crystallized, making it difficult to study the signal transduction mechanism.

In our research we used homology modelling to obtain complete structures for the TIR domain of the human TLR8. We combined homology modelling with advanced loop reconstruction. We obtained two models, which may represent two plausible conformations of this domain. Additionally, we used a complete model from the AlphaFold server. Next, we run molecular dynamics simulation for each monomer. Based on the initial models, we created homodimer structures and then simulated the association to validate the dimerisation mechanism.

Choosing the right model is very important in order to properly study the molecular mechanisms of Toll-like receptors. During simulations of individual monomers, we observed differences in potential energies between models and in the flexibility within the regions involved in dimerisation and adaptor proteins recognition. In the short time after simulation started proceeding we were able to observe proper dimer formation.

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[3] THE DUAL ROLE OF P53 IN REGULATION OF INTERFERONF SIGNALING PATHWAYS

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Introduction. The p53 protein coded by *TP53* is best-known for inhibition of cell cycle and activation of apoptosis. However, p53 participates in other biological activities. Interferons are cytokines, which regulate both innate and adaptive immunity and play major role in defense against viruses and bacteria. Interferons are strong activators of gene expression. Their activity is mediated by transcription factors from STAT family. There are three types of interferons, each signals through different receptors. The best-studied are interferons α (IFN-α, type I) and interferon γ (IFN-γ, type II). Their activity critically depends on STAT1 transcription factor. IFN-α induces activating phosphorylation of STAT1 and STAT2, which bind to IRF9 protein and, as a trimer, activate a set of interferon-regulated genes. IFN-γ induces the activating phosphorylation of STAT1, which forms homodimer and activates a different set of genes. These systems have negative regulators. One of them is SOCS1 protein, which prevents phosphorylation of STAT1. Previously, we found that *SOCS1* gene is positively regulated by p53. Thus, consequently, p53 should be able to modulate the cellular response to interferons. The goal of this work was to test how p53 modulates the gene activation induced either by IFN-α or by IFN-γ.

Methods. We employed lung cancer cell line A549 because it is a model of lung epithelial cells, which are frequently infected by respiratory viruses and during infection they are exposed to interferons. The p53 was activated by combination of actinomycin D and nutlin-3a (A+N). The cells were exposed to IFN-α or to IFN-γ concomitantly with A+N and the expression of genes was determined by Western blotting or RT-PCR. Moreover, we used p53-deficient cells (and controls) generated by CRISPR/Cas9 technology.

Results. Activation of p53 by A+N strongly inhibited phosphorylation of STAT1 induced either by IFN-α or by IFN-γ, hence, we expected that strong activation of p53 would inhibit activation of interferon-regulated genes. However, only minority of tested genes responded in this fashion to treatment with A+N and IFN-α, e.g. *IFI44*. In case of IFN-γ the interplay between its signaling and the p53 is more complicated. This interferon stimulates expression of some genes directly (first wave) through STAT1 (e.g. *IRF1*), while the expression of others is stimulated indirectly through IRF1 (second wave). Our results indicate that activation of p53 inhibits stimulation of IRF1 by IFN-γ but synergizes with this cytokine in activation of genes controlled by IRF1, e.g., *IFIT1*, *CASP1*.

Novel aspects. The p53 inhibits phosphorylation of STAT1, however, the consequence of this inhibition varies. The p53 inhibits stimulation of a subset of IFN-α-regulated genes. In case of IFN-γ, p53 inhibits activation of the first wave of genes, however, it synergizes with this cytokine in activation of the second wave.

Work supported by National Science Center, grant no. 2019/35/O/NZ5/02600.

[4] IMPACT OF IN VIVO AND IN VITRO HYPERICUM PERFORATUM L. ETHANOLIC EXTRACTS ON OXIDATIVE STRESS IN HUMAN MELANOMA CELLS

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Introduction: Increased oxidative stress can lead to multiple dysfunctions in the organism, including cancer. Both too high (hyperoxia) and too low (hypoxia) oxygen concentration can accelerate the production of reactive oxygen species (ROS). Normal cells often die in hypoxia, but cancer cells are better adapted to reduced oxygen levels. *Hypericum perforatum* contains numerous secondary metabolites with a wide variety of activities, including antioxidant hyperforin, hypericin and flavonoids.

Aim: In our study, we tested the effect of active substances derived from *H. perforatum* growing in natural sites and *in vitro* regenerants from the same populations on the production of ROS in five human melanoma cell lines under normoxia and hypoxia and on the expression of heme oxygenase (HO-1) and nuclear erythroid factor 2 (Nrf2).

Methods: The cell lines were isolated from the primary (WM115) and metastatic (WM266-4) tumor of the same patient. WM266-4 cells overexpressing *N*-acetylglucosaminyltransferase-III (GnT-III, WM266-4 3S), -V (GnT-V, WM266-4 5S) and mock-transfected control (WM266-4 IRES) were also included in the study. Donor plants for *in vivo* extracts and *in vitro* cultures were collected from wild populations occurring in the Krakow meadows. We used also a commercial product - *Tinctura Hyperici* by Herbapol with well-determined content of hyperforin as a reference. The cells were incubated in normoxic (21% O2) and hypoxic (0.5% O2) conditions for 24 hours with the different concentrations of extracts diluted in RPMI 1640 medium. The antioxidant effect was assessed by the colorimetric nitro blue tetrazolium (NBT) test. HO-1 and Nrf2 protein levels were evaluated by Western Blotting (WB).

Results: The obtained results indicate a reduced amount of ROS in samples treated with the highest concentrations of commercial extract (0.4 g 0.6 g of hyperforin /100 l medium) in comparison to the untreated cells, as well as the highest concentrations of *in vivo* and *in vitro* extracts (1.5 2.5 l/100 l medium). This effect was maintained both in normoxia and in hypoxia. WB analyses revealed the decreasing tendency of HO-1 and Nrf2 protein content in WM266-4, WM266-4 IRES, and 3S cell lines with the increasing dose of *in vitro* cultivated *Hypericum* extract.

Conclusions: Both commercially available and in-house prepared ethanolic *H. perforatum* extracts may exert anti-oxidant functions by declining ROS production and down-regulation of Nrf2 expression in the studied cells.

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[5] DOWNREGULATION OF PHLDA1 IN IMR-32 NEUROBLASTOMA CELLS AFFECTS EXPRESSION OF RECEPTOR TYROSINE KINASES AND CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE II

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Introduction

Neuroblastoma is the most common solid extracranial cancer in children. One of the available treatment methods of minimal residual disease 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 IMR-32 human neuroblastoma cell line caused up-regulation of Aurora A kinase and inhibition of apoptotic potential of the cells. We aim to unravel molecular networks of PHLDA1 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

Stable *PHLDA1* inhibition was obtained using shRNA, introduced to IMR-32 cells by lentiviral transduction or plasmid transfection. Phenotypic changes of cells were determined using light microscopy. To identify receptor tyrosine kinases (RTK) affected by *PHLDA1* silencing we performed Phospho-RTK kinase array analysis and results were verified by western blot. Additionally, IMR-32 cells were treated with ch14.18/CHO and/or EGFR-specific inhibitor, Gefitinib. To evaluate cell viability we measured levels of cellular ATP.

Results

IMR-32 cells with diminished *PHLDA1* expression display differentiation-like phenotype which is manifested by significant neurite outgrowth compared to control cells. Protein array analysis revealed that *PHLDA1* silencing up-regulates p-EGFR but down-regulates p-IGF-1R. Western blot analysis pointed out that *PHLDA1* inhibition affects total protein level of IGF-1R, EGFR, calcium/calmodulin-dependent protein kinase type II (CaMKII) but not DDB1- and CUL4-associated factor 7 (DCAF7), previously identified PHLDA1 binding partner. Treatment of IMR-32 cells with ch14.18/CHO decreases neuroblastoma cell survival in dose-dependent manner, and when used in combination with the aforementioned EGFR-specific inhibitor, potentiates cytotoxicity against IMR-32 cells *in vitro*.

Conclusion

Our findings expand knowledge of signaling pathways affected by PHLDA1 that relate to the cytotoxic impact of the therapeutic anti-GD2 antibodies on neuroblastoma investigated by us. We confirmed that PHLDA1 influences not only important tyrosine kinase receptors level but also CaMKII, which is one of the most abundant neuronal protein associated with cells proliferation, differentiation and synaptic plasticity. We also showed beneficial effect of combinatory treatment of neuroblastoma cells using ch14.18/CHO antibodies and the EGFR-specific inhibitor. We continue our studies aimed at further elucidation of PHLDA1 roles in neuroblastoma cells treated with ch14.18/CHO therapeutic antibodies and we plan to test cytotoxic effects of ch14.18/CHO treatment in combination with other pharmacological inhibitors of receptor tyrosine kinases.

[6] NEUTRALIZATION OF REACTIVE OXYGEN SPECIES IN CANCER CELLS AFTER IONIZING RADIATION AND THE ROLE OF miRNAs AS REGULATORS OF THIS PROCESS

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Cells maintain redox balance through generation and elimination of reactive oxygen species (ROS). Redox balance is disrupted in cancer cells and ROS levels are persistently high in these cells. To cope with these elevated levels of ROS, cancer cells have developed particular systems to adapt to these adverse conditions through activation of antioxidant pathways and efficient neutralization of ROS. The mRNAs for enzymes that are responsible for ROS neutralization can be directly targeted by micro RNAs (miRNAs), which are components of RNA-induced silencing complexes and play important roles in inhibiting translation of their targeted mRNAs.

Using microarrays, we compared expression of genes that are connected to pathways engaged in ROS neutralization and the responses to exposure of cells to 4Gy of ionizing radiation in human cancer cell lines (HCT116, K562 and Me45) originating from different tissues. From published studies we identified miRNAs which directly target mRNAs of ROS neutralizing enzymes, and for these miRNAs we compared microarray results for control and irradiated cells.

Ionizing radiation increased the levels of transcripts participating in ROS neutralization. The expression of peroxiredoxin and thioredoxin genes increased after irradiation in all cell types, but cell-line specific changes were seen for catalase and glutathione peroxidase gene expression, which suggests that cancer cells utilize various mechanisms for hydrogen peroxide neutralization after exposition to high doses of ionizing radiation [1]. Each cell type has their own specific optimal ROS thresholds which allow them to regulate their cellular processes, and these can be obtained through adjustment of levels of ROS neutralizing enzymes. Expression of their genes can be directly influenced by many miRNAs, thus indirectly influencing ROS levels. Expression of miRNAs differs in HCT116, K562 and Me45 cells and ionizing radiation change their levels. It is well established that ionizing radiation or hydrogen peroxide changes the levels of miRNAs, and therefore we propose that miRNA and ROS mutually regulate each other through creating a system of feedback loops in cell responses to oxidative stress.

[1] Bil P. et al. (2020) Antioxidants 9,701.

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[7] DEVELOPMENT OF SMALL-MOLECULE COMPOUNDS RESTORING THE GTPASE ACTIVITY OF THE RAS MUTANT PROTEIN.

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Introduction:

In a normal cell, signals from growth factor receptors cause RAS to attach to GTP. At this point, the signal is transduced to other proteins, which finally affect the regulation of, for example, cell division. However, the GAP protein causes RAS to rapidly hydrolyze GTP and the transduction of the signal that stimulates the cell to divide is interrupted. RAS activity is very frequently disrupted in cancer cells in a way that prolongs signal transduction. It is very commonly a consequence of a mutation in the gene encoding the RAS protein. Most often, these mutations render the mutant insensitive to GAP. As part of the analyzes, molecules that are able to induce the GTPase activity of the RAS mutant protein have been developed. These molecules represent a completely new class of compounds and can be considered as having therapeutic potential for tumors exhibiting the RASG12V mutation.

Methods:

The Promega GTPase GLO assay was used to test the GTPase activity of the wild type of RAS RAS WT and the mutant form - RAS G12V. The RAS G12V, RAS WT, and GAP proteins were purified by genetic engineering.

Results:

Two leader molecules can be considered as compounds restoring RAS G12V GTPase activity.

Conclusions:

Due to the ability to restore GTPase activity in the mutant RAS protein - RAS G12V, the molecules that will evolve from the studied molecules could potentially be used in therapies based on the restoration of GTPase functionality of this mutant in tumors.

This study was sponsored by The National Centre for Research and Development grant no. POIR.01.02.00-00-0034/15.

[8] ADIPOSE TISSUE-DERIVED STROMAL CELLS DRIVEN NEOVASCULARIZATION

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During wound healing, the formation of new blood vessels plays a major role. Neovascularization proceed in a process of angiogenesis and/or neovasculogenesis. Any disruption in consecutive stages of neovascularization can lead to chronic wounds or impairment of tissue repair. It was reported that adipose tissue-derived stromal cells (ADSCs) accelerate blood vessels formation in ischemic tissue and facilitate tissue regeneration.

The aim of the study was to explain the biological basis of accelerated blood vessels formation in ischemic tissue after ADSCs administration.

Experiments were performed on mouse models of hindlimb ischemia. Mice (C57BL/6NCrl strain 8-10 weeks of age) were divided into two groups: the first one represent a group where physiological processes of wound healing were observed. The second group received ADSCs injected into injured muscle. After 24 hours, 3, 7, 14, 21 days post injury (dpi) mice were sacrificed. The obtained muscles were subjected to either immunofluorescent or flow cytometric analysis.

Our research confirmed that recovery of the muscle following femoral artery ligation is accelerated when ADSCs are injected into the injury site. The new blood vessels appear faster and their density is higher. Our research revealed that ADSCs administration contributed to changes in morphology of macrophages infiltrating ischemic tissue. Initially, after ischemia induction, macrophages were large with irregular shape, scattered over tissue. Subsequently, they became more infiltrating, forming a clusters. At the end of the healing processes they acquired the shape of pericytes or cells which stabilize blood vessels. We have also examined bone marrow-derived stem/progenitor cells (BMDCs) involvement into neovascularization process in ischemic tissue. Notably, their contribution was lower when ADSCs were injected into ischemic site.

We conclude that accelerated ischemic tissue recovery after ADSCs administration might be due to influence of spatio-temporal distribution of infiltrating immune cells (macrophages, neutrophils and BMDCs), which are involved in each step of vascular formation, promoting effective ischemic tissue neovascularization.

This study was financed by National Science Center grant DEC-2018/02/X/NZ4/02331.

[9] CHANGES IN THE EXPRESSION OF THE MIRNAS RELATED TO THE HISTAMINERGIC SYSTEM IN ENDOMETRIOID ENDOMETRIAL CANCER

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Introduction/Rationale: Endometrial cancer (EC) is one of the most common malignant neoplasms in women in developed countries and is characterized by a steadily increasing incidence. The aim of this study was to evaluate changes in the expression pattern of miRNAs potentially regulating expression of the genes related to the histaminergic system in endometrial samples and whole blood in women with endometroid endometrial cancer. MiRNAs also regulate both suppressor genes as well as oncogenes. Moreover, miRNAs themselves can fulfill such roles in the process of carcinogenesis

Methods: The study group consisted of 30 women with endometroid endometrial cancer qualified for hysterectomy (G1 well-differentiated, 15 cases; G2 moderately differentiated, eight cases; and G3 poorly differentiated, 7 cases). The control group included 30 women with no neoplastic changes during routine gynecological examinations. The molecular analysis consists of extraction of the total RNA and next microarray analysis of miRNAs which regulating expression of the genes related to the histaminergic system.

Results: It was observed the strongest relationship between hsa-miR-33a-5p (G1 vs C up; G2 vs C down; G3 vs C down) and *HNMT*, hsa-miR-3915 (G1 vs C up; G2 vs C up; G3 vs C up) and *HRH4*, hsa-miR-1-3p (G1 vs C down ;G2 vs C down ; G3 vs C down) and hsa-miR-575 (G1 vs C down, G2 vs C down; G3 vs C down) and *EDN1* oraz, hsa-miR-27a-5p (G1 vs C up up; G2 vs C up; G3 vs C down) and *SLC23A2*.

Conclusions/Novel aspect: The conducted molecular analysis showed that miR-33a-5p, miR-3915, and miR-1-3p, miR-575, miR-27a-5p are connected with the histaminergic system in the endometroid endometrial cancer and may be treated as supplementary, diagnostic, molecular markers. It also seems that these results may be used in the evaluation of the severity of endometroid endometrial cancer.

presenting author: Michał Czerwiński

[10] NANO-STAR-SHAPED POLYMERS AS ANTIPSORIATIC PRODUCTS

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INTRODUCTION. Psoriasis is a chronic, autoimmune skin disease. Research shows possible correlation between psoriasis and low level of vitamin D3 in serum. Currently topical treatments with antipsoriatic drugs such as methotrexate (MTX) or acitretin (AC) is still considered as one of the major ways for treating psoriasis. The aim of our study was to prepare nanoparticles based on polymer-drug conjugates of MTX or AC, with encapsulated vitamin D3. Well defined star-shaped polymers used in the study possessed five or eight pH- and thermoresponsive arms composed of poly(*N*,*N*-dimethylaminoethyl methacrylate-*co*-hydroxyethyl methacrylate) and one biodegradable polyester arm composed of poly(ε-caprolactone) or poly(D,L-lactide-*co*-glicolide), respectively.

METHODS. pH- and thermoresponsiveness of polymeric carriers were investigated by determination of their cloud point temperatures (TCP). Nanoparticles with encapsulated vitamin D3 were prepared by nanoprecipitation method. Sizes of nanoparticles were determined by DLS measurements using Microtracs Nanotrac Flex .

RESULTS. Miktoarm stars solutions in PBS possessed TCP values in the range 55.5◦C-72.3◦C. During heating of some samples the increase of absorbance was observed in range of 5◦C-20◦C with decrease of absorbance above 20◦C, suggesting partial hydrolysis of ester groups in methacrylates and formation of schizophrenic polymers. Hydrodynamic diameters (Dh) of miktoarm polymers nanoparticles, after nanoprecipitation ranged between 146 nm and 1826 nm (with PDI = 0.04-0.50). In case of polymer-AC conjugates, mean Dh were around 175 nm (PDI = 0.12-0.18), and for samples with conjugated MTX mean Dh = 47 nm - 1337 nm (PDI = 0.2-2.0). Nanoprecipitation of polymeric prodrugs with vitamin D3 decreased PDI and Dh for corresponding bare polymer-drug conjugate samples. Hence, Dh for samples with polymer-AC conjugates were around 200 nm, with PDI at level 0.06, and for samples with polymer-MTX conjugates Dh = 195 nm - 270 nm, with PDI between 0.03-0.9.

CONLUSIONS. Nanoparticles of miktoarm polymers and polymer prodrugs with encapsulated vitamin D3 were obtained. Based on our previous results, such nanoparticles present novel approach for psoriasis treatment, due to their enhanced penetration through skin layers and effective delivery of both drug and vitamin D3 during degradation of polymeric carrier[1]. However, further analysis of nanopraticles properties, such as shape, encapsulation efficiency of vitamin D3 and cytotoxicity tests must be conducted.

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[11] THE EFFECT OF STING ACTIVATION ON TUMOR ASSOCIATED NEUTROPHILS IN 4T1 BREAST CANCER MODEL

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Stimulator of interferon genes (STING) is crucial component of innate immune response. Additionally, it appears as promise target for new anti-cancer therapies. The pathway activation results in type I interferons and pro-inflammatory cytokines production. One of the first effects observed in tumor microenvironment after STING activation is neutrophils infiltration. In tumors, neutrophils called T*umor Associated Neutrophils (TANs*) constitute heterogeneous population of cells. They display diversified functions depending on the tumor microenvironment circumstances. According to that, TANs can be divided into N1-like cells (anti-tumor) and N2-like cells (pro-tumor).

The aim of the work was to assess neutrophils activation state in the tumor microenvironment after intratumoral STING agonist administration in 4T1 breast cancer model.

