XXIIIrd Gliwice Scientific Meetings 2019

Gliwice, November 22-23, 2019

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Association for the Support of Cancer Research

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XXIIIrd Gliwice Scientific Meetings 22-23.11.2019

Friday 22.11.2019

Opening ceremony (9:00-9:10)

Session I (9:10-11:40) ***Non-coding RNA*** [chairperson: Wojciech Fendler]

Kevin Elias (Dana-Farber Cancer Institute, Boston): miRNAs as biomarkers for cancer screening and diagnosis.
Ashish Lal (NIH, Bethesda): When long non-coding becomes protein-coding.
Aleksandra Rusin (Baylor College of Medicine, Houston, Texas USA): Housekeeping RNA in homeostasis and tumorigenesis.
Anke van den Berg (University of Groeningen): NGS-based approaches in molecular diagnostics to guide treatment decisions for cancer patients.
Joost Kluiver (University of Groeningen): The role of noncoding RNAs in the MYC oncogenic network.

Coffee break (11:40-12:00)

Session II (12:00-14:30) ***Radiation Biology and Medicine***
[chairperson: Joanna Rzeszowska-Wolny]

Anna Czarnecka, Mateusz Spałek (Centre of Oncology, Warsaw): Radiotherapy as a boost for immunotherapy - how to obtain maximal benefit with minimal risk?
Marie Davidkova (Nuclear Physics Institute CAS, Prague): Quality of ionizing radiation as deteminant of the complexity and reparability of DNA damage at molecular and cellular level.
Valentin Djonov (University of Bern, Institute of Anatomy, Swizerland): Combined Preclinical Studies Using Microbeam Radiation Therapy.
Carmel Mothersill (McMaster University, Hamilton, Canada): Mechanisms operating after low radiation doses are different to and independent from those occurring after high doses: an explanation for non-linearity and a new therapeutic target
Sudha Sharma (Howard University College of Medicine, Washington, USA): Unraveling the Functions of RECQ1 Helicase in Breast Cancer.
Michael D. Story (University of Texas, Dallas, USA): Exploiting the conditional vulnerabilities caused by exposure to Tumor Treating Fields for cancer therapy.

Lunch (14:30-15:30)

Poster session (15:30-17:00)

Session III (17:00-19:00) ***Biotechnology in Medicine*** [chairperson: Marek Los]

Tim Forouzanfar, (Amsterdam University Medical Center): Innovative technologies in the development of bone implants for maxillofacial surgery.
Maciej Wiznerowicz (Poznań University of Medical Sciences): Machine Learning Identifies Stemness Features Associated with Oncogenic Dedifferentiation.
Tomasz K Wojdacz (Pomeranian Medical University) Discovery and clinical applications of methylation biomarkers.
Saeid Ghavami (University of Manitoba, Canada): Autophagy and Regulation of Cellular Phenotype.

Get together party in "Chata Polaka" restaurant (20:00-)

Saturday 23.11.2019

Session IV (9:30-12:00) ***Biomaterials and Drug Delivery*** [chairperson: Anna Kasprzycka, Wiesław Szeja]

Christopher Walkey (Baylor College of Medicine, Houston, Texas USA): Using mouse models to test novel CRISPR delivery methods.
Jerzy Gubernator (Wroclaw University, Wrocław): Modulation of activity and bioavailability of drugs by liposomal encapsulation.
Siddarth Agrawal/Wiesław Szeja (Wroclaw Medical University, Wrocław/ Silesian University of Technology, Gliwice): Prodrug delivery system. Increased effectiveness,selectivity and tolerability of glycoconjugates derivatives of metotrexate in cancer.
Joanna Jazowiecka-Rakus (Centre of Oncology, Gliwice): Mesenchymal stem cells for the delivery of oncolytic myxoma virus.
Marie-Nicole Theodorakis: Plasma-derived exosomes reverse epithelial-to-mesenchymal transition after immune therapy in patients with head and neck cancer.

Coffee break (12:00-12:30)

Session V (12:30-14:45) ***Genome editing*** [chairperson: Katarzyna Lisowska]

Bogna Wach (The Jacob of Paradies University, Gorzów Wielkopolski): Human genetic modification, between eugenics and medical treatment (therapy) - ethical and legal view (perspective).
Wiesława Widlak (Centre of Oncology, Gliwice): Application of the CRISPR / Cas9 system in cell culture - advantages and disadvantages.
Agnieszka Dzikiewicz-Krawczyk (Institute of Human Genetics, Polish Academy of Science, Poznan): CRISPR/Cas9 screens to identify functional non-coding elements in cancer cells.
Witold Konopka (Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw): Towards AgRP neuron selective gene manipulation in mice using AAV, Cre/loxP and CRISPR/Cas9.
Marek Kimmel (Rice University and Silesian University of Technology): Statistical Inference of growth and mutation patterns of tumors based on genomic data
Krista Rantanen (University of Turku): The importance of physiologically relevant oxygen concentration in research

Presentation of awarded posters (14.45-15.30)

Closing ceremony and lunch (15:30 -)

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Lecture Abstracts

Session I:
Non-coding DNA
(Chairperson – Wojciech Fendler)

miRNAs AS BIOMARKERS FOR CANCER SCREENING AND DIAGNOSIS

Kevin Elias

Assistant Professor, Harvard Medical School, Director, Gynecologic Oncology Laboratory, Division of Gynecologic Oncology, Brigham and Women's Hospital, Dana-Farber Cancer Institute, Boston, USA

Diagnosis of cancers at early stage is associated with improved overall survival, lower costs, and reduced morbidity for patients. However, many common and highly lethal cancers, such as ovarian and pancreatic cancer, do not have approved screening tests. This remains true even for individuals at high risk of developing disease, such as *BRCA* mutation carriers. Screening in high-risk populations is particularly challenging because these patients are at risk of multiple cancer types; tests must not only discriminate individuals with cancer from those without but also determine which cancer type is most likely to be present. Developing a simple, scalable cancer screening strategy for several different types of cancer into a single assay for these patients would save costs, improve compliance, and increase the number of cancers amenable to early diagnosis. To this end, our laboratory is investigating screening using serum microRNAs (miRNAs). Our approach combines advances in detection of rare circulating miRNA transcripts with a neural network machine learning approach. In our original paper, we showed that a model incorporating serum levels of 14 miRNAs had a high degree of accuracy (AUC 0.93, 95%CI 0.81-1.00) for distinguishing cases of invasive ovarian cancer from non-invasive lesions, benign tumors, or healthy controls. When applied to an independent dataset of 454 patients, the model had 100% specificity for identifying ovarian cancers versus 12 competing diagnoses. We have shown that the miRNAs are derived from the tumors themselves and can identify even microscopic lesions. In recent studies, we have shown how these risk probability algorithms can be adapted for high-risk patient populations. By utilizing a high throughput pipeline for serum miRNA analysis and cloud computing, we envision a rapid, flexible and scalable approach to cancer screening among high-risk individuals that can be used to manage the challenge of competing cancer risks over time.

WHEN LONG NON-CODING BECOMES PROTEIN-CODING

Xiao Ling Li[1], Bruna Muys[1], Wei Tang[2], Qinyu Sun[3], Qinyu Hao[3], Ana I. Robles[4], Sudipto Das[5], Kannanganattu V. Prasanth[3], Curtis C. Harris[4], Stefan Ambs[2], Thorkell Andresson[5], Ashish Lal[1]

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The human genome is transcribed into thousands of long noncoding RNAs (lncRNAs) of which the vast majority are not functionally characterized. Although a significant proportion of lncRNAs are known to be associated with polysomes and have the potential to be translated into micropeptides or small proteins, there are only a few examples where their existence in nature and functionality has been demonstrated in an endogenous setting. We have discovered a putative GI tract-specific lncRNA that encodes a small protein that we named FORCP. In today's presentation, I will share our unpublished data on *FORCP* and its role as a tumor suppressor and regulator of endoplasmic reticulum stress in colorectal cancer cells. Collectively, our findings indicate that some other lncRNAs that appear to be noncoding could be translated into small proteins that play vital roles in the pathogenesis of colorectal cancer.

HOUSEKEEPING RNA IN HOMEOSTASIS AND TUMORIGENESIS

Aleksandra Rusin, Christopher J. Walkey, Sandra L. Johnson, Jason D. Heaney, William R. Lagor, Salma Kaochar, Deborah L. Johnson

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Introduction: Non-coding RNAs are defined as RNA transcripts without protein-coding capacity. Such ncRNAs can be divided into two classes: housekeeping RNAs and regulatory RNAs. Housekeeping RNAs are constitutively expressed and necessary for cell viability. They include not only ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) but also small nuclear RNAs and small nucleolar RNAs implicated in diverse functions, including maturation of rRNAs and tRNAs, regulation of transcription, DNA replication, splicing of mRNA, and translocation of nascent peptide chain into endoplasmic reticulum during translation. Production of these RNA molecules is enhanced in highly proliferative cells, especially in cancer. Most housekeeping RNAs are transcribed by RNA Polymerase III (RNAPolIII), and therefore targeting its activity may become a novel anticancer strategy.

Methods: In order to manipulate RNA PolIII activity *in vivo,* we created a transgenic lox/stop/lox mouse model for conditional over-expression of Maf1 protein, a natural repressor of RNA PolIII. Overexpression of Maf1 in the liver was mediated by the virtue of cre recombinase delivered either by adeno-associated virus 8 (AAV8) or expressed in the liver under the albumin promoter.

Results: Here, we show that Maf1 overexpression in the liver reduces RNA PolIII activity, as measured by the levels of selected RNAPolIII products, including tRNAs, 7SK, 7SL, and RMRP. Reduced production of housekeeping RNA by RNAPolIII does not impair liver function, and does not impede liver regeneration after partial hepatectomy. However, overexpression of Maf1 in hepatocytes prevents development of hepatocellular carcinoma. Collectively, our results indicate that depletion of RNAPolIII products is well tolerated by normal hepatocytes in a mouse model, and prevents their oncogenic transformation.

Conclusion: This work delivers a proof of concept that targeting RNAPolIII transcription may be a safe and effective anticancer strategy.

keywords: RNA polymerase III, tRNA, hepatocellular carcinoma

NGS-BASED APPROACHES IN MOLECULAR DIAGNOSTICS TO GUIDE TREATMENT DECISIONS FOR CANCER PATIENTS

Jiacong Wei[1,4], Pei Meng[2,4], Miente Martijn Terpstra[1], Menno Tamminga[3], Mohamed Zma[1], Lennart Johansson[1], T. Jeroen N. Hiltermann[3], Harry J.M. Groen[3], Klaas Kok[1], Anthonie J. van der Wekken[3], Anke van den Berg[2]

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The number of genomic aberrations relevant for therapeutic decisions for non-small cell lung cancer patients has increased in the past decade. To reliably test the presence of these aberrations, often multiple molecular tests are required, which is a challenge due to the generally small tissue specimens. To optimize diagnostic testing, we (1) developed a transcriptome-based next generation sequencing (NGS) based on single primed enrichment technology and (2) determined whether PCR-based targeted NGS data on tumour tissue can be used to estimate presence of gene amplifications and explored the prognostic value of EGFR gene amplification in EGFR mutated NSCLC patients.