The experiments were conducted on 4T1 murine breast cancer model conducted on females of BALB/c mouse strain. 14 days after cancer cells inoculation, STING agonist (23-cGAMP) was administered intratumorally in a dose 5 g/mice. To assess time when neutrophils activation can be observed, luminol sodium salt solution was administered intraperitoneally. The images of mice were taken using IVIS Lumina System in four time points (0h, 6h, 12h, 24h) after PBS- or 23-cGAMP administration. The number and phenotype of neutrophils were determined by flow cytometry analysis in three time points (6h, 12h, 24h). N1-like activated neutrophils were characterized as CD11b+Ly6G+ICAM+ cells.

There was observed luminescence signal around tumors 6h and 12h after 23-cGAMP administration. The strongest signal was observed 12h after STING protein activation. There was no significant difference in neutrophils (CD11b+Ly6G+) percentage 6h after STING activation compared with control. In the next examined time points (12h and 24h) there was observed significant decrease in the percentage of neutrophils (CD11b+Ly6G+). However, there was observed significant increase in the percentage of N1-like cells (Ly6G+ICAM+) in each of examined time points.

The obtained results indicate that STING protein stimulation leads to neutrophils activation in 4T1 tumor model. STING activation results in gradual decrease in the percentage of neutrophils with simultaneously maintaining the increased percentage of ICAM-positive N1-like neutrophils.

The work is a result of the research project no. UMO-2019/35/N/NZ5/02506 financed by National Science Center.

[12] A NEW APPROACH TO IMAGING AND RAPID MICROBIOME IDENTIFICATION IN PROSTATE CANCER PATIENTS

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Little is known about the impact of urinary microflora and its effects on side effect after radiotherapy (RT). Until recently, any growth of bacteria grown from urine was considered invasive. A new look at the issue of the composition and significance of the urinary microbiome may bring the use of the mass spectrometry identification method MALDI. This study aims to use the MALDI technique to identify the microbiome of urine samples collected from pts treated with radiotherapy for prostate cancer.

We included 52 pts treated for prostate cancer irradiated with radical intent. Blood and urine samples were collected before the gold marker implantation, on the day of beginning RT, last day of RT, 1 month after RT. It is recommended to collect urine from the midstream, but not always pts do it correctly despite being instructed. Therefore, we decided to collect samples from first-void and midstream in 12 pts for MALDI, from remaining pts we collect two samples from midstream-void for MALDI and biochemical analysis.

Microorganisms were present in 145/186 urine samples. Using three different culture media the best microbial growth was observed on TSA (universal medium) and CLED (differential medium for urine specimens) the largest number of isolates was obtained. We found 33 different species 3 G(-) and 30 G(+). The most frequently isolated strains were: *Staphylococcus haemolyticus* (19%), *Staphylococcus epidermidis* (18%), *Staphylococcus hominis* (16%), *Enterococcus faecalis* (13%), and *Micrococcus luteus* (10%).

When comparing the type of urine samples, bacteria were more common in the samples from the first-void urine than from the midstream one. The absence of bacteria was found in 12.2% of samples from the first-void urine and 24.7% from the midstream. Overall, the ratio of the number of detected species per number of samples corresponding to the first and middle stream were 1.80 and 1.71%, respectively. The differences in the total incidence of species between streams did not present to be significant ($p=0.85$), but the number of bacterial species in the initial stream was generally greater.

The amount of bacterial species found in urine depended on the treatment. Before gold fiducial implantation the total number of detected bacterial species was significantly higher in comparison to the end of radiotherapy ($p=0.038$) indicating that the administered therapy results in depleting the local microbiome. One month after radiotherapy an increase in the number of isolated bacteria was observed. The number of bacterial species in urine did not correlate with the blood parameters. The presence of leukocytes ($p=0.013$) and proteins ($p=0.004$) in urine were related to a greater variety of bacteria found in urine specimens.

We obtained a similar spectrum of bacteria from the initial and middle urine streams. We also showed that there is a change in bacteria species is affected by the treatment of prostate cancer patients.

NCN 2020/39/B/NZ7/02733

[13] VARIANTS IN OPN AND CD44 GENES MAY INFLUENCE RECURRENCE AND PROGNOSIS IN NON-SMALL CELL LUNG CANCER TREATED WITH RADIOTHERAPY AND CHEMORADIOTHERAPY

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Osteopontin (OPN) is a multifunctional glycoprotein that mediates a variety of processes such as cell adhesion, proliferation, migration, angiogenesis, metastasis, apoptosis, and the immune response. It promotes cancer progression by binding to cellular receptors such as CD44. CD44-OPN signaling may be an important factor in the aggressiveness of lung cancer. In many solid tumors, elevated serum OPN levels and increased OPN and CD44 tumor expression are markers of poor prognosis. High OPN levels may also correlate with radioresistance and chemoresistance. Radiotherapy (RT), with or without chemotherapy (CT), remains part of the standard care in advanced lung cancer. In non-small cell lung cancer (NSCLC), large interindividual differences in drug and radiation resistance, as well as in recurrence rate are observed. Therefore, it is necessary to identify factors that may help predict treatment effects and survival in NSCLC patients. Polymorphisms in the *OPN* and *CD44* genes, especially in the promoter and regulatory regions, by altering the expression and activity of proteins, may influence disease progression and therapy results. In this study, the relationship between functional *OPN* and *CD44* polymorphisms and treatment outcome and prognosis was investigated in 307 NSCLC patients receiving RT and CHTRT.

There was no significant effect of the studied variants in the whole group. When patients treated with curative intent were analyzed, carriers of *CD44* rs187116 A allele had significantly longer locoregional recurrence-free survival (LRFS) ($p=0.039$) than the GG homozygotes. In multivariate models, *OPN* rs11730582 CC genotype was associated with significantly increased risk of death ($p=0.029$). In univariate and multivariate models, the *CD44* rs187116 A allele had a significant protective effect with respect to LRFS ($p=0.033$ and $p=0.016$). The final models revealed that the *OPN* rs11730582 CC genotype was an independent predictor of poor OS, whereas the CD44 rs187116 A variant was an independent protective factor against recurrence. Our preliminary data demonstrate for the first time that *OPN* and *CD44* genetic variants may affect clinical outcome of patients with inoperable NSCLC treated with curative intent. This highlights the role of the host-related genetic factors in modulating disease course and treatment effects in lung cancer.

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[14] INTRA-TUMOR HETEROGENEITY REVEALED BY MASS SPECTROMETRY IMAGING IS ASSOCIATED WITH THE PROGNOSIS OF BREAST CANCER

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Introduction/Rationale: Intra-tumor heterogeneity (ITH) results from the coexistence of genetically distinct cancer cell (sub)populations, their phenotypic plasticity, and the presence of heterotypic components of the tumor microenvironment (TME). Here we addressed the potential association between phenotypic ITH revealed by mass spectrometry imaging (MSI) and the prognosis of breast cancer.

Methods: Tissue specimens resected from 59 patients treated radically due to the locally advanced HER2-positive invasive ductal carcinoma were included in the study. After the on-tissue trypsin digestion of cellular proteins, peptide maps of all cancer regions (about 380,000 spectra in total) were segmented by an unsupervised approach to reveal their intrinsic heterogeneity.

Results: A high degree of similarity between spectra was observed, which indicated the relative homogeneity of cancer regions. However, when the number and diversity of the detected clusters of spectra were analyzed, differences between patient groups were observed. It is noteworthy that a higher degree of heterogeneity was found in tumors from patients who remained disease-free during a 5-year follow-up (n = 38) compared to tumors from patients with progressive disease (distant metastases detected during the follow up, n = 21). Interestingly, such differences were not observed between patients with a different status of regional lymph nodes, cancer grade, or expression of estrogen receptor at the time of the primary treatment. Subsequently, spectral components with different abundance in cancer regions were detected in patients with different outcomes, and their hypothetical identity was established by assignment to measured masses of tryptic peptides identified in corresponding tissue lysates. Such differentiating components were associated with proteins involved in immune regulation and hemostasis. Further, a positive correlation between the level of tumor-infiltrating lymphocytes and heterogeneity revealed by MSI was observed.

Conclusions/Novel aspect: We propose that a higher heterogeneity of tumors with a better prognosis could reflect the presence of heterotypic components including infiltrating immune cells, that facilitated the response to treatment.

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[15] USEFULNESS OF THE MYXOMA VIRUS CONSTRUCT ENCODING IL-15 IN DESTROYING MELANOMA CELLS IN VITRO IN COMBINATION WITH WP760

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Introduction: Oncolytic virotherapy is a relatively new approach to anti-cancer therapy, but due to its high curative potential resulting from the specificity of action and high effectiveness, as well as low level or even lack of side effects, it has quickly become an extremely promising strategy in oncology. One lead in searching for novel therapeutic strategies aims at improving the existing ones *via* innovative combinations. Examples of such an approach include a joint curative strategy based on the use of oncolytic myxoma recombinant viruses with therapeutic/reporter transgenes (MYXV-IL15, vMyx-IL15/tdTr) and a bisanthracycline-type chemotherapeutic agent dubbed WP760. The latter has shown high killing selectivity for melanoma cells *in vitro* and reduced side effects, when compared to the standard anthracycline drug i.e. doxorubicin. Combinations of novel treatment strategies have emerged as the key to success in destroying malignant neoplasms. For this reason, the concept of combining the action of an engineered myxoma virus construct and WP760, a novel selective chemotherapeutic seemed very interesting and particularly worthy of investigating.

Aim: Assessment of the effectiveness of the use of the myxoma virus construct (vMyx-IL15/tdTr) in combination with bisanthracyclineWP760 for destroying melanoma cells *in vitro*.

Methods: A genetically modified myxoma virus vMyx-IL15/tdTr, encoding a red fluorescent protein (tandem dimer Tomato red, tdTr) and interleukin 15, was used. Cytotoxicity of MYXV-IL15 for melanoma cell lines (murine B16-F10 and human WM35, WM793B, 451Lu and 1205Lu) and normal rabbit kidney epithelial cells was determined using MTS viability test. The infectiveness of MYXV-IL15 to melanoma lines was determined by analyzing single-step replication curves of MYXV-IL15 in melanoma cell cultures. Viability of B16-F10 melanoma cells following combination of the chemotherapeutic agent WP760 and MYXV-IL15administered either simultaneously or with an interval of 24 hours was tested by the MTS assay.

Results: The results confirmed that the examined melanoma cell lines are sensitive and permissive for the myxoma virus constructs used. Combination MYXV-IL15 and the bisanthracycline chemotherapeutic agent WP760 resulted in lowered survival *in vitro* of treated B16-F10 melanoma cells,as compared to treatment with virus alone or with WP760 alone. This result was noted for both simultaneous administration of the virus and the chemotherapeutic, as well as for infection followed after 24 hours with WP760.

Conclusion: Use of MYXV-IL15 myxoma therapeutic construct in combination with melanoma-selective agent WP760 is a potential therapeutic strategy for destroying hard-to-reach melanoma lesions *in vivo*.

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[16] DEFICIENCY OF THE HSPA2 GENE PROMOTES A MORE DIFFERENTIATED PHENOTYPE OF HUMAN EPIDERMAL KERATINOCYTES AND INDUCES ALTERATIONS IN ORGANIZATION OF THE THREE-DIMENSIONAL RECONSTRUCTED EPIDERMIS

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The human heat shock protein A2 (HSPA2) was originally described as a testis-specific member of the HSPA (HSP70) chaperone family involved in sperm cell differentiation and activity. Our recent studies have demonstrated that HSPA2 is also synthesized in human somatic tissues in a cell- and tissue-type specific manner. Among others, we have observed overrepresentation of the HSPA2 protein in stratified and pseudostratified epithelia. In the epidermis, a multilayered cornified epithelium, we have found HSPA2 in keratinocytes constituting the basal layer, but not in the fraction of stem cells. In searching for a functional significance of *HSPA2* expression in epidermis we have established human epidermal keratinocyte HaCaT line with selective inhibition of the *HSPA2* gene expression, using two genetic methods: the viral-mediated shRNA-induced knockdown and CRISPR/Cas9-mediated genome editing. The shRNA-mediated silencing of *HSPA2* expression resulted in reduced cell adhesion to the selected ECM components and caused a decrease in cell colony forming ability in monolayer culture *in vitro*. In turn, CRISPR/Cas9-mediated knockout of the *HSPA2* gene had no effects on cell adhesion but resulted in reduction of their clonogenic potential. Importantly, both *HSPA2* knockdown- and *HSPA2* knockout-cells formed epidermal equivalents showing similar alterations in spatial organization. Moreover, histological and immunophenotypic analysis of epidermal differentiation markers revealed that deficit in HSPA2 promoted a more mature phenotype of the 3D epidermal constructs. These dependences were observed irrespectively of the technique of the *HSPA2* gene targeting. Our results indicate that HSPA2 is essential for the ability to restore epidermal structure by HaCaT keratinocytes *in vitro*.

In the study we attempted to search for molecular mechanisms that rely on HSPA2 and are related to keratinocytes ability to form fully-developed epidermis. For this purpose, using RNAseq technique we analyzed HSPA2-dependent changes in global transcriptome in epidermal equivalents derived from HSPA2-deficient HaCaT cells. We examined two models of HSPA2 deficiency, namely lentiviral-mediated knockdown and CRISPR/Cas9-mediated knockout. Preliminary bioinformatics analysis revealed a list of differentially expressed genes and signaling pathways sensitive to HSPA2 deficit. Among identified pathways were those related to keratinization process, apoptosis regulation, inflammatory response and WNT signaling . The results provide further guidelines for more in-depth verification of HSPA2 significance for human epidermis physiology.

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[17] E2F2 Polymorphisms in Oral and Oropharyngeal Squamous Cell Carcinoma

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Introduction Oral squamous cell carcinoma (OSCC) and oropharyngeal squamous cell carcinoma (OPSCC) are subgroups of head and neck squamous cell carcinoma. E2F Transcription Factor 2 (E2F2) could contribute to cancer development, because it plays a critical role in many cellular processes, including the cell cycle, proliferation, differentiation, DNA damage response, and cell death. In the study, we assessed the associations of five E2F2 polymorphisms (rs6667575, rs3218121, rs3218211, rs3218148, and rs3218203) with OSCC and OPSCC and effect on the TNM staging and grading.

Methods The study included 94 primary tumour samples following surgical resection from patients, whereas the control group consisted of 99 healthy individuals. Genomic DNA was extracted from each tumour sample (20 mg) by DNeasy Blood Tissue Kits (Qiagen, Hilden, Germany) according to the manufacturers instructions, after tissue homogenization in a FastPrep-24 instrument using Lysing Matrix A tubes (MP Biomedicals, Solon, CA, USA). In the control group, the DNA was extracted from swabs taken from oral mucous membranes using a Swab-Extract DNA Purification Kit (EURx, Gdansk, Poland), according to the manufacturers instructions. The qualitative and quantitative analysis of all isolated DNA was performed by spectrophotometry in a Biochrom WPA Biowave DNA UV/Vis Spectrophotometer (Biochrom, Cambridge, UK). Genotyping was conducted with a QuantStudio 5 RealTime PCR System (Applied Biosystems, Foster City, CA, USA). DNA samples were genotyped by employing the 5 nuclease assay for allelic discrimination.

Results and Conclusion Our results suggested that the most significant difference between the control group and the cancer group was the A/G heterozygote for rs3218121. Samples containing this genotype were mostly found in the control group. In our samples, rs6667575, rs3218121, rs3218211, and rs3218148 polymorphisms may affect the course of OSCC and OPSCC, while rs3218203 was not associated with OSCC and OPSCC. However, further studies are warranted to confirm our findings.

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[18] IDENTIFICATION OF MOLECULAR PATHWAYS AND KEY GENES IN PROGRESSION OF CLEAR CELL RENAL CELL CARCINOMA BASED ON MICROARRAY ANALYSIS AND MIRNA-SEQUENCING DATA

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Introduction Clear cell renal cell carcinoma (ccRCC) is the most common pathological type and the main cause of death in renal cancer patients. With a 5-year survival rate of 10%, metastatic ccRCC is a fatal disease, highlighting the need to understand the cellular and molecular mechanisms in primary lesions that are predictive of relapse, both as biomarkers and as potential targets of the intervention. Due to the very high mortality rate of metastatic ccRCC, there is a need to understand the molecular mechanisms regulating disease development as well as to identify the biomarkers that indicate disease relapse and development.

Our group has already showed that during the development of ccRCC, the level of Monocyte Chemoattractant Protein-1 Induced Protein (MCPIP1) decreased together with the alteration of the WNT pathway. The loss of MCPIP1 RNase activity led to the upregulation of miRNA-519a-3p, -519b-3p, and -520c-3p, which inhibited the expression of Wnt pathway inhibitors. Moreover, we observed a decrease in the expression of the genes characteristic for the epithelial phenotype and an increase in the expression of genes associated with the mesenchymal phenotype. However, there are many unknown pathways altered during progression.

Method In this study, we analyzed data from microarrays analysis of whole transcriptome and Next Generation Sequencing of miRNA. We evaluated the variable expression at different stages of disease development, crucial for ccRCC progression. The selected genes were analyzed using NOD-SCID mice injected subcutaneously as a suspension of Caki-1 cells with stable overexpression and mutant form of the MCPIP1.

Results We identified specific miRNAs that change in the ccRCC line. Our results indicate that in ccRCC cells deficient in RNase activity, levels of miR-517a-3p, -517b-3p, -520c-3p, -517c-3p, -181a-2-3p, and -4484 are elevated compared to MCPIP1 overexpressing cells, which may be related to the development of ccRCC. These miRNAs regulate the expression of genes, which may be of key importance in the development of ccRCC. Based on microarray analysis of tissues from patients, our study suggests that the RNase activity of MCPIP1 is crucial in the several processes regulating tumor cell migration, morphology and proliferation leading in the consequence to local invasion and finally metastasis.

Novel aspect Our current results indicate the importance of MCPIP1 as a suppressor of tumor development and progression. We show that MCPIP1 is crucial in the regulation of cell fate and controls the acquisition of the mesenchymal phenotype and the activation of -catenin. Detailed analysis of the miRNAs could identify novel therapeutic targets critical to the progression of ccRCC. The results can be used in trials aimed at preventing tumor progression and developing resistance to ccRCC therapy. In our research we plan to identify ccRCC-specific molecular targets that regulate tumor progression and developed resistance to therapy.

[19] PHYTOCANNABINOIDS – ISOLATED FROM NATURE TO TREATING MELANOMA TUMORS

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Phytocannabinoids isolated from *Cannabis Sativa L.* act as exogenous ligands of the endocannabinoid system (ECS). ECS plays a relevant role in the organism physiology showing an effect on overall health, including the part of the natural immune system. Their function relies on the expression and direct interaction with G-protein-coupled membrane cannabinoid receptors. The function of phytocannabinoids is extended to other purposes, including the ability to modulate the activity of receptors not included in ECS, like GPR55 or TRPV1. Cannabidiol (CBD) and cannabigerol (CBG) indicate a non-psychotropic activity, and cannabinol (CBN) has a weak psychotropic effect. Therefore, those compounds are considered to have more clinical uses compared to psychoactive tetrahydrocannabinol (THC).

Recent therapeutic applications of CBD, CBG, and CBN have shown an anti-tumor activity, but their role in cancer pathophysiology is not completely clear. Each cannabinoid influences the intracellular pathways in its own manner. In this regard, there is still lack of information about their properties on various tumor cells.

Our research aims at determining the survival ability of neoplastic cells after their treatment with the selected phytocannabinoids (CBD, CBG, CBN) and at identifying possible apoptotic pathways. The study was performed on the human immortalized keratinocyte cell line (HaCaT), primary melanoma cells from the vertical growth phase (WM115) and the lymph node melanoma metastasis (WM266-4).

Our data revealed that the micromolar doses of phytocannabinoids exert potential *in vitro* anti-metastatic activity by inhibiting cellular metabolism more selectively against the metastatic melanoma cells (WM266-4) than for the primary melanoma cells (WM115) and keratinocytes (HaCaT). To understand the mechanisms underlying this effect, different cell features were studied, including: mitochondrial dehydrogenase activity (MTT), esterase activity (FDA), lactate dehydrogenase activity (LDH), influence on the reactive oxygen species (ROS) levels, the number of cells (CV) and the visualization of apoptotic-necrotic cells (AO/PI). To identify by which apoptotic pathway (extrinsic/intrinsic) do the phytocannabinoids influence the cells, the appropriate Caspase-8 and Caspase-9 assays were implemented. These data provide evidence to further support the potential of micromolar concentrations of phytocannabinoids as agents for use in the combined anti-tumor therapy.

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[20] ATR AND CHK1 INHIBITION ENHANCES GENOTOXIC EFFECT OF OLAPARIB IN P53MUT OVARIAN CANCER CELL LINE

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Poly (ADP-ribose) polymerase (PARP) inhibitors treatment has been successfully used in patients with ovarian cancer with a BRCA1/2 mutation. Olaparib was the first FDA approved PARP inhibitor for the treatment of BRCAMUT high-grade serous ovarian cancers (HGSOCs). Unfortunately, olaparib monotherapy is often insufficient, especially for the treatment of BRCA wild type (BRCAWT) ovarian cancer, although it may increase the dependence on other DNA repair pathways, to maintain genome stability. Activation of DNA repair pathways may include the stimulation of ataxia telangiectasia and Rad-3 related protein (ATR) and checkpoint kinase 1 (CHK1), which prevent further damage. Because of that, simultaneous targeting PARP and DNA repair checkpoint proteins may represent an alternative strategy for BRCAWT ovarian cancer treatment. The objective of the study was to evaluate the effect of ATR inhibitor (AZD6738, ATRi), CHK1 inhibitor (MK8776, CHK1i) and their combination with olaparib in the homologous recombination (HR) proficient, BRCAWT, p53MUT OV-90, ovarian cancer cell line. Cell survival and chromatin condensation were assessed up to 48 h after the treatment. Micronuclei (MN) formation was evaluated after 24 h, based on the doubling time of the OV-90 cell line. ATRi + PARPi combination was the most cytotoxic, as assessed by MTT assay. An increase in apoptotic cell fraction was observed after combined treatment, as based on chromatin condensation measurement. Statistically significant differences in the number of MN were observed after ATRi or combined treatment of PARPi + ATRi or PARPi + CHK1i. The study confirmed that combination treatment with PARPi and ATRi or CHK1i has a high cytotoxic and genotoxic effect on ovarian cancer cells, on the path of synthetic lethality.

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[21] CLINICAL OUTCOME AFTER STEREOTACTIC BODY RADIOTHERAPY (SBRT) FOR OLIGOMETASTATIC BREAST CANCER PATIENTS

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Introduction Treatment of metastatic breast cancer is mostly based on systemic therapy, while the exact role of radiotherapy is uncertain. During SBRT fewer fractions are used, shorter and faster treatment is more convenient for patients, moreover higher doses are potentially more effective. There is a greater emphasis not only on the effects of oncological treatment but also on the quality of life after the treatment is finished. In this context, radiotherapy may be important in the management of patients with metastatic breast cancer ameliorate clinical outcomes with limited toxicity.

We aimed to assess the effect of stereotactic body radiotherapy (SBRT) on survival, oncological outcomes, in patients with one to five oligometastatic lesions, and the potential delay to subsequent systemic therapy introduction or change (ST).

Methods Out of 247 metastatic breast cancer patients undergoing SBRT (1-5fx, df4Gy), treated between 2014 and 2020, 69 patients with oligometastatic disease with total dose 24 Gy were included in our single-institution study. Survival analysis was performed using the Kaplan-Meier method with log-rank test being used for evaluation of significance.

Results Majority of patients had single metastatic foci (57%), 2 (28%), 3 (10%), 4 (4%), 5 (1%). The most common site of metastases was bone 61%, remaining included lung 19% liver 19%, lymph nodes 1%. Total dose ranged from 24 to 60 Gy, usually delivered in 3 fractions 89% (1-5 fx). Systemic therapy before SBRT was applied 88% of the patients within this group hormonal therapy 74%, chemotherapy 30%, targeted therapy 17%. Also 83% got systemic therapy during SBRT mostly hormonal therapy 73%, chemotherapy 7%, targeted therapy 12%. Median follow-up was 28 months (range 2-68), with 67% of patients being alive at the time of analysis. Median time from primary treatment to SBRT was 2.7 years (range 9 mts - 18 years). Median time to further systemic therapy after SBRT was 13 months (1.3-55.5). The 1-, 3-, and 5-year OS were 94%, 64%, 62%, respectively. There was a trend that patients with bone metastases had better LC ($p=0.09$) and OS ($p=0.08$). In majority of patients as the first site of failure further distant metastases outside the irradiated volume was found. 1y LC was 84%, 3y was 75%, there were no LC after 25 mts. 1 year MFS was 60%, 3y was 30%, all first dissemination after SBRT had place before 27 mts. 1y, 3y, 5y FFST (freedom from ST) after SBRT were 76%, 39%, and 18%. Majority of patients got ST at the time of disease progression, median time to ST was 1.3 mts, but there were also patients with up to 42 mts FFST after progression. Those who did not get ST at the time of progression were treated with radiotherapy up to 13 courses.

Conclusions The use of radical radiation therapy to metastatic sites in oligometastatic breast cancer patients can be considered a valuable option and recommended to the appropriate candidates.

[22] PROLIFERATION RESPONSE OF THE YEAST CELLS SACCHAROMYCES CEREVISIAE AS A BIOLOGICAL RESPONSE TO THE APPLIED LOW-FREQUENCY ELECTROMAGNETIC FIELD

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The work deals with the possible influence of extremely low-frequency electromagnetic fields (ELF-EMFs) on Saccharomyces cerevisiae cells. The number of experimental studies dealing with non-ionizing EMF interactions with biological objects at various levels have grown rapidly and questions regarding the topic of its possible health risks are even more frequent and still full of uncertainty [1].

Even though the biological effect of ELF-EMFs was observed, the mechanism remains unclear. The goal of the work is to verify change of growth dynamics of exposed samples by application of time-varying magnetic field following the conditions valid for one of the possible prediction mechanisms to prognosticate biological response from exposure to ELF-MF called the ion parametric resonance (IPR) theory [2]. This theory is focused on targeting calcium Ca^{2+} cations bound in certain locations on cellular membranes of eukaryotic cells.

Experiments were performed in pairs and samples were cultivated for eight hours in two coils inside the incubator. The exposed samples were irradiated by ELF-EMF source represented by the coil situated within the incubator and another coil served as a control one. The results proof the frequency dependent selective biological response to externally applied time-varying ELF-EMF based on IPR model, needed to target specific ions, which evoke reaction of samples exposed to ELF-EMF and thus confirm that occurred biological effect could be predictable and could find its use in medicine.

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[23] RAB27A KNOCKOUT IN A MELANOMA CELL LINE

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Introduction: The growth, invasion and metastasis of cancer cells depend upon bidirectional cell-cell communication within a complex tumor microenvironment. Such communication involves the secretion of extracellular vesicles, including exosomes. Exosome biogenesis is a multistep process that engages several proteins including Rab GTPases. Members of Rab family are associated with vesicle trafficking, which makes them an interesting target to focus on while studying the role of extracellular vesicles. Rab27a is one of the key regulators of exosome secretion, therefore its functioning may be correlated with cancer progression. Rab27a overexpression was reported in melanoma patient-derived samples and associated with reduced survival. For that reason silencing Rab27 genes may reduce exosome production and release.

Methods: We applied the CRISPR/Cas9 technology to knockout Rab27a in the A375 melanoma cell line. We used a ribonucleoprotein transfection strategy to deliver Cas9 and three single guide RNAs that were targeting different exons to improve the likelihood of obtaining the desired mutations.

Results: We obtained Cas9 mediated cleavage in the targeted site, which we verified using the T7 endonuclease I assay. A monoclonal cell line was isolated by limiting dilution cloning. After screening over 80 clones successful loss of Rab27a protein was confirmed by Western blotting in two clones. Sanger sequencing showed the formation of insertion-deletion mutations at the targeted site. These results prove the effective gene editing.

Conclusion: We are now in a position to study the effects of Rab27a knockout in melanoma cells focusing on possible changes in exosome secretion as well as cancer cell biology. Moreover, we plan to knock out another protein participating in exosome biogenesis.

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[24] THE ROLE OF HSF1 IN THE REGULATION OF THE TRANSCRIPTIONAL RESPONSE TO ESTROGEN AT THE LEVEL OF CHROMATIN ORGANIZATION

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Heat Shock Transcription Factor 1 (HSF1) is an evolutionarily conserved transcription factor activated primarily in response to proteotoxic stress. It was recently linked to estrogen (E2) signaling through estrogen receptor (ER) and MAPK signaling. Here, to further investigate the cooperation of HSF1 and ER in estrogen signaling, we created a novel experimental model based on HSF1-deficient (HSF1) ER-positive breast cancer MCF7 cells in which HSF1 was stably inactivated using the DNA-free CRISPR/Cas9 method.

The RNA-seq analyses showed that 3,715 and 2,969 genes significantly changed the expression in HSF1+ and HSF1 cells after E2 stimulation, respectively. Among genes up- or down-regulated in both cell variants, approximately 68% or 81%, respectively, responded less effectively in HSF1 cells than HSF1+ cells (e.g. genes involved in the G2/M checkpoint and signaling pathways related to proliferation and migration). Interestingly, the weaker response to E2 in HSF1 cells (measured as a fold change E2 vs Ctr) was partially related to the higher basal expression of ER-dependent genes, which suggests the potential role of HSF1 in the suppression of these genes. This was consistent with the observation from the ChIP experiment in which a more efficient binding of ER in unstimulated cells was observed in the absence of HSF1.

Global analyses of ER and HSF1 chromatin binding patterns in E2-treated *wild-type* MCF7 cells combined with previously published ChIA-PET results allowed us to identify over 700 genes that could be co-regulated by both transcription factors. For these genes, in-depth bioinformatic analysis of ER and HSF1 binding sites was conducted for the presence of binding motifs (ERE and HSE, respectively), which allowed us to distinguish three modes of ER/HSF1 cooperation: canonical binding, cobinding, and possible tethering. In the last mode, it is also possible that ER and HSF1 bound to chromatin in different sites can interact leading to the formation of a chromatin loop. Moreover, various binding patterns were found in the regulatory region annotated to one gene. Further analyzes of the spatial organization of chromatin by chromosome conformation capture (3C) technique confirmed the role of HSF1 in the formation of ER-mediated chromatin loops while the proximity ligation assay (PLA) showed interactions between ER and HSF1 after E2 treatment.

Our results show the pleiotropic nature of HSF1, which, in addition to the regulation of *HSP* genes, may potentially constitute an additional tethering factor for ER and influence the organization of the chromatin loops created during estrogen signaling.

[25] ELECTROSPUN PACLITAXEL DELIVERY SYSTEM BASED ON BIODEGRADABLE POLYMERS

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Introduction

Polymer-based drug delivery systems for localized therapy have been extensively investigated over the last several decades among which implantable delivery vehicles can be mentioned. New strategies are being explored to obtain flexible, drug-eluting materials. The convenient method for obtaining soft materials is electrospinning. Nonwovens obtained during electrospinning possess high porosity and a large specific surface area and thus high loading capacity. This study aimed to develop biodegradable paclitaxel-loaded patches using electrospinning technique as soft, flexible and bioresorbable drug-eluting implants intended for postoperative, localized, anticancer therapy.

Methods

The poly(glycolide-ε-caprolactone) and poly(D,L-lactide-co-glycolide) were synthesized in the Centre of Polymer and Carbon Materials of the Polish Academy of Sciences. Obtained copolymers were mixed with paclitaxel (PTX) and then electrospun. Patches with 0.5 cm diameter were cut and sterilized with electron beam (25kGy). The patches were first tested *in vitro* using nuclear magnetic resonance spectroscopy, gel permeation chromatography, differential scanning calorimetry, scanning electron microscopy, high-pressure liquid chromatography. In the second stage, mice with developed tumors (6070 mm3, n= 5 per group) were treated with PTX-loaded patches or empty patches which were implanted directly into the tumor site (in the Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology). Tumor growth was monitored. Additionally, another group of mice with well-developed tumors (6070 mm3, n = 5 per group) were treated with PTX-loaded patches and with contact radiotherapy (brachytherapy). Brachytherapy was used at 3 doses of 2 Gy.

Results

Soft, flexible nonwoven materials loaded with the chemotherapeutic drug- paclitaxel intended for local anticancer therapy were obtained. The *in vitro* drug release and the degradation process of the PTX-loaded patches were characterized in details and on the basis of obtained results the best material was chosen for the *in vivo* study. The effect of the PTX-loaded patches on the tumor growth inhibition was investigated using mouse model of breast cancer. The growth of the tumors was slowed down in comparison with drug-free patches. PTX-loaded patches were also combined with brachytherapy. Such a combination was even more effective than either therapy alone.

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[26] HOW FAST CAN BE THE VINCRISTINE REMOVED BY WHITE-ROT FUNGI?

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Anticancer drugs, which are not effectively removed by wastewater treatment plants, may constitute an environmental hazard. Therefore, research into the elimination of cytostatics from water constitutes a necessity. Technologies based on white-rot fungi are worth particular attention in this respect.

The main aim of the project was to asses the applicability of five white-rot fungi in the removal of cytostatic drug – vincristine, from the environment. Selected organisms were: *Fomes fomentarius* (CB13), *Hypholoma fasciculare* (CB15), *Phyllotopsis nidulans* (CB14), *Pleurotus ostreatus* (BWPH), and *Trametes versicolor* (CB8). The test was conducted in 100 ml liquid organic medium and the mycelium were pre-cultivated for a week before adding the drug (at a concentration of 10 mg/l). The removal capacity was measured at regular intervals up to 21 days of incubation using Ultra-Performance Liquid Chromatography combined with Mass Spectrometry preceded with Solid Phase Extraction. All individual tests, and abiotic controls, were conducted in triplicate.

The results showed that biodegradation process of vincristine was fast. After two days, four fungi removed approximately 90% of the drug in the samples. Only *P. niulans* (CB14) showed no biodegradation ability. Such promising experiment results open up space for further research into the application of fungi in the elimination of pharmaceuticals from the environment.

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

key words: biodegradation; cytostatic drugs; white-rot fungi

[27] THE INFLUENCE OF STEREOCHEMICAL FORM OF PLA ON PROPERTIES OF MICELLES TARGETED WITH FOLIC ACID AND BIOTIN FOR SIMULTANEOUS DELIVERY OF DOCETAXEL AND RESVERATROL

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Introduction Block copolymers of polylactide (PLA) and polyethylene glycol (PEG) - PLA/PEG are commonly used for preparation of micelles. Biodegradable micelles may be applied for effective solubilization and delivery of hydrophobic anticancer drugs. However, the properties of micelles may be dependent on the kind of PLA, because poly(L,L-lactide) (PLLA) is a crystalline and poly(D,L-lactide) (PDLA) is an amorphous polymer. Additionally, the targeting moieties attached to the PEG may influence the characteristics of the nanoparticles. Therefore, the aim of the study was to analyze the effect of stereochemical form of PLA on morphology, size, drugs encapsulation efficiency and drugs release from micelles targeted with folic acid and biotin.

Methods Four kinds of micelles were obtained by co-solvent evaporation method: PLLA-PEG functionalized with folic acid and biotin, PDLA-PEG functionalized with folic acid and biotin, non-functionalized PLLA-PEG and non-functionalized PDLA-PEG. The micelles were co-loaded with docetaxel (DTX) and resveratrol (RES). The size of micelles was measured by means of the dynamic light scattering (DLS). Morphology was observed using transmission electron microscopy (TEM). The high-performance liquid chromatography (HPLC) was used for quantitative analysis of drug.

Results The TEM analysis revealed that all kinds of micelles characterized spherical shape. The size of PLLA/PEG micelles was higher compared to the PDLA/PEG micelles. Comparison of drug loading properties revealed that the encapsulation efficiency (EE) was higher in PLLA/PEG micelles. Moreover, the EE of DTX and RES was similar in PLLA/PEG micelles. In the case of PDLA/PEG micelles the EE of RES was higher than EE of DTX. *In vitro* analysis revealed that RES was released with similar rate from all kinds of micelles. The release of DTX was much slower in comparison to RES and the slowest release of DTX was observed from PLLA-PEG micelles.

Conclusions The influence of stereochemical form of PLA on the size of micelles, encapsulation efficiency and drug release was observed. This finding may be used for tailoring of properties of micellar drug delivery systems functionalized with folic acid and biotin and co-loaded with docetaxel and resveratrol.

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[28] COMPUTATIONAL PREDICTION AND BIOPHYSICAL VALIDATION OF SYNERGISTIC DRUG COMBINATIONS FOR BRAF-MUTANT MELANOMA

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Melanoma, which originates from melanocytes, after entering the metastatic stage causes the highest mortality among skin malignant tumors resulting from the lack of effective therapy due to common resistance to the applied drugs [1]. In the view of increasing worldwide prevalence the intensive search for successful therapy pattern is undertaken with the use of combined strategy. This includes the combination of recognized anti-cancer drugs with compounds acting on various vital cellular signaling pathways, particularly on the energy metabolism. In melanoma, the mutation of the proto-oncogene B-Raf cytoplasmic serinethreonine kinase (*BRAF*) gene is the most common (over 50% of patients), which is why most of the applied drugs are directed at inhibiting that signaling pathway. For the optimization of the choice of possible compounds, we performed the modified SynGeNet drug combination prediction study [2]. Experimental validation of effective combinations was done by monitoring the interaction of specific lectins with cellular surface glycans typical for various stages of melanoma progression in real-time experiments using quartz crystal microbalance with the dissipation monitoring [3]. Evaluation of the modification of the glycosylation process of metastatic melanoma cells as result of the applied combination of compounds could reveal potential usability in malignant melanoma treatment.

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[29] FUNCTIONAL DISSECTION OF IGH ENHANCERS AND ENHANCER RNAS IN B-CELL LYMPHOMAS

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Characteristic feature of B-cell non-Hodgkin lymphomas (NHL) are recurrent translocations juxtaposing an oncogene (e.g. *MYC, BCL2*) with immunoglobulin heavy chain (*IGH*) enhancers: E and 3regulatory regions (3RR1, 3RR2). Survival and proliferation of many B-cell lymphomas depend on the expression of the translocated oncogene. The function of *IGH* enhancers in B-cell maturation is well established, while the precise mechanisms of their involvement in oncogene expression and lymphomagenesis are yet to be determined. Enhancer RNA (eRNA) transcription is a feature of active enhancers. This class of non-coding RNAs plays a role in transcriptional regulation by recruitment and trapping of transcription factors, chromatin remodelers or RNA Pol II. Our goal is to identify functional elements in the *IGH* enhancers and eRNAs transcribed from those enhancers that are essential for oncogene expression and B-cell lymphoma cell growth.

Our tiling CRISPR interference screen in NHL cell lines with sgRNA library densely covering the *IGH* enhancers revealed three 500-700 bp regions, one in the E and two in the 3RR enhancers, whose targeting profoundly inhibited NHL cells growth. In parallel, we performed chromatin-enriched RNA-Seq in lymphoma and normal B cells, which confirmed transcription from CRISPRi-essential enhancer regions. Moreover, eRNA expression was validated in a large panel of NHL cell lines and in patient-derived samples. So far, subsequent validation of sgRNAs targeting the identified essential enhancer regions confirmed their inhibitory effect on eRNA and c-MYC expression in lymphoma cells. Functional studies of the eRNAs are ongoing.