For the first aim we interrogated cell lines and patient derived frozen biopsies, pleural effusion and FFPE samples. All clinical samples were selected based on previously identified mutations at the DNA level. In 26 FFPE samples with >50K unique reads and a DV200>50, all 17 SNVs/INDELs, 2 *MET* exon 14 skipping events and 14 fusion gene transcripts were detected at RNA level, giving a test accuracy of 100%. For the second aim we re-analysed data of 1586 NSCLC patients of which 134 had an EGFR mutation (8.2%) and clinical data being available for 66 of the patients. Depending on the amplification analysis strategy (within sample or relative to normal controls), 19% and 13% of the *EGFR* mutated group had an *EGFR* amplification, respectively. In *EGFR* wild type patients, amplifications were detected in 5% and 4% of the patients using the two methods, respectively. The sensitivity and specificity of the NGS based estimation of amplifications for *EGFR* were 94% (14/15) and 83% (29/35) for both data analysis approaches. Patients with concurrent *EGFR* mutations and amplifications (estimated within sample) treated with EGFR-TKI had a worse overall survival compared with those without concurrent EGFR amplifications.

In conclusion, we showed feasibility of an all-in-one RNA based NGS approach to identify different types of genomic aberrations at a high accuracy. Moreover, we showed that presence of *EGFR* amplification in *EGFR* mutant patients is predictive for a worse overall survival.

THE ROLE OF NONCODING RNAS IN THE MYC ONCOGENIC NETWORK

Joost Kluiver

Burkitt lymphoma (BL) is an aggressive form of B-cell lymphoma characterized by a hallmark translocation resulting in overexpression of the oncogenic transcription factor MYC. A large number of protein-coding genes are known to be regulated by MYC as well as a substantial amount of non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long ncRNAs (lncRNAs). MiRNAs are short RNA molecules that are known to act at the posttranscriptional level while lncRNAs have more recently emerged as a novel class of regulatory RNAs acting at the epigenetic, transcriptional or posttranscriptional level. Aberrant expression of both types of ncRNAs have been clearly implicated in various aspects of tumorigenesis. Here we identified short and long ncRNAs regulated by MYC and investigated how these ncRNAs contribute to BL pathogenesis.

Session II:
Radiation Biology and Medicine

(chairperson:

Joanna Rzeszowska-Wolny)

RADIOTHERAPY AS A BOOST FOR IMMUNOTHERAPY - HOW TO OBTAIN MAXIMAL BENEFIT WITH MINIMAL RISK?

Anna Czarnecka, Mateusz Spałek

Centre of Oncology, Warsaw

Numerous preclinical studies and early phases clinical trials have proven that the combination of immunotherapy with radiotherapy is a promising strategy for synergistic enhancement of treatment efficacy. Radiation delivered to the tumor site affects tumor cells, surrounding stromal cells, as well as increase systemic response. In some situations, the aforementioned combination may cause a phenomenon called abscopal effect. However, the underlying mechanisms of this synergy are unknown. What is more, the factors that might affect the efficacy of concomitant radioimmunotherapy are still under investigation. That comprises the optimal fractionation, sequence of treatment, timing, management of treatment toxicity, and the assessment of response.

QUALITY OF IONIZING RADIATION AS DETEMINANT OF THE COMPLEXITY AND REPARABILITY OF DNA DAMAGE AT MOLECULAR AND CELLULAR LEVEL

Lucie Ježková[1], Daniel Depeš[1], Iva Falková[1], Martin Falk[1], Alla Boreyko[2], Evgeny Krasavin[2], Nakahiro Yasuda[3], Kateřina Pachnerová Brabcová[4], Pavel Kundrát[4], Václav Štěpán[4], Marie Davídková[4]

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Ionizing radiation causes a wide panel of DNA damages. Single- and double-strand breaks, modified bases and sugars, abasic sites, DNA interstrand cross-links and DNA-protein cross-links are the main radiation-induced DNA damages. In aerobic conditions, the most important part of radiation action on DNA is the oxidative attack by $OH^{\cdot}$ radicals resulting from the radiolysis of water surrounding DNA. A primary damage, if it is unrepaired or missrepaired, may have serious consequences for living cells, depending on the type and the location of the damage. A lesion that modifies or suppresses a cellular function such as the inactivation of a tumor suppressor gene may be at the origin of the tumor development. If the damage affects a vital function of the cell, it may lead to the cell death.

Ionizing radiation of high linear energy transfer (LET) produces clustered DNA damages. Compared to isolated DNA damage sites, their biological consequences can be more severe. A model system frequently used to study the mechanisms of radiation DNA damage is DNA in a dilute aqueous solution. We investigated a clustered DNA damage induced by high LET radiation (namely C 290 MeV/n and Fe 500 MeV/n) in pBR322 plasmid DNA. The yield of double strand breaks (DSB) and non-DSB clusters was determined as a function of LET.