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[30] MICROBIOLOGICAL EVALUATION OF MILK FROM FARMS AND DOMESTIC CATTLE IN SELECTED REGIONS OF KHYBER PAKHTUNKHWA

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The presented study focuses on the prevalence of pathogenic microorganisms in dairy products specifically in the raw milk of farms and domestic cattle. The consumption rate of dairy products are increasing day by day among people of all ages i.e., infant, young and old due its main nutrient content. Apart from huge nutritious value of milk it also poses sever threats towards the people consume it. There are many pathogenic microorganisms which utilized various nutrient present in milk as a media for their growth. The growth of such pathogenic microorganisms leads to foodborne illnesses like food poisoning. The various pathogen commonly found in raw milk from are tick-borne encephalitis virus *Campylobacter*, *Brucella melitensis*, *Mycobacterium bovis, Salmonella*, and Shiga Toxin-producing *E. coli* (STEC). In this study various type of pathogenic microorganisms along with their microtoxin are found including *Salmonella spp, Escherichia coli* observed in 65% milk samples, *Salmonella* 66% *Proteus spp* 69%, *Bacillus subtilis* 60%, *Klebsiella pneumonia* 64%, Enterococcus faecalis 54%, *Staphylococcus aureus* 70%, *Streptococci* 68%, *Yersinia pestis* 66% were detected.

All these pathogens cause severe problems in those who consumed. It contains beneficial normal flora which improve the immunity and avoid diseases. As it has all basic and essential component therefore it provides best growth media for the growth of pathogenic microorganism. Current study is focus on the occurrence of pathogen microorganisms in raw milk from farms and domestic cattle. Such microorganisms used raw milk as media for their growth and nutrition as milk have all basic components (vitamins, fats, protein, mineral).

This study is carried out to provide information to veterinarians regarding selection of proper treatment strategies to prevent contamination of milk with life threatening pathogenic organisms.

[31] DEVELOPMENT OF SMALL-MOLECULE COMPOUNDS REPLACING THE NF1 GENE PRODUCT – INDUCING THE GTPASE ACTIVITY OF THE RASWT PROTEIN

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Introduction *NF1* mutations, including deletions of this gene, occur in various cancers and are also the cause of neurofibromatosis type I (von Recklinghausens disease). It is estimated that von Recklinghausens disease affects at least 1 out of 5,000 (five thousand) people. Half of the cases are due to inheritance, and half of the mutations occur in early embryonic development. In addition, the *NF1* mutations appear during carcinogenesis without being related to the inheritance of these mutations. Examples of neoplasms with the *NF1* mutations are: gliomas, sarcomas, breast cancers, endocrine tumors such as pheochromocytoma, melanomas, acute myeloid leukemias, ovarian cancers, and prostate cancers. Currently, there is no therapy aimed at replacing the GAP protein with small molecules. As part of the analyzes, molecules that are able to induce the GTPase activity of the RASWT protein have been developed.

Methods The GTPase-Glo Assay (Promega) was used to test GTPase activity of RASWT. The RASWT and GAP proteins were purified *via* methods of genetic engineering.

Results Derivatives of leading molecule are probably able to induce RASWT GTPase activity in the absence of GAP protein.

Conclusions The analyzed molecules can be considered as replacing the product of *NF1* gene in cells, and thus they may be precursors of compounds with therapeutic potential for patients with neoplasms exhibiting the *NF1* gene mutation.

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[32] A NOVEL NON-PATHOGENIC METHOD FOR ANALYZING THE VIRUS FILTERING ABILITY OF PROTECTIVE MASKS USING THE FLUORESCENCE PHENOMENON

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During the Covid-19 pandemic, wearing personal face masks has become a routine activity for people around the world. The main role of such masks should be protecting healthy people from different types of infections and helping the sick ones, to limit the widespread of viruses and bacteria. Therefore, the filtering abilities of commonly used personal masks were experimentally tested using the methodology developed by our team. This method uses polystyrene nanospheres coupled with a luminescent dye FITC, which was supposed to be a phantom of a real virus. For this purpose, a system for testing the filtration efficiency of masks was built, consisting of an aerosol generator in which nanospheres were suspended. The aerosol was then passed through the test mask, and then through the Andersen cascade impactor, where fluorescent nanoparticles were collected, through which qualitative results were obtained. As the analyzed materials, the hand-made masks made of cotton fabrics and different types of protective masks commonly available in retail and medical trade (eg. surgical, FFP2, FFP3) were chosen. In addition, specially prepared materials using nanofibers with metal admixtures were tested. These materials were applied to generally available masks as an additional protective layer increasing the filtering capacity of the mask. This method allows leading researches in a safe way without using potentially pathogenic organisms.

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[33] STUDIES OF TRANSDERMAL ENVIRONMENTAL MICRO- AND NANO-POLLUTANTS

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Introduction The impact of selected environmental and synthetic pollutants on human health is considered through the air and dermal contact, especially together with pathogen infections in the pandemic Covid-19 period. In environmental samples, we collected pollutants, including fine dust (2.5 m particle diameter) and added nano-plastic, for testing on model experimental setup for 3D human skin cell culture, initially developed in previous projects (students PBL 5th and 6th editions).

Aim The aim is to analyses environmental pollution and studies of penetration mechanisms with a particular focus on fine dust fraction (PM1 and PM2.5) and bioactive compounds in an experimental 3D dermal cell culture system.

Materials and Methods The collection of outdoor samples was performed using quartz microfibre filters (QM-A Whatman, diameter 47 mm) attached to Atmoservice PNS-15 aspirators (produced in Poland under the license of the low-volume sampler LVS 3.1, Comde-Derenda GmbH, Germany) with flow rates of 2.3 m3/h. In vitro studies were conducted by a live microscopic observation using JuliBr (NanoEntek; transit channel, magnification 100 x), and the cytotoxicity was evaluated for synthetic nano-particles (diameter of 100 nm) by 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Promega) viability assay.

Results and Conclusions Seasonally collected environmental pollutants showed impacts on human normal and cancer cell lines (ATTC, BEAS-2B, and A549) in cyto- and genotoxicity assays. Exposure of epithelial lung cells to factors contained in environmental dust decreased their viability and induced damage to the genetic material [1]. The possibility of penetration of micro- and nano-pollutants through human skin was investigated using in vitro 3D culture, which showed that pollutants penetrate intracellularly through endocytosis, similarly to pathogens [2], inducing damage and leading to cell death.

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[34] COVID-19 VACCINATIONS DECREASES SARS-CoV-2 INCIDENCES: SINGLE MEDICAL INSTITUTE REPORT

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Introduction. Since the outbreak of COVID-19 in December 2019, it has brought significant harm and challenges to over 200 countries around the world. The fast increasing number of cases highlighted the urgent development of COVID-19 vaccine. At the end of December 2020, the FDA granted approval for the PfizerBioNTech and for the Moderna vaccines. In Poland COVID-19 vaccinations with both these vaccines started in January 2021. The purpose of this study was to assess if vaccination has any effect in terms of COVID-19 incidence in the Maria Sklodowska-Curie National Research Institute of Oncology Gliwice Branch (NIO-PIB) employee population against the epidemiological situation in Poland and Silesia.

Methods. The study was focused on two pandemic periods that correspond to two consecutive waves of COVID-19 in Poland: one in fall 2020 (pre-vaccination) and one in spring 2021 (post-vaccination). Data comprised of the reported daily number of COVID-19 cases at NIO-PIB (internal data) and in Poland/Silesia (sources: https://ourworldindata.org/coronavirus-source-data, https://koronawirusunas.pl/), as well as the data on the number of vaccinated employees. Due to big disproportion among populations (NIO-PIB workers n=1800, Poland n38M, Silesia n4.5M) both the number of cases and the incidence rates were compared (using The Chi-square test or Wilcoxon rank sum test). Also linear regression was used to make predictions about what the situation would be in the NIO-PIB without reaching the vaccination threshold 70%.

Results. Analyzing the differences of COVID-19 incidence rates in I vs II wave we found significance difference in case of NIO-PIB (p0.001) and as well in case of Silesia rates (p=0.002) but no difference on country level of analysis. When assessing the difference on level of number of cases we found significance difference in all three comparisons (NIO-PIB: p0.001, Silesia: p0.001, Poland: p0.001). Surprisingly we found that only in case of NIO-PIB in II wave vs I we had 78% decrease in COVID-19 incidence (OR=0.22). On the contrary in Poland and Silesia weve observed increase in COVID-19 cases (respectively OR=1.23, OR=1.49). Analysis of prediction with assumption on how II wave would look like at NIO-PIB without any vaccination in comparison to actual data showed that with no vaccination we might have 5.39 times more COVID-19 incidence (OR=5.390, p0.001).

Conclusions. COVID-19 vaccines were developed in an exceptionally short time, which aroused a lot of skepticism. Simultaneously after the vaccination campaign in NIO-PIB a large decrease in COVID-19 incidence among employees was observed. Despite the limitations of simplifying and basing the calculations only on the reported incidence, ignoring other factors that affect the dynamics of the pandemic, there is a clear improvement in the NIO-PIB situation in post-vaccination wave compared to the pre-vaccination one. The observed effect is most likely related to COVID-19 vaccination.

[35] c-Myc protein level affected by unsymmetrical bisacridines plays a role in apoptosis induced by these compounds in HCT116 colorectal and H460 lung cancer cells

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Unsymmetrical bisacridines (UAs) are new antitumor compounds patented in Europe (EP 3070078 B1) and in the USA (US 10202349 B2). In their structure they contain previously synthesized in our Department drugs: C-1311 and C-1748. Importantly, UAs exhibit different properties than their monomer components. They do not intercalate to dsDNA, but interact with quadruplex DNA. G-quadruplex structures are present in promoter regions of oncogenes, such as: *MYC, KIT, RAS* genes, *BCL2, VEGF* and in telomeric repeats.

The aim of the presented studies was to evaluate whether UAs can affect the expression and protein level of c-Myc in living cells and what is the consequence for the cellular response induced by UAs treatment.

The effects of four UAs: C-2028, C-2041, C-2045 and C-2053 on human colorectal HCT116 and lung H460 cancer cells were examined. MTT assay showed that all compounds exhibited high cytotoxicity against studied cancer cells. The concentrations of UAs corresponding to IC90 doses ranged from 0.04 to 0.4 M and were similar for both cell lines. Real-time PCR was used to establish the mRNA level of *MYC* gene, while Western blot was applied for analysis of c-Myc protein level. In HCT116 cells UAs treatment resulted in increase in the mRNA amount of *MYC*, but the protein level did not change compared to control. On the other hand in H460 cells slight decrease in expression of *MYC* was observed after UAs exposure. What most important, in lung cancer cells bisacridines caused complete inhibition of c-Myc protein level after 72 h of incubation. The analysis of cell cycle progression in HCT116 and H460 cells exposed to UAs revealed some changes in the distribution of cells in each of the phases. The most profound alterations after treatment with compounds were transient accumulation of cells in S phase of the cycle in H460 cells and appearance of the sub-G1 fraction in both HCT116 and H460 cells. Number of cells with degraded DNA increased during the time of treatment and was the highest for C-2041-HCT116 treated cells and C-2045- and C-2053-H460 treated cells. HCT116 and H460 cells exposed to UAs underwent apoptosis what was confirmed by observation of nucleus morphology, determination of changes in asymmetry and permeability of the cell membrane, analysis of presence of active caspase-3, cleaved PARP and changes in mitochondrial transmembrane potential. Importantly, the apoptosis was induced by UAs earlier and to a greater extent in H460 (especially in cells exposed to C-2045 and C-2053) compared to HCT116 cells (except C-2041). Necrosis was observed only in a small percentage of HCT116 and H460 cells. Concluding, strong c-Myc inhibition by UAs in H460 cells seems to contribute to induction of apoptosis in these cells and the most effective compounds were C-2045 and C-2053.

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[36] THE ROLE OF MCPIP1 IN CHANGE THE PHENOTYPE OF LIVER CELLS

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MCPIP1 (Monocyte Chemoatractant Protein-Induced Protein 1) protein, coded by ZC3H12A gene, is one of the negative regulator of inflammatory response. The MCPIP1 protein is composed of several domains, including the zinc finger domain CCCH and the PIN domain with RNase activity. As a result of RNase activity MCPIP1 protein regulates levels of many transcripts, including pro-inflammatory cytokines: IL -1β and IL-6 as well as several miRNAs level important in tumor growth and metastasis.

The MCPIP1 protein plays an important protective role in the development and progression of breast cancer and clear cell renal cell carcinoma. Our results indicate the participation of MCPIP1 protein in the regulation of tumor proliferation, survival and metastasis. Decrease in the level of MCPIP1 protein triggers a surge in cell mobility and the growth in level of mesenchymal markers, such as: vimentin and β-catenin. Furthermore, our last research showed that the level of MCPIP1 protein may have an impact on the induction of the epithelial-mesenchymal transition (EMT) in the liver.

In ongoing research, we used an unique mouse model with knockout of the Zc3h12a gene, which leads to the depletion of MCPIP1 in the hepatocytes. To examine the role of MCPIP1 in the change of liver phenotype, we analyzed the livers of 42 weeks old mice using RT-PCR, Western blot and immunohistochemical staining methods.

Our studies have shown differences between the liver phenotype of wild-type mice and mice with the depletion of MCPIP1 protein. Knockout of the Zc3h12a gene in mouse livers increases the level of transcription factors responsible for the development of the mesenchymal phenotype e.g. Snail. In addition, we observed an increase in the level of β -catenin and structural proteins such as fibronectin or N-cadherin. Interestingly, knockout of the Zc3h12a leads to an increase in the level of intracellular cleavage fragments (CTF) of N-cadherin. It's worth stressing that CTFs may participate in the regulation of the expression of certain genes, like metalloproteinase MMP9.

Our research indicates that the MCPIP1 protein plays an important role in development of EMT in the liver. Depletion of MCPIP1 might be an important factor leading to the development of liver cancer. In addition, our results indicate that MCPIP1 may regulate level of CTFs of N-cadherin which can fulfil important functions other than being just the structural protein.

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[37] miR-625-5p IMPLICATED IN REGULATION OF APOPTOSIS AS NOVEL PUTATIVE ONCOMIR IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction Acute lymphoblastic leukemia (ALL) is the most common cancer in children. T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive subtype of ALL, arising from T lymphocyte precursors. miRNAs are non-coding RNAs, contributing to leukemogenesis by their involvement in key cellular functions such as proliferation, apoptosis and signaling. We previously found in miRNA transcriptome analysis that hsa-miR-625-5p is overexpressed in T-ALL patients, thus is a potential oncomiR in T-ALL.

Methods T-ALL JURKAT cells (with high endogenous miR-625-5p level) were transduced for stable inhibition of this miRNA. To evaluate the influence of miR inhibition on growth of T-ALL cells, we used flow cytometry GFP competition assay and CCK8 proliferation assay. To identify the genes mediating the impact of miR-625-5p on cell growth, we subjected JURKAT cells upon miR inhibition to Ago2-RIP-seq. We used Magna RIP magnetic beads coated with anti-AGO2 antibody to immunoprecipitate RNA bound to RISC complexes. Ago2-IP and total RNA fractions were sequenced (polyA RNA-seq, 150 bp reads, 60M paired end reads/sample, Illumina NovaSeq 6000). Transcripts depleted in Ago2-IP fraction upon miR inhibition were screened for the presence of miR-625-5p binding sites.

Results Inhibition of miR-625-5p decreased the growth of JURKAT cells. In RIP-seq analysis we identified 1530 transcripts depleted in RISCs (by at least 20%) upon miR-625-5p inhibition, 384 of these genes having at least one putative 3UTR binding site for this miRNA, thus being direct targets of miR-625-5p. Over-representation analysis (OVR) performed for these direct miR-625-5p targets showed high overlap with processes revealed by OVR of differentially expressed genes (DEGs) between JURKAT cells upon miR-625-5p inhibition and control cells, including processes known to be implicated in T-ALL (e.g. PI3K-Akt, NOTCH and MAPK signaling). Moreover, OVR for DEGs revealed processes, such as cell death, negative regulation of lymphocyte apoptotic process, negative regulation of thymocyte apoptotic process, indicating that miR-625-5p affects apoptosis of T-ALL cells.

Among transcripts depleted in RIP-seq, with predicted 3UTR binding sites for miR-625-5p, we found genes previously reported to have pro-apoptotic activity (*TP53AIP1*, *IER3*, *HRK*, *STK17A*). Silencing of these genes by overexpressed miR-625-5p may contribute the decreased of apoptosis and to growth advantage of T-ALL cells.

To further confirm that miR-625-5p affects growth of T-ALL cells via regulation of apoptosis, we performed apoptotic assay of JURKAT cells and showed that inhibition of this miRNA increased apoptotic events, in line with our hypothesis on oncogenic potential of miR-625-5p.

Conclusions. miR-625-5p is a novel putative oncogenic miRNA in T-ALL which may regulate apoptosis by silencing pro-apoptotic genes *TP53AIP1*, *IER3*, *HRK, and STK17A.*

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[38] DIAGNOSTIC SIGNIFICANCE OF TOTAL CIRCULATING DNA (CFDNA).

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Introduction: Currently, the basis for the application of appropriate oncological treatment is histopathological examination. It requires the collection of a tissue sample that is difficult to access and strains the patient. Hence the need to find a readily available tissue equivalent. The purpose of this study was to evaluate diagnostic estimates of total circulating DNA (cfDNA) in cancer.

Methods: Systematic search was performed in Medline and PubMed Central databases. All statistical analyses were performed using R version 4.0. Statistical significance was set at p 0.05. Pooled sensitivity, specificity, diagnostics odds ratio (DOR) and their 95% confidence interval were calculated with mada and meta packages. I2 test was used to evaluate the heterogeneity.

Results: Finally, 62 articles were included in the analysis, in which the concentration of cfDNA in 5699 cancer patients and 3323 controls was examined. The calculated pooled sensitivity, specificity and diagnostic odds ratio (DOR) was 0.798 [95% CI 0.764-0.832] and 0.864 [95% CI 0.816-0.888], 20.913 [95% CI 16.184-28.803] respectively. The funnel plot showed the asymmetry (p0.001) with publication bias and study heterogeneity as a possible causes (pooled sensitivity 93%, pooled specificity 89%, DOR 82%). In order to find the sources of heterogeneity, we performed subgroup analyzes for tumors of various localizations, cfDNA detection methods, plasma / serum as a source material. In some of these subgroups we managed to obtain a symmetrical funnel plot, but in all analyzes the sample heterogeneity was always higher than 80%. To explain the problem better, we compared the raw cfDNA concentration data from the analyzed articles. The median value of the cfDNA concentration was 1277.5 ng / ml for the control group and 2712.5 ng / ml for the cancer group, and these values were different by Mann-Whitney U test (p = 0.03). However, these values do not fully reflect the actual situation, an overlapping range of values was found in the control and cancer group (0-968 ng / ml for the control group and 0-33 250 ng / ml for the cancer group). The analysis of the sources of heterogeneity again showed that in the previously mentioned subgroups the range of the obtained values was equally large. While in the case of the cancer group, the influence of tumor localization, histological type and disease stage on the cfDNA concentration cannot be ruled out, the large dispersion of cfDNA values in the healthy (control) population indicates the influence of pre-analytical and analytical conditions on the obtained values.

Conclusions: Despite the promising high values of the sensitivity and specificity of the cfDNA test, the lack of standards for isolation, detection and reporting of test results causes a large dispersion of the results, and consequently the impossibility of its clinical application.

[39] THE ROLE OF MCPIP1 PROTEIN IN TUMOR INITIATION AND STEMNESS OF NORMAL EPITHELIAL CELLS

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Introduction Despite many years of searching for effective therapies, tumors are still one of the most common causes of death in the world. Developing new treatment strategies is extremely difficult due to the problem of identifying cancer stem cells (CSCs) which are responsible for tumor initiation and progression. The inflammatory response is a key element in tumor development. The MCPIP1 protein (Monocyte Chemotactic Protein-1 Induced Protein) is involved in the negative regulation of inflammation due to its RNase activity, which allows mRNA degradation of pro-inflammatory cytokines. A growing number of publications suggest that the MCPIP1 protein may influence the development of cancer by direct or indirect regulation of factors involved in the processes of angiogenesis, proliferation and cell death.

Aim The main aim of our research is to study the importance of MCPIP1 for the presence of CSCs markers, factors involved in tumor initiation as well as acquirement of stemness features. Moreover, we investigate whether MCPIP1 level or activity will change phenotype of normal cells into cancerous.

Materials and methods To examine the effect of MCPIP1 in normal epithelial cell line TCMK-1 we used two different viral vectors. To stably inhibit MCPIP1, cells were transduced with lentiviral vectors (shCtrl, shMCPIP1). For stable overexpression MCPIP1, TetON system was used (pLIX PURO and mutant form pLIX D141N). We analyzed cells proliferation and clonogenicity, as well as the levels of CSCs markers by western blot and qPCR. Next, cells were injected subcutaneously into NOD-SCID mice to check if low level or mutation of MCPIP1 will induce tumor growth in vivo. Mice blood was analyzed for the presence of circulating tumor cells (CTCs) with flow cytometry.

Results We have shown that the low level of MCPIP1 or mutation in PIN domain increases the proliferation and clonogenicity of TCMK-1 cells compared to control. Moreover, cells with decreased level of MCPIP1 protein or with the D141N mutation are characterized by higher levels of the c-Met receptor, CD44 and c-Myc phosphorylation, which are among the markers of CSCs. In addition, low level of MCPIP1 results in increased expression of the major marker of mesenchymal cells vimentin and transcription factors Twist and Snail involved in epithelial-to-mesenchymal transition. The obtained results suggest that the loss of the MCPIP1 protein causes the acquisition of the characteristics of neoplastic cells. Next, we checked whether MCPIP1 low level in normal cells predisposes them to proliferate when administered to mice. We found that, D141N mutation caused the growth of large tumors with higher number of CTCs, while the control cells injected into mice developed only local fibrosis.

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

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[40] TREHALOSE-RELEASING NANOGELS: A POTENTIAL TREHALOSE DELIVERY SYSTEM FOR AUTOPHAGY STIMULATION

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Introduction Autophagy is a fundamental process in cells that plays a critical role in maintaining intracellular check-and-balance mechanisms. The dysfunction of autophagy has been linked to various human diseases, e.g., neurodegenerative diseases. Most studies indicate that a relatively high concentration of trehalose is needed to induce autophagy. The poor pharmacokinetics profile of trehalose might be the main issue, which is linked to its high hydrophilic nature, affecting poor penetration into cell membranes and rapid excretion from the body. In addition, the presence of intestinal trehalase, an enzyme that can hydrolyze trehalose, impedes its oral absorption. Therefore, there is a need for seeking effective trehalose delivery that can overcome biological barriers and deliver trehalose to the targeted tissues.

Aims This study aimed to fabricate trehalose-rich nanogels that are stable in serum-containing media, have a moderate positive surface charge to provide electrostatic interactions with negatively charged membranes for brain targeting, has low cytotoxicity, and can sustainably release trehalose at physiologically relevant condition to induce autophagy.

Methods A series of trehalose-rich nanogels have been successfully synthesized *via* free radical polymerization (FRP) in reverse miniemulsion with the use of Span 80 as surfactant and lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP) as photo-initiator. The designed strategy toward trehalose-rich nanogels is based on covalent incorporation of 6-O-acryloyl-trehalose within polymer network, from which trehalose could be released at pH 7.0 by the cleavage of ester bond which is boosted by 1 and 2 acrylamide-type comonomers. The actual conjugated trehalose was assessed enzymatically by measuring the amount of glucose produced after enzymatic degradation of trehalose by trehalase. The colloidal stability of nanogels was tested in various media (water, PBS (pH 7.4), 5% dextrose-in-saline solution (D5NS), DMEM + 10% FBS, RPMI + 10% FBS, and low serum primary cell media). Cytotoxicity study and cellular uptake were tested in primary human umbilical vein endothelial cells (HUVECs).

Results We successfully fabricated trehalose-rich nanogels with high trehalose content (50% w/w). Nanogels could sustainably release trehalose at physiological conditions. Interestingly, the increased trehalose feeding could improve the stability of nanogels in serum-containing media. Ultimately, stable nanogels were non-toxic to primary HUVECs up to 1 mg/mL and were efficiently uptaken during short incubation.

Conclusions Trehalose-rich nanogels showed splendid properties, specifically high colloidal stability in serum-containing media, high cytocompatibility, and the ability to sustainably release trehalose at physiologically relevant conditions, which make them potentially applicable as trehalose delivery vehicles for autophagy stimulation.

[41] INFLUENCE OF THE TUMOR MICROENVIRONMENT ON THE EFFECTIVENESS OF RADIOTHERAPY OF HEAD AND NECK SQUAMOUS CELL CARCINOMAS IN VITRO

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Interactions between cells in the tumor microenvironment have a significant impact on the effectiveness of cancer treatment. The tumor microenvironment consists of cancer cells, fibroblasts, endothelial cells, stromal mesenchymal cells and immune cells. Cells other than cancer cells in the head and neck squamous cell carcinomas (HNSCC) may account for up to 80%. HNSCC is the sixth most common cancer. After treatment, about 50% of local recurrence is observed after 3 years. Therefore, more research into new therapies is needed.

The aim of the study is to investigate the impact of radiotherapy on the co-culture model of cancer cells with normal cells present in the tumor microenvironment.

We examined two HNSCC cell lines: FaDu (hypopharyngeal carcinoma cells), A253 (salivary gland squamous carcinoma cells) and two normal cell lines: HUVEC and Wi38. Cells were placed in 24- or 48-well plates and were irradiated with single-dose: 2 Gy or 10 Gy. Photon radiation X 6 MV, with a beam power of 400 MU/min, was used. Plates with cells were placed in a phantom, at a depth of 10 cm. Studies were carried out on monocultures and co-culture with cancer cells and normal cells. Immediately after irradiation the number of DNA double-strand breaks (DSB) was determined using anti-H2AX antibody by flow cytometry. Three days later, cell proliferation and radiosensitivity were analyzed using the MTS assay. Apoptosis and cell death were determined using Annexin V/7-AAD staining and caspase-3 staining. Also one day after radiation, cells migration was analyzed using wound-healing assay.

Co-culture with FaDu cells and HUVEC reduced the number of DSB compared to the FaDu monoculture after irradiation with a dose of 2 or 10 Gy. Co-culture with FaDu cells and HUVEC slightly reduced cancer cell radiosensitivity after 10 Gy radiation. Co-culture with either A253 or FaDu cells and HUVEC cells or A253 cells and Wi38 fibroblasts highly increased the percentage of cells in early phase of apoptosis after 10 Gy radiation compared to cancer cells monocultures. The area of caspase-3 positive cells decreased in all co-cultures groups compared to cancer cells monocultures after 10 Gy irradiation. In co-culture with either A253 or FaDu cells and Wi38 fibroblasts faster cell migration was observed after 2 Gy radiation during the first 6 hours of the test compared to cancer cells monocultures. The opposite effect was observed in co-culture with A253 or FaDu cells and HUVEC after irradiation with the dose of 10 Gy.

Co-culture of head and neck squamous cell carcinomas with endothelial cells and fibroblasts increased the percentage of cells in the early phase of apoptosis. Simultaneously, decreased the percentage of cells in the end stage of apoptosis. Radiosensitivity of HNSCC requires further research, especially in co-cultures with other cells present in the tumor microenvironment.

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[42] BIORESORBABLE, ELECTROSPUN NONWOVEN AS RADIOSENSITIZER DELIVERY SYSTEM

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INTRODUCTION: Approaches directed towards the hypoxic tumor environment may be a considerable promise in improved clinical outcomes of solid tumors radiotherapy. One of them concerns the application of radiosensitizers, such as nitroimidazoles, to enhance the killing of tumor cells by radiation while exerting a lesser effect on normal cells. Among all nitroimidazoles, only nimorazole has been clinically tested and is used in Denmark with encouraging results. **This study aimed** to obtain the drug delivery system (DDS) in the form of a bioresorbable nonwoven wafer for the controlled release of nimorazole as a proposal for the future local treatment of solid tumors.

MATERIALS AND METHODS: The aliphatic polyester and polyester carbonate were synthesized from L-lactide, glycolide, -caprolactone, and trimethylene carbonate and used in electrospinning to obtain layer-by-layer nonwoven wafers. DDSs differed in terms of fibre structure that contained nimorazole monolithic or core-shell fibres and the surface that was covered by electrospray or uncovered. The wafers were tested using nuclear magnetic resonance spectroscopy, gel permeation chromatography, differential scanning calorimetry, and scanning electron microscopy. The amount of released nimorazole was evaluated with the use of high-pressure liquid chromatography.

RESULTS: Two types of bioresorbable polymers were investigated as nimorazole delivery systems: poly(L-lactide-co-glycolide-co-trimethylene carbonate) (PLAGATMC) and poly(L-lactide-co--caprolactone) (PLACap). The high values of encapsulation efficiency revealed that a significant amount of nimorazole has been incorporated into the fibres, regardless fibre structure. In the case of uncovered DDSs that contained monolithic fibres, almost all of the nimorazole contents were eluted after 6 and 9 weeks from PLAGATMC and PLACap, respectively. The wafers composed of core-shell fibres were covered to prevent the fast release of nimorazole. A three-phase release profile of the drug was observed for PLAGATMC.

CONCLUSIONS: The use of core-shell fibres and the covered surface of polymeric nonwovens seems to be the best choice for obtaining controlled delivery systems of nimorazole with a delayed and prolonged release profile. Such drug-loaded wafers may be implanted into a tumor resection cavity or directly into the tumor tissue (in case of unresectable cancers), as a postoperative therapy combined with radiation. Poly(L-lactide-co-glycolide-co-trimethylene carbonate) has great potential in this regard.

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[43] DIFFERENCES IN CYTOTOXICITY, PROLIFERATION, MIGRATION AND TOTAL PROTEIN AMOUNT IN HUMAN SKIN CELLS INDUCED BY SELECTED ORGANIC SOLVENTS

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Potentially active compounds should be tested on cell lines *in vitro* prior to their use in drugs or cosmetics. Obtaining accurate concentrations often requires dissolving them in appropriate organic solvents. However, the organic solvents themselves can also affect the cell properties by inducing cytotoxic effect or altering their proliferation, migration or total protein amount, that could interfere the results of the experiments.

In our study the most frequently used organic solvents were represented by ethanol and methanol (protic solvents), acetone and dimethyl sulfoxide (aprotic solvents), 1,4-dioxane and toluene (non-polar solvents). Their influence on cell cytotoxicity, proliferation, migration and total protein amount was examined in a simple biological model a monoculture of the immortalized human keratinocyte cell line (HaCaT). Biological tests like the metabolic activity assay (MTT) and the crystal violet staining (CV) were performed for the range of concentrations 0.1-5% (v/v). The proliferation assay (BrdU), the wound healing assay and the determination of total proteins in cells were examined for the one pre-selected concentration of these solvents: 1% (v/v). Moreover, some tests were also performed using various culture media (like Dulbeccos Modified Eagle Medium and Minimum Essential Medium), or medium with decreasing concentrations of the Fetal Bovine Serum (0-10% v/v, FBS) to show differences in the selected cell features.

The results show an increasing dose-dependence of the solvents on the studied HaCaT cells, where the highest doses of the examined solvents have the adverse effect (in most cases). A slight dependency related with the polarity index of the applied organic solvents and belonging to the proper solvent group is also noticeable. The results also presents that the cell reaction to the solvent is dependent on the cell growth media composition.

In conclusion, these data confirm that a proper selection of type and dose of the organic solvents for the dissolution of the active substance is a crucial step to obtain reliable results.

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[44] CHANGES IN THE EXPRESSION PATTERN OF TNF-A AND ITS RECEPTORS IN ENDOMETROID ENDOMETRIAL CANCER DEPENDING ON THE DEGREE OF CANCER DIFFERENTIATION

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Introduction/Rationale: Tumor necrosis factor (TNF-α) is a pleiotropic cytokine involved in a wide range of processes within the tumor microenvironment. The aim of this study was to evaluate changes in the expression of TNF-α and its receptors, TNFRI and TNFRII, in endometrial cancer compared to control.

Methods: The study involved 30 women diagnosed with endometrioid endometrial cancer (study group) and 30 women without neoplastic changes (control group) were qualified for the study. The study group was further divided according to the endometrial cancer grade: G1, 15 cases; G2, 8 cases; G3, 7 cases. The expression profile of TNF-, TNFRI and TNFRII was determined by microarrays and then validated by qRT-PCR. Changes in the concentration profile of TNF-α, TNFRI and TNFRII proteins were also evaluated using the enzyme immunoassay test ELISA.

Results: The analysis revealed TNF-α and TNFRII overexpression regardless of the endometrial cancer grade, both at the gene and protein levels. In the case of TNFRI, there was a significant increase in its expression with the exception of G3 cancer, while the concentration of this protein increased with the progression of endometrial cancer.

Conclusions/Novel aspect: The observed overexpression of TNF-α, TNFRI and TNFRII may indicate their participation in the initiation of endometrial cancer and activation of signaling pathways targeted at the survival of tumor cells, which further promotes tumor progression.

presenting author Robert Nowakowski

[45] ANTIPROLIFERATIVE ACTIVITY OF STYRYLQUINAZOLINE AND THIOSEMICARBAZONE COMPOUNDS AGAINST GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is one of the most aggressive and difficult to treat cancers. GBM is the most common cancer among primary tumours of the brain and central nervous system. Currently the prognosis of diagnosed patients with this type of cancer is up to a several months. Only small percentage of patients survive longer than 2 years with standard treatment consisting of surgical resection, radio- and chemotherapy. Hence, recent research in medicinal chemistry and medicine has focused on finding new effective compounds to combat GBM, as well as new therapeutic approaches. Our group has recently been exploring the use of styrylquinazolines and thiosemicarbazones (TSC) as effective and selective compounds for the treatment of GBM. Here, we present the antiproliferative activity of the tested derivatives against three glioblastoma cell lines: T98G, U87 and U251 that differ in their molecular landscape. The data obtained show that the highest antiproliferative activity was recorded for the quinoline TSC derivative, where the IC50 was 34 nM against U251 cells. This cell line also proved to be the most sensitive to the tested compounds.

In addition, we decided to investigate the possibility of using the tested derivatives in combination therapy with noscapine and temozolomide on U251 cell line. Our preliminary results are promising, and some of the tested combinations of compounds appear to work better together than in monotherapy. In further studies, we also plan to investigate more deeply the mechanism of action of selected compounds, including the cell cycle inhibition and apoptosis induction.

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[46] ESTABLISHING SKIN CELL SPHEROIDS AS A MODEL OF THE ARTIFICIAL SKIN

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Skin consists of three layers (epidermis, dermis and the subcutaneous tissue), where each layer is built by various cells. Due to its large surface, this organ is exposed to contact with the variety of external factors. The main cell types present in the epidermis are keratinocytes (90% of cells) and melanocytes (production of melanin), whereas dermis is built mostly by fibroblasts (secretion of many growth factors) and mast cells (immune cells). Traditionally, cells are grown as a monolayer (2D models). However, this model does not fully reflect the cell behavior in our organism. In the human body different cell types interact with each other and also with the extracellular matrix (ECM), like fibronectin, collagen or laminin. The three-dimensional (3D) cell culture is a more relevant model with a typical organ microarchitecture and microenvironmental signaling, but the usage of scaffolds makes it difficult to apply for various viability assays. At the moment, there is no ideal and cheap skin substitute that could be widely used for basic research. Thus, easier intermediate models are cell spheroids, which can be applied in the *in vitro* research to imitate better the natural environment of a living organism.

The aim of our research is to create multi-cell spheroids, which can be utilized in numerous analyzes, including checking the cytotoxicity of active medical ingredients, as well as tests of the cosmetics and individual cosmetic ingredients effectiveness. Our spheroids were created thanks to the hanging drop method with the use of keratinocytes and fibroblasts. The conditions were optimized according to the following parameters: density of cells, droplet size, incubation time, spheroids collection method and addition of ECM proteins (concentration and type). We determined the best conditions to growth the single-cell spheroids by means of the morphometric analysis with the optical microscope, live-dead cell visualization with the fluorescent microscope and metabolic activity analysis of cell spheroids. The multi-cell spheroids of keratinocytes and fibroblasts were also prepared. Before the creation of these spheroids, each cell type was stained with an appropriate fluorescent tracer, which allows their differentiation in the spheroid under the microscope.

To sum up, our approach led to the precise and effective creation of stable spheroids of the desired size. The procedure was optimized to be relatively easy, cheap, reproducible and possible to implement in most laboratories (no special equipment is mandatory).

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[47] FOLIC ACID-QUANTUM DOTS PLATFORMS FOR EFFICIENT DELIVERY OF UNSYMMETRICAL BISACRIDINES TO LUNG AND PROSTATE CANCER CELLS

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Nanotechnology-based drug delivery provides a promising area for improving the efficacy of cancer treatments. Nanoparticles (NPs), thanks to their unique properties, can be modified *e.g.* by conjugation with proteins or enzymes. Chemotherapeutics can be delivered with engineered NPs targeting surface proteins, which are overexpressed on the membrane of cancer cells. One of the most intensely studied classes of protein in drug delivery is the folic acid receptor, which has a high affinity for folic acid (FA) [1]. C-2028 is the representative of a new class of antitumor agents - unsymmetrical bisacridines (UAs), patented in Europe and the USA. These compounds, synthesized in our laboratory, are highly active against many experimental cellular and tumor models, including lung, colon, pancreatic, and prostate [2,3]. Here, we investigated the influence of using folic acid (FA) as a linker between quantum dots (QDs) and C-2028 (QDs-CD-FA-C-2028 conjugates) on cytotoxicity, cellular uptake, and mechanism of internalization in human lung H460 and prostate Du-145 and LNCaP cancer cells as well as in normal MRC-5 cells.

The cytotoxicity against tested cells was assessed by the MTT assay after 72 h of incubation. The cellular uptake and mechanism of internalization (using endocytosis inhibitors) following treatment with mentioned above compounds were performed using Confocal Laser Scanning Microscope (CLSM).

Conjugation of C-2028 with QDs or QDs-FA did not significantly change the cytotoxic activity of this compound. CLSM images indicated that the use of FA as a linker between C-2028 and QDs (QDs-CD-FA-C-2028 conjugates) significantly increased the amount of delivered compound (C-2028) to the cells, especially in the case of cancer Du-145 and H460 cells. The internalization of QDgreenCD-FA-C-2028 to cells of all lines was highly energy-dependent and was taken up by three endocytosis pathways: CME (clathrin-mediated endocytosis), CavME (caveolae-mediated endocytosis), and MP (macropinocytosis). However, the level of these endocytosis pathways was different depending on cell lines.

Summing up, conjugation of C-2028 with QDs-CD-FA did not change the cytotoxic activity of this compound. However, the amount of delivered compound to the cells significantly increased, especially in the case of Du-145 and H460 cells. QDgreenCD-FA-C-2028 was taken up by the cooperation of three endocytosis pathways: CME, CavME, and MP in all of the studied cell lines.

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[48] INTRAMUSCULAR, INTRAVENOUS OR CONTRALATERAL? WHICH IS THE BEST ROUTE OF ADIPOSE DERIVED STROMAL CELLS ADMINISTRATION IN MURINE MODEL OF HINDLIMB ISCHEMIA THERAPY?

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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 with the use of growth factors or cells that secrete proangiogenic factors which stimulate new blood vessels formation. Recently the most promising cells in the treatment of critical limb ischemia are mesenchymal stromal cells (MSCs). MSCs have been used to treat critical limb ischemia in preclinical and clinical trials but still, there is little known about their optimal delivery strategy.