At cellular level, the efficiency of cell killing increases for high-LET radiation. Surprisingly, the characteristics of DNA damage and repair upon exposure to different particles with similar LET significantly differ. High-resolution confocal microscopy has been explored to examine phosphorylated histone H2AX (γH2AX)/p53-binding protein 1 (53BP1) focus streaks at the microscale level, focusing on the complexity, spatiotemporal behavior and repair of DNA double-strand breaks generated by B and Ne ions with similar LET (~135 keV/μm). Both high-LET radiation types generate highly complex γH2AX/53BP1 focus clusters with a larger size, increased irregularity and slower elimination than low-LET γ-rays. Ne ions produce even more complex γH2AX/53BP1 focus clusters than B ions, consistent with DSB repair kinetics. Although cells exposed to γ-rays and B ions eliminated a vast majority of foci (94% and 74%, respectively) within 24 h, 45% of the foci persisted in cells irradiated with Ne. Our results suggest that the complexity of DSB damage critically depends on (increases with) the particle track core diameter.

The goal of the lecture is to overview and analyze the complexity and reparability of DNA damage in relation to the quality of ionizing radiation.

COMBINED PRECLINICAL STUDIES USING MICROBEAM RADIATION THERAPY

Valentin Djonov

University of Bern, Institute of Anatomy, Swizerland

The Microbeam Radiation Therapy (MRT) technique uses synchrotron-generated X-rays to produce a spatially and periodically alternating dose distribution in the tissue. The underlying radiobiology of MRT appears to follow a different paradigm of radiation tissue interactions. In parallel to the excellent tumor control, normal, healthy tissues show a remarkably high resistance even when irradiated with hundreds of Grays in MRT mode.

Our preliminary data, employing different animal models, indicates that (i) in a range of 400-600 Gy 'peak' dose, MRT could be used as completely novel anti-angiogenic, tumor-vascular disrupting strategy, because it is a unique method to destroy immature tumour-vessels while sparing surrounding mature normal vessels. (ii) MRT in a range of 100-150 Gy causes a partial disintegration of the endothelium which leads to a temporally significant increase in tumor blood vessel permeability. An MRT induced "transpermeability window" has been identified as potent drug delivery system. The experimental use of the therapeutic window proved to be highly effective: a combined/double treatment, i.e. MRT followed by chemotherapy (cisplatin) or Au Nanoparticle, dramatically reduced the tumor progression in different experimental murine models. This treatment strategy seems be very efficient - 18 months after treatment in more that 50% of the experimental melanomas a complete tumor remission have been achieved. The best explanation for these positive results lies in the disruption of the vascular barrier leading to extravasation of the nanoparticles and chemotherapeutic agents and their penetration into the tumor during the permeability window.

The described drug delivery strategy has a broad spectrum of potential applications: in addition to tumor therapy, it could be useful for the treatment of different pathologies using different compounds (nanoparticles/gold/antibodies, etc.).

MECHANISMS OPERATING AFTER LOW RADIATION DOSES ARE DIFFERENT TO AND INDEPENDENT FROM THOSE OCCURRING AFTER HIGH DOSES: AN EXPLANATION FOR NON-LINEARITY AND A NEW THERAPEUTIC TARGET

Carmel Mothersill, Colin Seymour

McMaster University, Canada

The "non-targeted effects" of ionizing radiation including bystander effects and genomic instability predominate after low dose exposures and dominate response outcomes. These effects are unique in that no classic mutagenic event occurs in the cell showing the effect. In the case of bystander effects, cells which were not in the field affected by the radiation show high levels of mutations, chromosome aberrations, ROS and membrane signalling changes (horizontal transmission of mutations and information which may be damaging) while in the case of genomic instability, generations of cells derived from an irradiated progenitor appear normal but then lethal and non-lethal mutations appear in distant progeny (vertical transmission). The phenomena are characterized by high yields of mutations and distant occurrence of events both in space and time. This precludes a mutator phenotype or other conventional explanation and appears to indicate a generalized form of ROS mediated stress induced mutatgenesis, which is well documented in bacteria. The nature of the signal travelling between irradiated and unirradiated cells and organisms is currently unknown but recent evidence suggests that there may be a physical component such as a vibration wave involved. UV photon mediated transmission has also been documented and the latter mechanisms can induce the release of exosomes, which by themselves can induce bystander effects when added to recipient cells. This presentation will discuss the phenomenology of non-targeted effects both in vitro and in vivo, including recent data suggesting that excitation decay-induced photons in the UVA range lead to exosome release and consequent mitochondrial malfunction and elevated ROS in recipient cells. Photons, calcium, and neurochemicals are important in signal production while the exosome cargo, and cytokine mediated pathways especially TGFb determine response to the signal. By highlighting some key challenges and controversies, concerning the mechanisms and more importantly, the reason these effects exist, we will discuss current ideas about the wider implications of non-targeted effects in evolution and biology.

UNRAVELING THE FUNCTIONS OF RECQ1 HELICASE IN BREAST CANCER

Xing Lu[1], Swetha Parvathaneni[1], Xiao Ling Li[2], Bayan Bokhari[1], Ashish Lal[2], Sudha Sharma[1,3]

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RecQ-like helicase 1 (RECQ1, also known as RECQL or RECQL1), a DNA repair helicase, is critical for genome stability. Loss-of-function mutations in the RECQ1 gene are associated with increased susceptibility to breast cancer, however, the underlying mechanisms are unclear. We are investigating how the genetic or functional loss of RECQ1 compromise cellular ability to deal with genotoxic stress and promote tumorigenesis. In addition to elucidating specific new details of biological roles of RECQ1 helicase, our findings provide critical evidence to support further exploration into the potential therapeutic implications of RECQ1 expression and functions in breast cancers.

EXPLOITING THE CONDITIONAL VULNERABILITIES CAUSED BY EXPOSURE TO TUMOR TREATING FIELDS FOR CANCER THERAPY

Michael D. Story

University of Texas, Dallas, USA

EXPLOITING THE CONDITIONAL VULNERABILITIES CAUSED BY EXPOSURE TO TUMOR TREATING FIELDS FOR CANCER THERAPY

Michael D. Story, Narasimha Kumar Karanam, Debabrata Saha, Liang-hao Ding, Asaithamby Aroumougame, Kalarisian Srinavasan

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Tumor treating fields (TTFields) is a noninvasive physical modality of cancer therapy that applies low-intensity, intermediate frequency, and alternating electric fields to a tumor. Interference with mitosis was the first mechanism describing the effects of TTFields; however, TTFields was shown to not only reduce the rejoining of radiation-induced DNA double-strand breaks (DSBs), but to also induce DNA DSBs. The mechanism(s) by which TTFields generates DNA DSBs is related to the generation of replication stress including reduced expression of the DNA replication complex genes *MCM6* and *MCM10* and the Fanconi's Anemia pathway genes, which were identified by gene expression analysis and western blotting. When markers of DNA replication stress as a result of TTFields exposure were examined, newly replicated DNA length was reduced with TTFields exposure time and there was increased R-loop formation. Furthermore, as cells were exposed to TTFields a conditional vulnerability environment developed which rendered cells more susceptible to DNA damaging agents or agents that interfere with DNA repair or replication fork maintenance. The effect of TTFields exposure with concomitant exposure to cisplatin or PARP inhibition, the combination of TTFields plus concomitant PARP inhibition followed by radiation, or radiation alone at the end of a TTFields exposure were all synergistic. Finally, gene expression analysis of 47 key mitosis regulator genes suggested that TTFields-induced mitotic aberrations and DNA damage/replication stress events are likely initiated independently of one another. This suggests that enhanced replication stress and reduced DNA repair capacity are also major mechanisms of TTFields effects, effects for which there are therapeutic implications.

Session III:
Biotechnology in Medicine
(chairperson: Marek Los)

INNOVATIVE TECHNOLOGIES IN THE DEVELOPMENT OF BONE IMPLANTS FOR MAXILLOFACIAL SURGERY

Tim Forouzanfar

Amsterdam University Medical Center

MACHINE LEARNING IDENTIFIES STEMNESS FEATURES ASSOCIATED WITH ONCOGENIC DEDIFFERENTIATION

Maciej Wiznerowicz

Poznań University of Medical Sciences

DISCOVERY AND CLINICAL APPLICATIONS OF METHYLATION BIOMARKERS

Tomasz K Wojdacz

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Epigenetic mechanisms of gene expression regulation alter the expression of genes without changing primary gene sequence. Enzymatic addition of methyl group to cytosines in DNA strand is referred to as DNA methylation and this modification is one of the most important epigenetic mechanisms of gene expression regulation.

In general terms methylation of cytosines in gene promoter (promoter hypermethylation) represses gene expression. In normal conditions methylation dependent gene expression regulation is essential for organism development and tissue differentiation. At the same time, changes of the normal methylation status of genes (hypomethylation of methylated and hypermethylation of non-methylated) significantly contribute if not initiate not only malignant transformation but also other diseases. Thus, the phenotype of the disease to a large extant depends on the methylation changes acquired during disease development. Identification of the physiological processes disrupted by methylation changes during disease development allows not only for the discovery of new treatment targets but also biomarkers for clinical disease management.

There is large research evidence indicating that methylation biomarkers provide clinically useful information at all stages of the clinical disease management: from risk assessment through early diagnosis and treatment personalization to post treatment surveillance. Still, the use of methylation biomarkers in clinical practice is marginal and needs to be improved.

In my talk I will review the process of discovery and clinical validation of the methylation biomarkers using examples from my research in clinical oncology and studies of the influence of the effects of environmental exposures on the methylation pattern of cells.

AUTOPHAGY AND REGULATION OF CELLULAR PHENOTYPE

Saeid Ghavami

Department of Human Anatomy and Cell Science, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada

Macroautophagy (hereafter autophagy) is a very tightly regulated catabolic process for the degradation of cytosolic proteins and organelles via lysosomes. Autophagy is a cellular mechanism which is responsible for degradation of misfolded proteins and damaged organelles inside the cells. It plays essential role in determining cellular fate and linked to other cellular pathways like apoptosis and unfolded protein response (UPR). Autophagy occurs at low basal levels in most cells as a part of cellular homeostasis such as turnover of long-lived proteins and selective removal of damaged organelles, however, it can be rapidly increased in response to different forms of metabolic stress, including nutrient starvation, hypoxia and/or in increased metabolic demands related to the rapid cell proliferation to maintain cellular homeostasis.