The aim of the present work was to compare three different routes of human adipose derived stromal cells (ADSCs) administration in a murine model of hindlimb ischemia.

Unilateral femoral artery ligation was performed on males of C57BL/6NCrl mouse strain. ADSCs were administered (1) into gastrocnemius muscles, (2) intravenous and (3) contralateral (into contralateral limb, where unilateral femoral artery ligation was absent). Gastrocnemius muscles were excised, fixed in liquid nitrogen and stained using immunofluorescent antibodies. Microscopic observations were performed using a confocal microscope.

Immunofluorescent assessment of the gastrocnemius muscles extracted 14 days post injury (dpi) showed that after intramuscular administration of ADSCs twice more new blood vessels were observed compared to intravenous and contralateral administration groups. Arterioles diameter on the 7th dpi was significantly increased in the intramuscular group and control groups compared to the intravenous and the contralateral groups. On the 7th dpi also more muscle fibers and the greater muscle regeneration were observed when ADSCs were injected intramuscular into ischemic limb compared to control and others ADSCs groups. The highest area occupied by macrophages was observed in the group where ADSCs were administered intramuscular both on the 7th and the 14th days post injury.

Our results revealed that muscle regeneration, angiogenesis, arteriogenesis and proangiogenic M2 macrophage infiltration in murine model of hindlimb ischemia differ depending on ADSCs administration route. We demonstrate that intramuscular route (directly into ischemic limb) of ADSCs administration is more efficient in functional recovery after critical limb ischemia than intravenous or contralateral route.

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[49] IDENTIFICATION OF PARTNERS INTERACTING WITH N4BP1 PROTEIN AND DETERMINATION OF ITS CELLULAR FUNCTIONS AND LOCALIZATION

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Introduction: *Nedd4 Binding Protein 1* (N4BP1) consists of NYN (*N4BP1, YacP-like Nuclease domain*) domain with ribonucleolitic activity, that shares common protein fold with PIN (PilT N-terminus) domain found in Regnase-1, K-homology domain (KH domain), responsible for interaction with RNA, and so-called CoCUN (Cousin of CUBAN) domain, that binds ubiquitin residues. Studies proved that N4BP1 is an essential factor in many important cellular processes, such as immunological response, signal transduction to NFB transcription factor and degradation of viral mRNA species.

The aim of this study was to find optimal conditions for immunoprecipitation of N4BP1 complexes, identification of N4BP1 binding partners and determination of cellular localization of this protein in cells as well as its role.

Methods: For the purpose of this project a numerous plasmids coding different variants of N4BP1 including IgG1-Fc-tagged (IgG1 heavy chain fragment) and sfGFP-tagged variants were generated. These vectors were then used for the transfection of HEK293 cells in order to produce high amounts of tagged versions of N4BP1 that were pivotal for immunoprecipitation. After immunoprecipitation of N4BP1 complexes, the samples were analyzed using mass spectrometry in order to identify proteins interacting with N4BP1. The sfGFP-tagged variant of N4BP1 was also used to determinate the cellular localization of overexpressed N4BP1 using fluorescence microscopy.

Results: Immunoprecipitation of N4BP1 complexes followed by mass spectrometry showed numerous proteins interacting with N4BP1, although these interactions still require a Western Blot validation. Obtained data also allowed us to distinguish few non-coding RNA species that may be a part of N4BP1-based protein complexes. We have also shown, that N4BP1-sfGFP preferentially localizes in granular structures in cytoplasm. The amount of such granular structures increased after stimulation of cells with sodium arsenite to mimic cellular stress conditions. These results suggest the role of N4BP1 in processes taking place in cytoplasmic granules e.g. stress granules and processing bodies (P-bodies).

Conclusions: In summary, all of our observations shed a new light on the role of N4BP1 in cellular processes. Identified novel targets of N4BP1 suggest that this protein may be an important factor in sustaining a genomic integrity via the regulation of non-coding RNAs levels.

[50] THE IMPACT OF DGCR8 INHIBITION ON MICRORNA BIOGENESIS IN B-CELL LYMPHOMA

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MicroRNAs are a class of small, non-coding RNAs that inhibit gene expression at the post-transcriptional level. Differential miRNA profile is often observed in many types of cancer including B-cell lymphoma. miRNA biogenesis consists of several regulated steps including transcription of miRNA genes to long primary miRNA transcripts (pri-miRNAs) that are processed by Drosha-DGCR8 complex to precursor miRNAs (pre-miRNAs) and exported to the cytoplasm to be processed by Dicer to mature miRNAs. In this study, we aim to determine the effect of DGCR8 inhibition on miRNA biogenesis. We used two short hairpin RNAs, shDGCR8-1 and shDGCR8-2, in lentiviral vectors to inhibit DGCR8 expression in Hodgkin lymphoma L1236 cells and Burkitt lymphoma ST486 cells. We showed that the levels of both *DGCR8* transcript and DGCR8 protein were significantly decreased upon shDGCR8-1 and shDGCR8-2 compared to levels upon scrambled negative control (SCR). Interestingly, DGCR8 inhibition caused death of L1236 cells as the percentage of living cells determined by flow cytometry decreased 3 folds for shDGCR8-1 and 1,5 fold for shDGCR8-2 within 10 days. Moreover, DGCR8 inhibition prevented biogenesis of several miRNAs, including miR-155 and miR-19b. The levels of mature miR-155 and miR-19b decreased, whereas the levels of the pri-miR-155 and pri-miR-17~92 (pri-miR-19b) increased upon shDGCR8 compared to SCR introduction to L1236 cells. Interestingly, not all miRNAs were equally affected by DGCR8 inhibition, since the levels of both miR-146a and pri-miR-146a were not altered compared to SCR. Further studies that involve analysis of additional miRNAs and pri-miRNAs are ongoing.

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[51] IDENTIFICATION OF IRRADIATION-INDUCED ATM-DEPENDENT LNCRNAS

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Keywords: lncRNAs, ATM, DNA damage response, DNA double-strand breaks

DNA damage response (DDR) is a complex process, essential for cell survival. Especially deleterious type 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 signaling 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. To further prove that ATM is involved in regulation of selected lncRNAs we utilized a specific ATM inhibitor (KU-60019). Functional analysis were performed on cells from healthy donors with knockdown of selected lncRNAs.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. 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. The primary study indicated that detected lncRNAs play essential role in DDR.

In conclusion, we identified lncRNAs induced in response to DNA damage in an ATM-dependent manner.

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[52] THE USEFULNESS OF MURINE MESENCHYMAL CELLS AS A MYXOMA VIRUS CARRIER FOR THE PLANNED THERAPY OF MURINE GLIOBLASTOMA

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Introduction: Glioma (glioblastoma) is a malignant tumor with a high remission rate owing to the presence of the so-called brain tumor initiating cells (BTIC). Due to lesion localization it is very difficult to treat. A promising therapeutic perspective in the development of novel targeted anti-cancer therapies is offered by oncolytic viruses (OV) which possess great potential to treat malignancies due to their dual anti-cancer effects - oncolytic and immune-stimulating. To enhance virus-mediated apoptosis of BTIC we used a recombinant myxoma construct Myx-M011L-KO which bears M011L gene knockout. Systemic drug administration appears a justified approach in the treatment of metastatic tumors such as glioma. Since effective systemic delivery of OV benefits from the use of protective carriers which can secure against premature destruction of the therapeutic cargo in this study we examined mesenchymal stem cells derived from mouse adipose tissue (mADSCs) as a prospective carrier for myxoma therapeutic viral construct in the planned treatment of experimental murine glioma.

Methods: mADSCs were isolated from mouse subcutaneous adipose tissue, and their phenotype was confirmed by flow cytometry. We evaluated M011L knockout virus (vMyx-M011L-KO) versus wild-type MYXV (vMyx-WT) in terms of infectiveness and single-step growth curve in mouse (GL-261) as well as human (LN18 and U-251MG) glioma cell lines as well as mADSCs carrier cells. Cytotoxicity of both myxoma virus variants towards the cell lines studied was determined using MTS viability test.

Results and conclusion: We confirmed correct morphology and immunophenotype of the isolated murine ADSCs. In the course of *in vitro* experiments, permissiveness was demonstrated for the cell lines examined infected with either the therapeutic myxoma construct vMyx-M011-KO or vMyx-WT. Both used myxoma viruses killed, to a large extent, GL-261 murine glioma cells and, to a lesser degree, the human glioma LN-18 and U-251 MG cell lines. Unfortunately, lack of robust viral replication, as well as the low survival rate of infected murine ADSC cells eliminates them as a potential carrier of the targeted therapeutic construct. In the planned therapy of murine experimental glioblastoma we will replace mADSCs with weakly immunogenic adipose-derived or bone marrow-derived human mesenchymal stem cells (ADSCs and BM-MSCs, respectively).

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[53] GLUTATHIONE CONJUGATION OF THE ANTITUMOR-ACTIVE UNSYMMETRICAL BISACRIDINE C-2028 IN HUMAN LIVER SUBCELLULAR FRACTIONS – THE MAJOR ROLE OF GLUTATHIONE S-TRANSFERASE M1-1

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Introduction: Unsymmetrical bisacridine derivatives (UAs) are a novel class of antitumor-active agents of extraordinary structures, patented in Europe (EP 3070078A1) and the USA (US 10202349B2), that may be useful in the treatment of tumors that are usually resistant to therapy [1]. Glutathione S-transferases (GSTs) participate, through direct detoxification, in the development of tumor cell resistance against chemotherapeutic agents as their increased expression has been found in various types of tumors [2]. Thus, the affinity determination of a specific GST to a potential antitumor drug may reduce the phenomenon of drug resistance of neoplastic cells to the extent that allows effective therapy.

Objective: This work aimed to estimate the selectivity of various human GSTs towards a representative UA, compound C-2028.

Methods: To determine GST-mediated GSH conjugation of C-2028, *in vitro* experiments were conducted with human liver subcellular fractions (microsomes and cytosol) as well as using three selected recombinant human GST isoforms (*i.e.*, hGSTA1-1, hGSTM1-1, and hGSTP1-1). The chemical structures of the generated metabolites were identified by liquid chromatography/mass spectrometry.

Results: We demonstrated that C-2028 was extensively metabolized to one GSH monoconjugate mainly by hGSTM1-1 a GST isoform implicated in colorectal and breast tumors [3-4]. Metabolite formation rate was linear with respect to both time (up to 120 min) and enzyme concentration (0.01 0.1 mg/mL) while changing the pH of the reaction buffer from 6.5 to 7.4 had no effect on the progress of the reaction. No GSH S-conjugates were observed when using human liver microsomes and cytosol.

Conclusions: Our results indicated the importance of GSH conjugation of the compound in tumor cells exhibiting the high GSTM1-1 activity level. In a clinical aspect, this may strongly affect the patients drug bioavailability. On the other hand, GST-mediated depletion of cellular GSH may increase tumor cell exposure to the putative reactive products of the UA metabolic transformations. Consequently, future works should include detailed measurements of the biological activity of the obtained GSH species in cellular detoxification pathways. (project Mini-grant R/D subsidy 2021, Faculty of Chemistry, Gdańsk University of Technology).

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[54] ANALYZING THE LINC00116/MITOREGULIN FUNCTIONS IN B-CELL LYMPHOMA

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Long noncoding RNAs (lncRNAs) are defined as RNAs longer than 200 nucleotides that are not translated into proteins. However, several lncRNAs were shown to encode for peptides with a biologically significant functions. LncRNA deregulation is related to the pathogenesis of lymphomas. LINC00116 is a lncRNA shown to be upregulated in B-cell lymphoma compared to normal B-cells. Recently, LINC00116 was shown to encode a peptide called Mitoregulin that was localized in the inner mitochondrial membrane. In this study, we focus on the function of LINC00116 and Mitoregulin in Hodgkin lymphoma KHM2 cells. Therefore, we inhibited LINC00116 using two short hairpin RNA, shRNA-1 and shRNA-2 in lentiviral vectors. We showed that both LINC00116 levels and Mitoregulin protein levels were decreased upon shRNA-1 and shRNA-2 compared to negative (NT) and scrambled (SCR) controls. Additionally, we analyzed cell cycle distribution and reactive oxygen species (ROS) production by flow cytometry, yet we did not observe any significant changes upon shRNA-1 and shRNA-2. In conclusion, we showed that Mitoregulin protein is expressed from LINC00116 in KMH2 cells. Further functional studies needed to determine LINC00116 and Mitoregulin functions in lymphoma are now ongoing.

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[55] ANALYSIS OF THE APPLICABILITY OF ANTIBODIES RECOGNIZING EGFRvIII IN THE CASE OF PRIMARY GLIOBLASTOMA CELL LINES

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Introduction Glioblastoma (GB) is considered one of the most deadly tumors. The median survival of patients with GB is 12-14 months from diagnosis and despite the subsequent years of research, the life expectancy of patients does not extend. Amplification of the epidermal growth factor receptor (EGFR) is detected in approximately 50% of GB patients. Overexpression or amplification of EGFR in 35-60% of cases is accompanied by expression of a mutant oncogenic receptor, termed EGFRvIII. Due to its presence only in neoplastic cells, EGFRvIII seems to be an ideal target in anti-cancer targeted therapy. One of such therapies is CAR-T. In order to classify GB patients to anti-EGFRvIII therapy and to monitor its effects, patients stratification should be unquestionable. In our research, we verified whether EGFRvIII specific L8A4 antibody can serve such diagnostic tool, especially in immunohistopathological examination. GB cells with and without expression of this gene confirmed with other methods were analyzed.

Methods Analyzes were performed on primary glioblastoma cell cultures that were derived from 20 tissue samples obtained from patients during neurosurgical resection and corresponding formalin-fixed and paraffin-embedded tissue (FFPE) sections. Real-time reverse transcription PCR and MLPA analyzes were performed to evaluate EGFRvIII mRNA gene expression and gene copy number, respectively. The L8A4 antibody detecting EGFRvIII protein was used during immunocytochemical and immunohistochemical analyzes.

Results Tissue sections from 20 patients were selected for the study, EGFRvIII expression was found during the real-time PCR analysis in a total of 13 sections (high EGFRvIII expression detected in 6 and low EGFRvIII expression detected in 7 sections), which was also confirmed at DNA level in the MLPA analysis. Further, immunocytochemical analysis of both cells with confirmed real-time PCR expression of EGFRvIII and negative for expression of this gene, indicated that the L8A4 antibody bound to cells with expression of EGFRvIII confirmed in real-time PCR. Finally, during immunohistochemical analysis, non-specific binding of L8A4 antibody to EGFR negative sections was observed, which was a false positive result. On the basis of the analysis, it would be impossible to determine whether the cells in the analyzed preparations express EGFRvIII.

Conclusions Study indicated discrepancies in EGFRvIII detection in samples derived from the same patient, depending on the selected method of analysis. EGFRvIII is a specific antigen of GB cells, but its analysis with the use of antibodies still remains questionable. Despite the existence of commercially available antibodies capable of detecting EGFRvIII, the specificity of these scientific/diagnostic tools is yet not sufficient. Also analysis of previously published data with the use of this antibody suggested nonspecific results.

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[56] HPV-POSITIVE HEAD AND NECK SQUAMOUS CELL CARCINOMA CELLS RELEASE EXOSOMES CARRYING HPV PEPTIDES.

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Background. Human papillomavirus (HPV) is an etiologic factor in head and neck squamous cell carcinoma (HNSCC). In comparison to HPV(-) tumors, HPV(+) tumors respond favorably to therapy, mediate more effective anti-tumor immune responses, and ultimately have better prognosis. We previously demonstrated that HNSCC cell lines release small extracellular vesicles (sEVs), which are also referred to as tumor-derived exosomes (TEX). TEX carry tumor-associated antigens (TAAs) as well as various immunoinhibitory and immunostimulatory proteins and modulate anti-tumor immune responses *in vitro* and *in vivo* in orthotopic oral carcinoma mouse models. TEX from HPV(+) or HPV(-) cancers are characterized by distinct mRNA and miRNA cargo components and only TEX from HPV(+) HNSCC cells carry oncogenic proteins and mRNA encoding the E6/E7 transcripts.

We hypothesized that TEX from HPV(+) or HPV(-) HNSCC cells show a distinct modulation of anti-tumor immune responses, which is in part mediated by the presence of viral antigens in HPV(+) TEX.

Methods. TEX were isolated from supernatants of HPV(+) cell lines (UMSCC-2 and UMSCC-47) or HPV(-) cell lines (PCI-13 and PCI-30) by size exclusion chromatography (SEC). Vesicles recovered in fraction #4 were characterized as sEVs according to the ISEV criteria. sEVs underwent proteomic analysis using a modified FASP protocol [1,2]. Each sample was divided into two peptide fractions eluted at pH 5 and 2, and analyzed individually using UltiMate 3000 RSLC nano-LC System connected to Q Exactive Orbitrap mass spectrometer. Data analysis of human sEV proteins identified numerous differences in the proteome of HPV(+) vs. HPV(-) TEX [3]. The raw data was examined for the presence of viral peptides. For this propose, protein identification was performed using Swiss-Prot viral database with a precision tolerance 10 ppm for peptide masses and 0.02 Da for fragment ion masses.

Results. The sEV proteome of TEX produced by HPV(+) HNSCC cells contained three viral peptides (IKGFEFPHPFPMKPDNTPQFQLTDQSWKSFFER, EIANAAKAIK, VEGDTLADR) deriving from three different HPV proteins: Minor capsid protein L2, Probable protein E5 and Replication protein E1. Interestingly, recent reports indicate that E2/E5 viral proteins and not E7/E6 are immunogenic and induce antigen-specific T cell responses in HPV(+) individuals.

Conclusions. For the first time HPV proteins have been detected in TEX produced by HPV(+) tumor cells. The presence of immunogenic viral proteins in TEX together with TAAs suggests that TEX may mediate anti-viral and anti-tumor immune responses in HPV(+) HNSCC patients.

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[57] ANTICANCER EFFECT OF VASCULAR DISRUPTING AGENT (CA4P) IN COMBINATION WITH STING AGONIST (CGAMP) IN MURINE BREAST CANCER AND MELANOMA 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 and B16-F10 melanoma models. In the study, CA4P (vascular disrupting agents) was combined with 23-cGAMP (STING agonist) 23-cGAMP was administered one day prior to CA4P administration. CA4P was administered intraperitoneally in a dose 50 mg/kg mice body weight. 23-cGAMP was administered intratumorally in a dose 5 g/mice.

Tumor growth inhibition was observed after combination therapy. The highest number of CD8 cytotoxic T lymphocytes was observed in tumors after 23-cGAMP monotherapy and in combination therapy. However, no difference was observed between these groups. The highest number of natural killer cells (NK) was observed in tumors after combination therapy. Additionally, there was observed decrease in tumor blood vessel after CA4P monotherapy. We have also observed the changes in M1 and M2 macrophages populations after conducted therapies.

The proposed therapeutic strategy where CA4P was combined with 23-cGAMP has a greater effect than either alone.

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[58] ANALYSIS OF MIRNAS IN SERUM AND SERUM-DERIVED EXOSOMES – PUTATIVE BIOMARKERS FOR EARLY DETECTION OF CANCER IN PARTICIPANTS OF LUNG CANCER SCREENING STUDY

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Introduction Circulating microRNA is a promising source of molecular biomarkers that could support the early detection of lung cancer in screening programs based on low-dose computed tomography. Although several miRNA signatures of lung cancer were proposed based either on the whole serum or serum-derived exosomes, a direct comparison of both specimens collected from high-risk smokers was not performed yet. The study aimed to assess the level of 12 selected miRNAs present in previously proposed lung cancer signatures in the whole serum and serum-derived exosomes from participants of lung cancer screening.