Cell phenotype plays a crucial rule in determining the cell structure and function and a key factor for organism function. Therefore cellular phenotype is involved in both health and disease condition. Here we will describe how autophagy is involved in regulation of cellular phenotype in different models including primary airway mesenchymal cells, primary atrial fibroblast, idiopathic pulmonary disease patient derived fibroblasts, and lung adenocarcinoma cells. We will describe how autophagy regulates proliferative, synthetic and epithelial to mesenchymal phenotype of the cells in lung fibrosis and lung cancer disease model.

Session IV: Biomaterials and Drug Delivery

(chairpersons: Anna Kasprzycka, Wiesław Szeja)

USING MOUSE MODELS TO TEST NOVEL CRISPR/CAS DELIVERY METHODS

Christopher J. Walkey, Ayrea E. Hurley, Denise G. Lanza, Jason D. Heaney, William R. Lagor

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Over the past few years, CRISPR/Cas gene editing has emerged as the leading method to introduce targeted changes to genomic DNA. Following its original discovery as an anti-viral defense mechanism in bacteria, the system has been simplified and adapted to function in mammalian cells in order to introduce mutations at specific genomic loci by double-stranded DNA cleavage and error-prone repair. In addition, the guide RNAs and Cas nuclease enzymes involved in gene editing have undergone extensive modifications that allow alternative functions, including base replacement, DNA insertions and transcriptional activation or repression. At this point, CRISPR/Cas-based therapies are beginning to enter the clinic.

Despite its power and relative ease of use, leading CRISPR/Cas-mediated gene editing into therapeutic use is not without its challenges. One of these challenges is the delivery of the CRISPR/Cas machinery into its target tissue in an effective manner, without affecting non-targeted tissues and organs. Therefore, we are beginning to develop mouse models that will allow researchers to test novel delivery systems. Our method is based on transgenic mice that express a fluorescent protein following CRISPR/Cas-mediated excision of upstream transcriptional and translational stop signals. In this manner, we can detect CRISPR/Cas events in individual cells. Using modern fluorescence microscopy techniques, rare events can be detected in large sections of tissues and organs. Our initial results presented here will demonstrate the effectiveness of our approach. Using adeno-associated viruses to deliver guide RNAs and Cas9 nuclease specifically to the liver, we detected fluorescent protein expression in ~13% of hepatic cells, indicating a significant level of gene editing events. An initial examination of other tissues by standard fluorescence microscopy did not reveal gene editing events in non-hepatic organs. However, a closer examination of tissues following CLARITY clearing and lightsheet microscopy revealed rare gene editing events in the pancreas and stomach of one mouse, as indicated by weak fluorescence. These results confirm the utility of our fluorescence-based reporter mouse for testing the effectiveness and specificity of CRISPR/Cas delivery.

Currently, we are generating new derivative strains of the reporter mice in order to simplify the requirements for the guide RNAs used by CRISPR/Cas editing. We are also adding bioluminescence as a second reporter for preliminary screening. These new mouse strains will be used to test a variety of novel delivery methods, including custom adeno-associated viruses, protein shuttles and lipid-based nanoparticles. This research constitutes a component of the NIH's Somatic Cell Genome Editing initiative.

Keywords: CRISPR/Cas, transgenic mice, fluorescent reporter

MODULATION OF ACTIVITY AND BIOAVAILABILITY OF DRUGS BY LIPOSOMAL ENCAPSULATION

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Liposomes are one of the best candidates for Paul Ehrlich's concept of a 'magic bullet', as their structure is similar to this of cell membranes, they are non-toxic or of low toxicity, they are small, and it is relatively easy to direct them at a selected molecular target using antibodies, peptides, folic acid or other ligands. The use of liposomes results in a prolonged drug circulation life, reduced drug toxicity (by bypassing healthy tissues) and often increased efficacy of a therapy (Torchilin, 2006). Liposomal anthracyclines (epirubicin and mitoxantrone) are expected to have a very strong potential in the treatment of several human cancer types including breast, ovarian, prostate, pulmonary and skin cancers. In our studies two novel formulation on EPI have been assessed as anti-breast cancer agent. The method involving EDTA ion gradient giving faster drug release rate following the liposome accumulation within tumour tissue, and thus better drug efficacy than the method based on ammonium sulphate gradient (used for Doxil®). For example, low drug release rate in the tumour, was reported as a drawback of Doxil® The second liposomal formulation of EPI was based on vitamin C ion gradient method. In this case the ability of the ascorbate to generate the ion gradient for active drug loading and additionally to increase the cytotoxic abilities of the anticancer drugs toward cancer cells was utilized. Both liposomal constructs were tested against human MDA-MB-231 and murine breast 4T-1 cancers in vitro and in vivo models. Obtained results indicate that liposomal EPI exhibited excellent pharmacokinetics with remarkably increased antitumor activity and reduced toxicity for animals. In next study, in order to increase the antitumor potential of mitoxantrone (MTX) the drug was encapsulated by vitamin C ion gradient in order to increase the drug activity by ROS mediated toxicity. Additionally, anacardic acid, a NF-κB pathway inhibitor was incorporated in the liposomes bilayer in order to achieve slectevity toward cancer cells. As a result one of tested MTX formulations exhibited remarkably decreased toxicity towart normal human skin fibroblasts (NHDF cell line) and high toxicity to tested melanoma cell lines. The use of natural substances as a liposomes components can be not only be a tool (for example) to pH gradient generation, but also a method to switching off or switching on selective pathways supporting cancer cell survival. Such liposomes exhibit more specific activity toward cancer cells and decreased toxicity toward healthy tissues.

Torchilin, VP, 2006 Multifunctional nanocarriers. Adv. Drug Deliver. Rev. **58**, 1532-1555.

keywords: liposomes, active loading, anthracyclines, anacardic acid, vitamin C

PRODRUG DELIVERY SYSTEM. INCREASED EFFECTIVENESS, SELECTIVITY, AND TOLERABILITY OF GLYCOCONJUGATE DERIVATIVE OF METOTREXATE IN CANCER

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The clinical management of cancer requires the development of molecules that target specific cell types with high selectivity. One of the leading strategies is based on the conjugation of an established therapeutic agent to a targeting ligand that can deliver the attached pharmacophore selectively to the diseased cells. The elevated glucose intake and GLUTs overexpression are frequent in neoplasms and provide clinically corroborated strategies for cancer diagnostics. In recent years glycoconjugation has emerged as an appealing strategy for targeted delivery of anticancer drugs. The use of methotrexate, a clinically-incorporated drug applied in the treatment of cancer patients as well as autoimmune diseases, is frequently limited by the toxicity of the compound. The project represents the first attempt to systematically evaluate the anticancer activities of a novel methotrexate conjugate *in vitro*. The results indicate that the compound exhibits potent anticancer activity against a range of solid tumor cell lines with IC50 values significantly lower compared to free methotrexate. It preferentially annihilates cancer cells while showing low toxicity in noncancerous cells. Moreover, cellular uptake is GLUT-specific and 100-times more efficient than that of free methotrexate. The results highlight the rationale for the development of glucose conjugated anticancer drugs to increase their effectiveness, cellular selectivity, and tolerability.

Keywords: drug design, glycoconjugates, methotrexate, Warburg effect, in vitro anticancer activity

MESENCHYMAL STEM CELLS FOR THE DELIVERY OF ONCOLYTIC MYXOMA VIRUS

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Oncolytic viruses are anti-cancer agents potentially capable of targeting disseminated neoplastic lesions triggering lytic action and adaptive immune responses. However, intravenous administration of viruses triggers antiviral immune response and mobilizes the phagocytic system leading to sequestration of the viral payload. Effective delivery of oncolytic viruses into tumors is, therefore, a challenge. Use of cellular carriers might provide a solution to this problem. Mesenchymal stem cells (MSCs) are a promising cellular-type carrier candidate. They can be preloaded *ex vivo* with oncolytic virus and infused iv. to the recipient. Upon completion of transit to the tumor site the infected MSCs are postulated to release their oncolytic cargo. During the lecture I will discuss our studies of MSCs loaded with recombinant constructs of a promising oncolytic virus (myxoma virus, MYXV). Some of these constructs encoding fluorescent proteins were used to investigate *in vitro* transfer of virus in co-cultures from MSCs to recipient cancer cells. Involvement of input and/or viral progeny in such transfer will be discussed. Challenge of animals (e.g. mice) with intravenously injected cancer cells previously co-cultured with such MYXV-infected MSCs allows studying inhibition of neoplastic foci formation in specific tumor models. Bioluminescence imaging (BLI) of tissues resected from animals treated with therapeutic MYXV constructs encoding also luminescent protein and loaded into MSCs will be presented. Treatment of animals bearing experimentally-induced cancer lesions using MSCs-MYXV therapeutic oncolytic system featuring immunomodulatory cytokine will be discussed along with innate and adaptive immune responses observed in extracted tissues and blood from examined animals. The presented studies demonstrate that MSCs allow for efficient ferrying of therapeutic MYXV constructs to disseminated neoplastic foci.

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PLASMA-DERIVED EXOSOMES REVERSE EPITHELIAL-TO-MESENCHYMAL TRANSITION AFTER IMMUNE THERAPY IN PATIENTS WITH HEAD AND NECK CANCER

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Photodynamic therapy (PDT) is a palliative treatment option for head and neck squamous cell carcinoma (HNSCC) patients which induces local inflammation and alters tumor cell morphology. Exosomes are virus-sized microvesicles and present an effective way of intercellular communication. We and others have shown that exosomes have the potential to serve as liquid biomarkers for disease stage and activity. Here we show that exosomes in plasma of HNSCC patients undergoing PDT reprogram tumor cells towards an epithelial phenotype. Exosomes were co-incubated with cancer cells, and changes in expression of EMT markers were evaluated as were proliferation, migration, chemotaxis and invasiveness of tumor cells. We showed a PDT-mediated conversion from the mesenchymal to epithelial tumor phenotype. Hence exosome may serve as non-invasive biomarkers of this transition.

Session V:
Genome Editing
(chairperson: Katarzyna Lisowska)

HUMAN GENETIC MODIFICATION, BETWEEN EUGENICS AND MEDICAL TREATMENT (THERAPY) - ETHICAL AND LEGAL VIEW (PERSPECTIVE)

Bogna Wach[1]

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Techniques used in genetic engineering have become a source of new therapies that enable the treatment of previously incurable diseases. In addition to the hope for patients and their families which has been aroused by the use of somatic cell gene therapy, there are doubts associated with the use of new techniques, especially those associated with the change in the germ line.

Has science predicted the consequences of interference in the human genome? If we decide to change the germ line, what will be the long-term characteristics (properties) of the organism that will be inherited by future generations? Because the essence of germline change is related to research and experiments on human embryos, will this change the perspective of clinical research involving humans? Who should give the consent for experiments and future therapy? Doctors, gametes donors or future parents? At what point does the therapy end and the specific production of individuals with the characteristics desired by parents or employers begins? Will the fascination with new technologies in genetics not turn into discrimination against people who will not be able to reach for them? Do we not put ourselves in Gods place when we use genetic solutions to create new beings we desire?