Methods Exosomes were isolated using mini-size exclusion chromatography (mini-SEC) from the serum of participants of the MOLTEST-BIS screening study organized by the Medical University of Gdańsk in 2016-2018; exosomes were characterized and confirmed by Western blot, dynamic light scattering, and transmission electron microscopy. Three groups of participants were included: with no changes in the lung, with benign lung nodules, and with lung cancer. The level of 12 miRNA species (namely: hsa-miR-103a-3p, hsa-miR-122-5p, hsa-miR-142-5p, hsa-miR-144-3p, hsa-miR-148a-3p, hsa-miR-148b-3p, hsa-miR-15b-5p, hsa-miR-21-5p, hsa-miR-23a-3p, hsa-miR-23b-3p, hsa-miR-30c-5p, hsa-miR-425-5p) was analyzed by quantitative RT-PCR using the miRCURY LNA approach in the whole serum and serum-derived exosomes.

Results The level of two oncomirs, miR-21-5p and miR-30-5p, correlated with the stage of cancer in a group of patients. However, no significant differences in the level of analyzed miRNA species were detected in exosomes among three compared groups of screening participants (n=3x50). Moreover, hypothetical differences between patients with screen-detected cancer (n=36) and individuals with no lung nodules (n=49) were compared using RNA purified from the whole serum and serum-derived exosomes. Some differences between groups (small effect size) were observed in the case of miR-23b-3p, miR-122-5p, and miR-142-5p. However, only miR-23b-3p showed a similar pattern of changes in both types of specimens (slightly reduced level in cancer patients).

Conclusions In the material collected from participants of the lung cancer screening study, no significant differences between participants with no malignancy and patients with screen-detected cancer were observed if miRNA species present in previously proposed signatures of lung cancer were analyzed in either whole serum specimens or serum-derived exosomes. This suggested that miRNA panels based on clinically-diagnosed lung cancers (compared to a generally healthy population) should be re-evaluated when aimed to be used in the context of lung cancer screening. Also, we failed to reveal any advantage of using exosome miRNA instead of total serum miRNA as a biomarker of early lung cancer.

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[59] p53-DEPLETION ENHANCES COLON CANCER CELLS' RESISTANCE TO MANUMYCIN A

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Manumycin A (MA) is a well-tolerated natural antibiotic synthesized by *Streptomyces parvulus* showing pleiotropic anticancer effects in various preclinical *in vitro* and *in vivo* models. Previously MA was well-known as a selective and competitive inhibitor of Ras farnesyltransferase, however available evidence shows that MA has much wider pleiotropic effect on the activity of other transcription factors. In our previous study, we have shown that MA induces HSF1 phosphorylation in lung and breast cancer cells and its anticancer effects can be counteracted by heat shock proteins belonging to the HSPA (HSP70) family. Manumycin polyketides, as a molecular glue can induce interactions between proteins that typically do not interact, it was found that one of them is p53. MA can also affect the cell cycle distribution. Bearing in mind that cell-cycle arrest is one of the most prominent outcomes of p53 activation we decided to evaluate the potential influence of p53 on the response of cancer cells to MA.

We found that MA caused cell type-specific alterations in the total levels of p53. MA treatment resulted in overproduction of wild-type p53 in A549 and MCF7 cell lines but not of p53 mutant in NCI-H23 cells. Therefore, we decided to study if MA treatment efficiency depends on the levels of p53wt in cancer cells.

To evaluate the influence of wild-type p53 on cells response to MA we used three cancer cell lines: A549 (lung), HCT116, and RKO (colon). Using RNAi-mediated technology we stably downregulated the *TP53* gene expression in A549 cells. Using MTS assay we found that p53 knockdown did not change the sensitivity of A549 cells to MA. Our other models were colon cancer cell lines, namely HCT 116 p53-/- with deleted *TP53* gene or RKO E6 cells that overexpress E6 oncoprotein (this modification results in p53 degradation). Results of the MTS assay indicate that p53-deficient colon cancer cells were more resistant to MA than parental cell lines expressing wild-type p53.

Our results suggest that MA may activate the p53wt-dependent pathways, nevertheless the final outcome is cell type-dependent, e.g. depletion of p53 in p53wt colon (HCT116, RKO) but not lung (A549) cancer cells can abolish the anticancer effect of MA.

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[60] ANALYSIS AND MODELING OF BIOLOGICAL PROCESSES WITH THE USE OF THE MICRO-FLOW SYSTEM

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Cancer, as a disease, has an enormous impact on society all over the world. Therefore, its development is the subject of numerous scientific studies. Understanding this issue has a great influence on the optimization of the treatment process. Unfortunately, extracorporeal research involves the cultivation of tumor cells under completely different conditions than in the hosts organism. Hence, the aim is to provide a near-real cell culture environment, which is one that can be ensured by a microreactor. Its main premise was the use of a hydropneumatic system to control the microcircuit by manipulating the channels supplying fluids to the reaction chamber. The functional principle of this solution was to produce a microchip. The first channel was used for liquid flow. Its regulation was possible owing to the overpressure created by the air channel. In our project, a microchip created from a PDMS gel was used as part of the environment for cell growth. Carefully prepared HELA tumor cells with fluorescently labelled histone H2B were introduced into the system to assess their cell cycle length and to record monitoring of the movements of individual daughter cells. Cells from the research sample were treated with doxorubicin. During the experiment observations were made using a fluorescence microscope with automatic image acquisition software. The resulting images were analysed using an algorithm which was created in Matlab.

[61] ANALYSIS OF SELECTED GENES ABNORMAL PROMOTER METHYLATION IN EGFRvIII GLIOBLASTOMA CELL LINES

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Introduction: Epidermal growth factor receptor (EGFR) is considered as an important therapeutic target in glioblastoma (GB). *EGFRvIII* mutation accompanies half of the *EGFR* amplified glioblastomas and occurs in 28% of GB patients. Although *EGFRvIII* undergoes amplification, it may not result in increased protein or mRNA expression but may be associated with the fact that extrachromosomal amplicons possibly enable flexible regulation of gene expression. One of the hypotheses states that EGFRvIII gradually leads to epigenetic reprogramming of cells. Since CAR-T therapy may have a great chance to be effective in patients with EGFRvIII positive tumors, it is especially important to investigate the biology of this mutated protein. To answer the question how EGFRvIII may contribute to epigenetic reprogramming of cancer cells, profiling of DNA methylation should be conducted. Here, we studied GB methylation (DNA methylation level and expression of selected genes encoding demethylase and methyltransferase enzymes) in DK-MG glioblastoma sublines. Analyses were conducted on a model of four DK-MG sublines, significantly differing in *EGFRvIII* expression (very high, high, low, and very low) from extrachromosomal amplicons as well as aggressive cancer phenotype or lack thereof. Importantly, the introduction of *EGFRvIII* cDNA with genetic engineering method into DK-MG low subline did not regain an aggressive phenotype of DK-MG high cells, which further emphasizes the need to study the mechanism of EGFRvIII action.

Methods: To investigate the biology of EGFRvIII mutated protein, the methylation-specific PCR (MSP) and real-time MSP were applied. A methylation curve was used to estimate the percentage of methylation in MSP and real-time MSP. Expression of mRNAs encoding selected demethylases and methyltransferases as well as *EGFRvIII* were analyzed by qPCR.

Results: We found complete methylation of promoter region CpG islands of the *RASSF1A*, *RUNX*, and *MGMT* genes and no detectable methylation in the *PTEN*, *VHL*, *RB1*, *GATA6*, *CASP8*, and *TIMP3* genes. Interestingly, we observed partial methylation of the *EGFR* and *CD133* gene promoters. These results correlated to some extent with the expression of *EGFRvIII in analyzed sublines, with the lowest methylation in DK-MG high cell line*. The data presented in the demethylases/methyltransferases expression studies do not clearly illustrate whether they play a crucial role in the methylation states controlling EGFRvIII expression status.

Conclusions: Here, we demonstrate that the cancer-specific DNA hypermethylation in the promoter of *CD133* and *EGFR* genes may occur in EGFRvIII-positive glioblastoma. Since the difference in the DNA methylation of these genes between individual DK-MG sublines (differing in aggressive phenotype) was demonstrated, methylation may partially be responsible for flexible regulation of these genes expression.

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[62] ECTOSOMAL TRANSFER OF AVB3 AND AVB5 INTEGRINS FROM MELANOMA CELLS TO RECIPIENT VASCULAR ENDOTHELIAL CELLS PROMOTES THEIR MIGRATION

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Introduction/Rationale Tumor-derived ectosomes enhance proangiogenic phenotype of endothelial cells, what might be mediated by intercellular transfer of v3 and v5 integrins *via* ectosomes. The aim of this research was to evaluate whether v3 and v5 integrin-bearing ectosomes released by melanoma cells could act a potential proangiogenic factor during cancer progression.

Methods Ectosomes were isolated from conditioned media from primary WM115 and metastatic WM266-4 melanoma cell cultures. Media was concentrated by low-vacuum filtration dialysis (1000 kDa cut-off membrane) followed by differential centrifugation at 18 000 x g to obtain ectosome pellet. Melanoma cells and isolated ectosomes were analysed for v3 and v5 integrin expression by Western Blot. Furthermore, 60 g of WM115- or WM266-4-derived ectosomal protein was added to confluent HUVEC cells. After 18 of incubation changes in total and surface expression of v3 and v5 integrins in HUVECs were assessed by Western Blot and flow cytometry. Finally, migratory properties of HUVECs were analyzed in wound healing assay after incubation with ectosomes with or without the presence of anti-v3 and anti-v5 integrin antibodies or RGD motif-containing peptides that blocked activation of v3 integrin (Echistatin) or both v3 and v5 integrins (Cilengitide).

Results: Western Blot revealed higher expression of v3 integrin in WM266-4 cells and WM266-4-derived ectosomes *versus* WM115 cells and WM115-derived ectosomes, whereas v5 integrin expression was higher in WM115 cells and WM115-derived ectosomes. Both total and surface expression of v3 integrin was increased in HUVECs after incubation with WM266-4-derived ectosomes but not with WM115-derived ectosomes. An increase in total v5 integrin expression was observed only after incubation with WM115-derived ectosomes, while the surface expression of this receptor remained unchanged despite HUVECs being treated with ectosomes. In wound healing assay incubation of HUVECs with WM115- or WM266-4-derived ectosomes caused 1,6 and 2,3-fold increase of the wound closure rate, respectively. However, migration of HUVECs was significantly reduced (approx. 20-35%) when Cilengitide, Echistatin, anti-v3 or anti-v5 integrin antibodies were used for incubation in addition to ectosomes. It suggests that v3 and v5 integrins transferred by ectosomes can act synergistically with v3 and v5 integrins expressed by HUVECs.

Conclusions/Novel aspect: Melanoma cells are able to propagate migratory phenotype of HUVECs by transferring v3 and v5 integrins *via* ectosomes. Functional effect exerted by ectosomal integrins on recipient endothelial cells points to their clinical relevance in development of novel, integrin-targeted antiangiogenic therapies for melanoma.

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[63] IMMUNOHISTOCHEMICALLY DETECTED LOX EXPRESSION IS HIGHER IN METASTASES THAN IN PRIMARY TUMORS IN OVARIAN CANCER.

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Introduction/ Background*: In our previous microarray study we identified a set of genes (multigene signature) related to different survival of patients with high-grade serous ovarian cancer (HG-SOC) [1]. One of the genes from this signature was LOX, coding for lysyl oxidase. LOX is engaged in the cross-linking of the extracellular matrix proteins. Various studies have indicated that LOX may act as either an oncogene or a tumor suppressor, depending on the type of tissue/tumor. As our previous study indicated that higher LOX mRNA level was negatively correlated with survival of ovarian cancer patients, we aimed to check whether LOX level determined by immunohistochemistry shows the same dependency and could be useful in clinical practice.

Methodology: Two commercially available anti-LOX antibodies (ab174316-Abcam, NB-2530-Novus Biologicals) were validated by western blotting, using protein extracts from eight ovarian cancer cell lines and recombinant LOX protein as a control (NBP-59887-Novus Biologicals). Then, immunohistochemical analysis was performed (tissue samples of HG-SOC) and on tissue arrays containing spectrum of different FIGO stages, histological types, etc. (US Biomax). The results were analyzed using Statistica (version-13.1, StatSoft Poland).

Result(s)*: Only one antibody (ab174316-Abcam) was positively validated as specific toward LOX and it was used for further analysis. However, Kaplan-Meier analysis showed no correlation between LOX protein level and the patients survival time. Further analysis revealed that LOX level was correlated with primary/metastatic tumor difference: higher stromal LOX expression occurred eight times more often in metastatic than in primary tumors. When LOX expression was assessed in cancer cells, there was no such correlation. In addition, detailed inspection of tissue slides revealed that LOX is strongly expressed in nerve ganglions in normal ovary and tumor adjacent normal tissue.

Conclusion*: Although our previous observations indicated that higher *LOX* mRNA level was related to shorter survival of ovarian cancer patients, protein level of LOX does not demonstrate prognostic value in the analyzed group of patients with HG-SOC. However, we observed significantly higher LOX expression in the stroma of metastases compared to that of primary tumors. This observation is consistent with the assumption that LOX is associated with a more aggressive tumor phenotype. Intense staining of ganglions with anti-LOX antibody can have diagnostic value and will be further investigated.

[64] MCPIP1 IN SKIN TUMORIGENESIS

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Introduction: Skin cancer is the most frequent form of malignancy. There are three major types of skin cancers: basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma, of which the first two are grouped together as non-melanoma skin cancers. Actinic Keratosis (AK) is the most common precursor of SCC. MCPIP1, encoded by the *Zc3h12a* gene, possesses a PIN domain that has RNase properties and regulates stability of transcripts coding for inflammatory cytokines (e.g. IL-6, IL-12p40) and transcription factors (e.g. c-Rel, C/EBP). MCPIP1 functions as an essential regulator of inflammation, but also plays a critical role in cell differentiation, proliferation and cancer development. Importantly, MCPIP1 localizes predominantly in the differentiated suprabasal layers in normal human epidermis. Our recent study showed that keratinocyte-specific Mcpip1-deficient (Mcpip1eKO) mice develop spontaneous skin pathology and systemic inflammation.

Methods: We analysed distribution of MCPIP1 in skin biopsies of patients with actinic keratoses (AKs) and SCCs by immunohistological staining. To deepen our knowledge on the role of MCPIP1 in skin pathophysiology, we utilized a well characterised DMBA/TPA approach to induce skin carcinogenesis in mice with keratinocyte- and myeloid-specific ablation of Mcpip1. Then, in order to characterize the phenotype of the obtained tumors, we performed histological and immunostaining studies, NGS analysis and real-time PCR.

Results: Our immunohistological analysis showed that while in AK the expression of MCPIP1 was high, in fully developed SCC the overall expression was low and mostly limited to keratin pearls, the most characteristic feature of invasive SCC tumors. Next, our *in vivo* studies showed that keratinocyte-specific Mcpip1-deficient (Mcpip1eKO) mice developed tumors faster and then with increased quantity, compared to the control mice. At the histological level, we observed more aggressive tumors. This was indicated by abundant keratinization, infiltration of tumors cells into dermis and the presence of keratin pearls. This was also correlated with enhanced expression level of genes encoding squamous cell carcinoma antigens. On the other hand, our preliminary studies showed than none of mice with myeloid-specific Mcpip1 deficiency (Mcpip1mKO) developed SCC-like tumors. However, those mice developed numerous melanocytic nevus, indicating the possible early onset of melanoma development.

Conclusions: Based on our data, we hypothesize that MCPIP1 functions as a significant factor in the development and progression of both non-melanoma and melanoma skin cancer.

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[65] 89Zr-DFO-Atezolizumab for measuring PD-L1 expression level in glioblastoma (GBM)

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Introduction: Despite rigorous therapeutic regimens patients with glioblastoma (GBM) eventually relapse. Recently, immune checkpoint inhibitors (ICPIs) targeting programmed cell death 1 receptor (PD-1) and its ligand (PD-L1) have been approved for the treatment of multiple tumour types. Regrettably, for GBM patients only modest and unpredictable responses have been reported so far. This is most likely due to a relatively immune-depleted (cold) GBM microenvironment characterised by high level of immunosuppressive cytokines which inhibit T cells activity. There are still no reliable biomarkers to accurately predict ICPIs therapy response; and the PD-L1 expression in tumours is mostly evaluated via immunohistochemistry (IHC) using a snapshot of tissue dissected post-operatively or during biopsy. **Therefore, we radiolabelled atezolizumab, the anti-PD-L1 mAb, with zirconium-89 to quantify dynamic changes in PD-L1 expression which might help to select patients for the treatment with ICPIs.**

Methods: Atezolizumab was radiolabelled with 89Zr (*t1/2* = 78.4 h), and desferrioxamine (DFO) was used as the chelator for the radiometal. Since atezolizumab cross-reacts with murine PD-L1, the pharmacokinetics studies performed on mice are considered representative of the clinical setting. 89Zr-DFO-Atezolizumab was prepared in house following a method published by Vosjan et al. in 2010 (1). The PD-L1 expression level was measured by flow cytometry (FC). The specificity assay of the radioimmunoconjugate binding was performed using murine (BL6-NPE-GFP) and human (U87-MGvIII, U87-MG) GBM cell lines (4oC, 1 h) w/o stimulation with IFN. Mice bearing BL6-NPE-GFP brain tumours were injected with 89Zr-DFO-Atezoizumab (co-injection: 10 g labelled + 210 g non-radiolabelled atezolizumab) and imaged 72 h post-injection.

Results: The 89Zr-DFO-Atezolizumab was obtained with a 98% radiochemical purity and specific activity of 10-2 MBq/g (2.7*10-4 mCi/g). All studied cell lines demonstrated specific, but relatively low radioimmunoconjugate binding. The measured cell-associated radioactivity was in agreement with the PD-L1 expression level measured by FC. There was a marked increase in 89Zr-DFO-atezolizumab binding after 24 h treatment with proinflammatory cytokine IFN. Pre-incubating the cells with 100-fold molar excess of non-radiolabelled atezolizumab significantly reduced the radiotracer binding. PET/CT images showed preferential and focal accumulation of the radiotracer in the tumour mass 72 h post-injection.

Conclusion: Our data shows that 89Zr-DFO-Atezolizumab targets with high specificity PD-L1 positive human and murine GBMs providing real time information about the protein expression level.

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[66] AMIDES AND THIOAMIDES AS PROSPECT DRUG MOLECULES

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The significant development of organic chemistry and chemoinformatics have contributed considerably to the introduction of many new drugs in pharmacy and medicine. As a matter of fact, the amides and thioamides are the group of drugs that are widely implemented in the pharmacotherapy due to their antibacterial, antifungal or anticancer properties. Hence, a novel set of amides and thioamides of ethyl esters and methyl amino acids was synthesised and analysed as well. On the whole, the amino acids can be valuable precursors to generate compounds with potential/desired biological activity; therefore some amino acids were selected as the substrates for producing hydrochlorides of methyl and ethyl esters. The obtained subproducts were subsequently applied in the generation of the amides of the chosen amino acids as well as in the final synthesis of thioamides [1].

Moreover, the generated amides and thioamides of methyl and ethyl esters of the selected amino acids were tested *in vitro* on murine melanoma tumor line. The widely known cell culture methods, including incubation and passage of cells and measurements of the degree of cytotoxicity of formulation were employed (the Alamar Blue assay). Evaluation of tumor cell survival was performed as a function of concentration and length of exposure to the tested formulations. Fluorescence signals were recorded at excitation wavelength at 560 nm and emission wavelength at 590 nm.

The Alamar Blue assay is an important redox indicator that is usually engaged in order to evaluate metabolic function and cellular health, apoptosis, cell cycle function and control. Resazurin is the active substance, which is stable in culture medium and water-soluble. The dye acts as an intermediate electron acceptor in the electron transport chain without interference of the normal transfer of electrons. Thus, the dye indicator changes from the oxidized, non-fluorescent, blue state to the reduced, fluorescent, pink state. The observed transition allows to measure quantitatively (by colorimetric and fluorometric readings) or qualitatively (by a visible change of color) the presence or absence of viable cells [2].

The Alamar Blue assay ensures precise time-course measurements due to its high sensitivity and linearity. What is more, the test is non-radioactive, non-toxic, safe for the user and eco-friendly to the environment; therefore it can be used with different cell models. Unfortunately, *in vitro* bioassays may not approach effectively highly *in vivo* effects, hence, the further development of other multiple parameters is needed [3].

The expected, positive findings of the feasible studies in destroying melanoma cells would provide basis for further investigations on more effective melanoma treatment, a type of cancer that is resistant to chemotherapy so far.

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[67] IgG N-GLYCOSYLATION REFLECTS THE SEVERITY OF INFLAMMATION IN HASHIMOTO'S THYROIDITIS

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Introduction: Class G antibodies (IgG) are the most abundant glycoproteins in human blood serum, whose structure, half-life and effector functions are modulated by the attachment of various sugar forms called *N*-glycans. The composition of the IgG glycome is influenced by both genes and environmental factors, making it reliable biomarker of overall human health. Regulation of IgG *N*-glycosylation is a part of homeostasis but also can be a reflection of its disruption. Changes in IgG *N*-oligosaccharide patterns have been implicated in remission and disease progression, representing both predisposition and functional mechanisms involved in illness pathogenesis, including autoimmunity. One of the most common autoimmune diseases is Hashimoto thyroiditis (HT), which is the most frequent cause of hypothyroidism.

The aim of our study was to evaluate changes in IgG *N*-glycosylation in donors with the elevated autoantibody (anti-thyroglobulin, anti-Tg and/or anti-thyroid peroxidase, anty-TPO) levels without symptoms of hypothyroidism (n=35), and patients with HT during L-thyroxine therapy (n=38) (study groups) compared to healthy donors (control group; n=41).

Methods: IgG was isolated from human blood serum by affinity chromatography with protein G. IgG *N*-glycans were then released by digestion with PNGase F (*N*-glycosidase F), fluorescently labeled with 2-aminobenzamide (2-AB), and analyzed by ultraperformance liquid chromatography combined with mass spectrometry (UPLC-MS). The resulting chromatograms were integrated into 25 peaks to which glycans were assigned based on the glucose unit values (GU) and mass-to-charge ratio (m/z) of the detected ions, using the Waters UNIFI scientific information system. Statistical analysis was performed using the Kruskal-Wallis test in Origin 2021b software (Origin Lab). Statistically significant differences between groups were determined at p0.05.

Results: The study showed a statistically significant increase in the amount of the two-antennary complex-type *N*-glycans mono- and disialylated with a bisecting *N*-acetylglucosamine (GlcNAc), and core fucose on IgG from HT patients compared to the donors showing the elevated levels of anti-Tg and/or anti-TPO without hypothyroidism and healthy donors.

Conclusions: IgG *N*-glycosylation is essential for antibody function in inflammatory reactions aimed at elimination of pathogens or own antigens in autoimmune processes. We have shown that the structure of IgG sugars is altered in Hashimotos disease indicating that the process of IgG glycosylation may have an important role in the development of thyroid autoimmunity. Our results may be important to better understand the pathology of HT Further studies are needed to explore the role of the altered *N*-glycan structures in IgG activity in HT inflammation.

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[68] HSF1 functional knockout may result in a better response to 4-hydroxytamoxifen and palbociclib in ER-positive breast cancer cells

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Breast cancer is the most frequent malignancy in women worldwide. Four clinically relevant molecular types are characterized based on the expression of estrogen receptors (ER), progesterone receptors (PR), and ERBB2 (HER2). Among them, ER-positive (ER+) luminal adenocarcinoma constitutes the majority of breast cancer cases. They are firstly treated with anti-hormonal therapy using selective estrogen receptor modulators (SERMs), such as tamoxifen. More recent therapeutic options of ER+ breast cancers include inhibitors of CDK4/6, among them palbociclib.

We found that 17-estradiol (E2) stimulates activation of Heat Shock Factor 1 (HSF1), a well-known regulator of the stress response, in ER+ breast cancer cells via MAPK signaling. Activated HSF1 cooperates with ER in the regulation of estrogen action and supports the E2-induced cell proliferation and migration. As there are premises that high levels of HSF1 may be associated with resistance of estrogen-dependent breast cancers to hormonal therapies based on antiestrogens, we decided to investigate the influence of HSF1 on the response of ER+ breast cancer cells (MCF7 and T47D) to this kind of therapy. We treated cells, either HSF1-proficient (HSF1+) or HSF1-deficient (HSF1), with 4-hydroxytamoxifen (4-OHT), either alone or in combination with E2. We found that treatment of HSF1+ cells with 4-OHT resulted in slightly enhanced proliferation, which may be a consequence of ER activation (estimated by ER phosphorylation at Ser118). Nevertheless, 4-OHT inhibited E2-stimulated cell proliferation of HSF1+ and HSF1 cells. Then, we tested whether HSF1 loss enhances the anti-proliferative effect of palbociclib. We found that MCF7 cells were more sensitive to this compound than T47D cells. IC50 of palbociclib was more than two-fold lower in HSF1 than in HSF1+ MCF7 cells, while the difference between HSF1+ and HSF1 was insignificant in T47D cells. Palbociclib also inhibited E2-stimulated cell proliferation, yet it was slightly more effective in the absence of HSF1 only in MCF7 cells. These results suggest that ER-positive breast tumors with low HSF1 expression may be more sensitive to treatment with 4-OHT and palbociclib than cases with high HSF1 levels, but this is also dependent on other stronger factors, e.g. p53 mutation.

[69] DEVELOPMENT OF AN APPROPRIATE IN VITRO MODEL TO STUDY THE FQAD SYNDROME

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Introduction: Fluoroquinolones (FQs) are a broad-spectrum class of synthetic antibiotics used to treat common infections. FQs are derivatives of topoisomerase inhibitors used as chemotherapeutics. Many serious adverse effects of FQs similar to those observed after chemotherapy have been reported, and finally classified by the FDA as fluoroquinolone-associated disability (FQAD.) The etiology of FQAD syndrome is not fully understood, but it is associated with impaired mitochondrial and genomic DNA maintenance. FQAD patients struggle, among others, with damage to connective tissue, tendons, as well as drowsiness, dizziness, liver or pancreatic dysfunctions and arrhythmias.

Methods: In this study, we attempted to isolate epithelial cells from the urine of a patient diagnosed with FQAD and, in parallel, from a healthy donor. Cells were isolated according to the protocol described previously (Zhou T et al., 2012). Subsequently, the cells were reprogrammed towards induced pluripotent stem cells (iPSc) with episomal plasmids (Epi5 Episomal iPSC Reprogramming Kit, Life Technologies), 400 ng of each: pCE-hOct3/4, pCE-hSK pCE-hUL, pCE-mp53DD, pCXB-EBNA1 and 1 g of pCE-mCherry-miR302/367, as described by Drozd et al, 2015. IPS cells were differentiated into induced neural stem cells (iNSc) with the use of PSC Neural Induction Medium Kit, according to the manufacturers protocol. For the immunocytochemical analyzes, cells were seeded on Geltrex-coated glass coverslips and then fixed in 4% paraformaldehyde in PBS for 20 min (iPSc) or 15 min (iNSc), and permeabilized with 0.25% (iPSc) or 0.1% (iNSc) Triton X-100 in PBS for 10 minutes at room temperature. Next, preparation was performed as already described (Drozd et al, 2015). INSc from a healthy donor and FQAD patient were used to determine the IC50 of selected fluoroquinolones moxifloxacin and ciprofloxacin. The viability of cells was evaluated by the MTS assay.

Results: The obtained iPS cell lines were characterized by the presence of pluripotency markers (Oct3/4, SOX2, TRA-1-60, TRA-1-81). IPSc differentiated into iNSc expressed SOX2 and Nestin. The IC50 for selected antibiotics was significantly lower for FQAD patient iNSc than for healthy donor iNSc.

Conclusions: The results suggest that FQs display dose and time-dependent cytotoxic activities in vitro, and FQAD patient-derived cells are more susceptible to their side effects. The development of an appropriate in vitro experimental model for the FQAD study will enable the broadening of knowledge in this field. An established iPS cell line provides an opportunity to analyze FQs impact on cell biology. Moreover, due to organ damage, i.e. liver, kidneys or the nervous system, iPS cells and derivatives may also become a potential therapeutic tool for FQAD patients. The use of iPSc technology for patients recovering from chemotherapy should be considered in general.

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[70] NOVEL BIOMARKERS SELECTION FOR ONCOLOGICAL PATIENTS STRUGGLING WITH HORMONE-DEPENDENT TUMORS – IN SILICO ANALYSES

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Introduction. Hormone-dependent malignancies include ovary, cervical, breast, testis, prostate, and uterine tumors. Hormones, being involved in multitude signal transduction pathways, highly impact various cellular processes such as apoptosis, proliferation, cell cycle or cytoskeleton organization. Currently, with a steadily high rate of cancer mortalities, there is a need for novel biomarkers which would help to efficiently estimate patients condition and prognostication.

Aim of the study. The purpose of our research was to investigate cell cycle and cytoskeleton-related signaling pathways in cancers dependent on hormone homeostasis via *in silico* analyses to find novel potential biomarkers and evaluate their efficacy in predicting patients clinical prognosis and survival.

Materials and methods. The gene expression data of patients suffering from cancers of interest was acquired from The Cancer Genome Atlas. Initial analysis of 54 gene sets implicated in pathways related to cell cycle and cytoskeleton organization from MSig database allowed to specify differentially expressed genes among chosen tumors. Subsequently, impact of the potential gene biomarkers on patients disease free survival was assessed via Gene Expression Profiling Interactive Analysis 2 (GEPIA2). Lastly, beanplots visualizing cell cycle and cytoskeleton-related risk signatures in regard of the gene expression were generated using a BoxPlotR webtool.

Results. Significant differences in gene expression patterns were identified and presented on heatmaps enabling formation of a subset containing top hub gene and close interactors for each cancer type. Obtained subsets of genes were found to be bearing a prognostic potential in certain cancers included in the analysis. Majority of these possible biomarkers exhibited a noticeable negative impact on patients survival, though some of them appear to reduce the hazard ratio. Also, particular gene expression seems to co-depend on the prognostic signature associated with cell cycle and cytoskeleton regulation, such as various *TNR* levels influencing the expression of signature genes in Uterine Carcinosarcoma (UCS), or *GTF2A1* level impacting certain cell cycle- and cytoskeleton-related genes in Ovarian Serous Cystadenocarcinoma (OV).

Conclusions. To conclude, our preliminary *in silico* analyses indicate that *COL6A3*, *TNR*, *GTF2A1*, and *NKX3-1*, along with certain interacting neighbors may be a potential biomarkers in malignancies dependent on hormones. Additionally, selected genes were associated with regulation of cell cycle and/or cytoskeleton. They were also found to have influence on specific clinical features of the patient such as cancer type, cancer stage, or histological type.

[71] ASSESSING GTPASE ACTIVITY OF KRAS WILD-TYPE, KRAS G12V AND KRAS G12D DEPENDING ON THE PRESENCE OF GTPASE STIMULATING PROTEINS

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Introduction. The highest prevalence of mutations of KRAS protein is observed among patients with pancreatic, colorectal and lung cancer. To maintain homeostasis, KRAS occurs in an active state when bound to the GTP and an inactive state when bound to the GDP. Mutations that alter KRAS protein structure lead to the decline of GTPase activity, therefore KRAS stucks on its active state constantly. KRAS G12V and KRAS G12D are observed to have the highest prevalence in regard to codon 12. In terms of the localization of alterations, both mutations are supposed to impair GTPase activity and further cellular processes similarly. In this study in vitro testing model was used to express three analyzed KRAS proteins (wild-type, G12V and G12D) for further analysis.

Materials and methods. To obtain cell lines with the expression of analyzed proteins, a special plasmid with KRAS wild-type sequence was used to prepare two other plasmids for KRAS G12V and KRAS G12V. Using designed plasmids, lentiviral vectors were prepared and used in the transfection of CHO-S and U-87 MG cells. U-87 MG cells were used as a model for the assessment of the effectiveness of designed plasmids by means of immunofluorescent signal standing for the presence of KRAS proteins. CHO-S cells were used as an expression model of KRAS proteins. Proteins of interest were isolated from the cell cultures and tested for their GTPase activity.

Results. Sequencing of the plasmids indicated that constructs were properly designed and prepared. Immunostaining of U-87 MG cells 9 days after transfection showed the presence of the protein of interest and therefore positive result of transfection procedure. Further isolation of the KRAS proteins from CHO-S cell culture let to confirm the presence of recombinant protein. Analyzing GTPase activity, wild type of KRAS showed the lowest luminescence in case of presence and absence of GAPs when compared to KRAS G12V and KRAS G12D. Both analyzed mutants were observed to have comparable luminescent signals, stimulated with GAPs or not.

Conclusions. Transfected CHO-S cells seem to be an effective in vitro model for expressing recombinant KRAS proteins and for their further analysis. In regard to the results, KRAS G12V and KRAS G12D possess significantly lower intrinsic and GAP-stimulated GTPase activity than KRAS wild-type. Both analyzed mutants seem to be insensitive to the addition of GTPase stimulating proteins.

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[72] ANTIPROLIFERATIVE ABILITY OF NEW TERPYRIDINE COMPLEXES

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Terpyridines and their derivatives are often explored during anticancer studies because of their cytotoxic properties. Thanks to the presence of three aromatic rings in their molecules, these compounds possess an ability to chelate metal ions. Addition of the central metal atom increases activity of the molecule, however, high anticancer activity of free ligands was also reported. The mechanism of action of terpyridines is based on DNA intercalation, generation of reactive oxygen species (ROS) and increase in oxidative stress levels, to which cancer cells are particularly susceptible. Here we present the activity of the new terpyridine complexes studied on the several cancer cell lines. The results indicate great antiproliferative properties, therefore we investigated tests with cell cycle analysis on two cancer cell lines: human lung adenocarcinoma (PC-9) and human glioblastoma (U-251). An ability of the compounds to induce apoptosis was additionally investigated. Results of cell cycle analysis indicated that inhibition occurred at the G0/G1 phase. Number of apoptotic cells significantly increases in higher concentrations of tested compounds. This initial studies are promising in the context of more in depth analysis.

[73] AN OPENING-REINFORCING COLLECTIVE BEHAVIOR OF THE BK CHANNELS IN HUMAN GLIOBLASTOMA CELLS

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Introduction. Glioblastoma multiforme (GBM) is a particularly malignant type of cancer which exhibits almost complete resistance to currently available therapies (surgery, chemotherapy, radiotherapy, and even immunotherapy). The large-conductance voltage- and Ca2+-activated K+ channels in GMB cells (gBK) play important physiological roles i.e., they regulate cell growth and extensive migration. However, the complete molecular picture of this phenomena remains unclear. Most of reports in literature summarize the single-channel characteristics of gBK channels, but the question of their possible cooperativity has been never analyzed in literature hitherto.

Methods. The single- and multichannel (2-4) patch-clamp recordings on U-87 MG cells were obtained using operational amplifier (Axopatch 200B, Axon Instruments) and converter (Clampex 11, Axon Instruments). The signals were sampled at 5 kHz and lowpass filtered with a corner frequency of 1 kHz. Symmetrical solution was used in our experiments, which included 130 mM potassium gluconate, 5 mM KCl, 10 mM HEPES, 2 mM MgCl2, 20 M EGTA, and CaCl2 at two different concentrations 20 M and 200 M. The membrane potential (Um) was fixed at -60 mV and +60 mV. For each combination of number of channels, Um and [Ca2+], 3-6 independent traces were recorded, for which we performed the kinetic analysis.

Results. Open state probability increases with the number of gBK channels present (and active) in analyzed membrane patch. The effects of cooperation are more evident at high Ca2+ concentration (over 150 M) in comparison to low Ca2+ regime, and when the channels are voltage-activated. The presence of clusters of cooperative gBK channels shift the typical activation curve towards more negative potentials. The cooperation of the gBK channels results in relative shortening the dwell-times of closed states for each successive channel that cooperates within the gBK channel cluster.

Conclusions/Novel aspect. Our results suggest that the gBK channels exhibit strongly enhancing cooperation which is Ca2+-and voltage-dependent. We are convinced that the cooperation of BK channels is possible due their overexpression in GBM cells and their spatial proximity (10 nm) which allows the channels to directly interact and gate in a cooperative manner. This leads us to another question about the mechanism of such a collective behavior, what is going to be the next step in our research and considerations. Moreover, the collective behavior ought to deeply affect the physiological meaning of gBK channels (the cooperativity can result in persistent channel firing). The possibility of its regulation can indicate a new direction in GBM-related research.

[74] PROGNOSTIC POTENTIAL OF NANOG EXPRESSION FOR PATIENTS WITH SQUAMOUS CELL CARCINOMA OF OROPHARYNX IN RELATION TO IMMUNOHISTOCHEMICAL SCORE

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Introduction: Several reasons for conflicting results concerning prognostic potential of Nanog expression in squamous cell carcinomas of oropharynx can be identified. One of them may be related to differences in immunohistochemical staining procedure, particularly in scoring systems and cut-off points used to distinguish tumors with Nanog overexpression or its lack. Therefore, the aim of the present study was to compare prognostic potential of Nanog expression analysed by three immunohistochemical scores in the group of 63 squamous cell carcinomas (SCC) of oropharynx.

Methods: Immunohistochemical assessment of Nanog expression was performed on formalin fixed and paraffin-embedded sections. Primary rabbit monoclonal antibody (Cat. No 3579, Cell Signalling Technology) and BrightVision system (Immunologic, Duiven, Netherlands) as a visualization system were used. To distinguish lack of overexpression or overexpression three scoring system were applied: (1) semiquantitative score (SQS), (2) immunoreactive score ((IRS) and (3) histological score ((HS). SQS include three categories of staining intensity: (1) lack of positive cytoplasamatic staining, (2) week or moderate cytoplasamatic staining in tumours areas and (3) strong cytoplasmatic staining in tumours areas. IRS is a result of multiplication of percentage of positive stained cells (five categories: 0 - 0%, 1 - 25%, 2 2550%, 3 50-75%, 4 - 75%) and staining intensity (three categories, similar like in SQS), giving a range from 0 to 12. HS was calculated according to the formula: H-score = (1percentage of weakly positive cells) + (2percentage of moderately positive cells) + (3percentage of strongly positive cells), giving a range from 0 to 300. For all three scores, the cut-off points for Nanog overexpression, allowing for optimal separation of overall (OS) and disease free survival (DFS) curves, were search by minimal *P*-value method.

Results: In semiquantitative score, the best separation of OS and DFS curves was obtain by cut-off point: lack of staining *vs* week/moderate/strong staining, although statistical significance was not reach (OS: HR = 1.016, $P = 0.081$, DFS: HR = 6.876, $P = 0.061$). The cut-off points for IRS and HS were, respectively: 1 (OS: HR = 6.977, $P = 0.014$, DFS: HR = 6.002, $P = 0.019$) and 50 (OS: HR = 6.977, $P = 0.014$, DFS: HR = 6.002, $P = 0.019$). The cut-off points found for these two scores allow to identify the same subgroups of patients with lack of Nanog expression (11.1%) and its overexpression (88.9%). All patients with tumors characterized by lack of Nanog overexpression identifying by IRS and HS survived 5 years without evidence of cancer progression. In multivariate analysis Nanog expression analysed by IRS and HS was independent prognostic factor for OS (HR = 10.195, $P = 0.024$).

Conclusion: Immunohistochemical score using to distinguish Nanog overexpression or its lack has influence on prognostic potential of this biomarker.