There are opinions to introduce a total legal ban on the use of techniques associated with the modification of human germ lines , or at least a moratorium, until further research is done. It is well known that scientific progress is inevitable, and existing ethical and legal principles may not provide an answer to new problems. Therefore, in addition to in-depth research into new technologies in biology and medicine, a very broad debate is needed, not only with the participation of scientists, ethicists and lawyers, but also with the participation of the whole of society, because the problem affects everyone. There is a clear need to develop new ethical and legislative solutions, both at national and international level, for the application and control of the effects of new technologies.

APPLICATION OF THE CRISPR/CAS9 SYSTEM IN CELL CULTURE – ADVANTAGES AND DISADVANTAGES

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The CRISPR/Cas9 system is a type of "adaptive immunity" which allows bacteria to retain a genetic memory of viruses and plasmids it has encountered. This prokaryotic system is also functional when introduced into eukaryotic cells. It is most widely used to generate gene knockouts (via insertion/deletion). This ground-breaking technology for genome editing based on RNA-guided nucleases has great potential due to its simplicity, efficiency, and versatility.

CRISPR/Cas9 gene targeting requires a custom single guide RNA (sgRNA) that contains a targeting sequence (crRNA) and a Cas9 nuclease-recruiting sequence (tracrRNA). The crRNA region is a 17-20-nucleotide sequence that is homologous to a region in the gene of interest and will direct Cas9 nuclease activity. Cas9 makes a double-strand break which, when repaired, generates insertion/deletion, thus the frameshift resulting in a knockout of protein-coding genes. The system components can be delivered to the cell in different forms, separately or in one vector, using different delivery ways. The final results depend largely on the quality/specificity of sgRNA, Cas9 effectiveness, efficiency of the transfection.

We have tested several available systems to edit coding or regulatory DNA sequences. sgRNA can be made *in vitro* or *in vivo* from a plasmid DNA template or can be chemically synthesized as one molecule or crRNA and tracrRNA separately. Also, the Cas9 nuclease can be delivered in vector-based (plasmid, lentivirus) or DNA-free (protein, mRNA) formats. Plasmid- and lentiviral-based delivery enables the initial selection/enrichment of transfected cells (antibiotic selection or flow cytometry when vector encodes a fluorescent protein), but there is a risk of introducing plasmid DNA into the genome. The use of the Cas9/EGFP protein should also allow visualization of the transfected cells. However, using live imaging microscopy we found it as not useful. For difficult-to-transfect cells, the lentiviral sgRNA/Cas9 delivery method can be used which generates side effects resulting from the cell's response to a viral infection. Usually, a clonal selection is necessary to obtain a homogenous population with a fully characterized loss-of-function mutation for the gene of interest. Individual clones may differ from each other regardless of the side effects of sgRNA introduction (i.e. off-targets). It is therefore advisable to obtain several clones for further analysis.

For different purposes, different strategies can be recommended. We found chemically synthesized sgRNAs (or crRNAs and tracrRNA) with the Cas9 protein (containing nuclear localization sequences) or the Cas9D10A nickase (to minimize off-target effects) delivered by the Viromer® CRISPR reagent as the most effective way to perform the gene knockout. Better results are obtained when several sgRNAs are used simultaneously.

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CRISPR/CAS9 SCREENS TO IDENTIFY FUNCTIONAL NON-CODING ELEMENTS IN CANCER CELLS

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Over 98% of the human genome does not encode proteins. The noncoding elements include e.g. noncoding RNAs, enhancers, promoters and transcription factor binding sites. Development of the CRISPR/Cas9 technique provided a tool for functional analysis of the noncoding genome. MYC is a proto-oncogene with a well-documented essential role in the pathogenesis and maintenance of several types of cancer. As a transcription factor, MYC binds to specific E-box sequences in the genome to regulate expression of adjacent genes. However, there is no universal set of MYC targets, as many of them are cell type- and developmental stage-specific. To date a comprehensive analysis of direct MYC targets with essential roles in different types of cancer is missing. Here we used CRISPR/Cas9 to destroy E-box sequences and perform a genome-wide screen to identify functional MYC binding sites and corresponding target genes essential for growth of MYC-addicted cancer cells. Based on publicly available MYC-ChIP-Seq data in different cancer types we designed a MYC-CRISPR sgRNA library targeting 25,448 E-boxes. Next, we performed a high-throughput screen in K562 chronic myelogenous leukemia with the MYC-CRISPR library to disrupt E-boxes and in parallel with the Brunello library to knock down protein-coding genes. Cells were harvested at T0 (after selection with puromycin) and T1 (after 27 days culture). Constructs significantly depleted from the cell pool were determined by next generation sequencing and DESeq2 algorithm. We identified 152 E-boxes essential for growth of K562 cells ($p<0.001$). For 45% of the E-boxes adjacent genes were also significantly depleted in the genome-wide Brunello screen, and 25% of these genes were well-known MYC targets. Gene ontology analysis revealed that the genes localized near essential E-boxes were involved in processes crucial to cancer cell growth such as RNA and DNA biosynthesis, ribosome biogenesis, cell migration, translation and metabolism. For seven selected E-boxes we confirmed their disruption by CRISPR/Cas9 and for five we observed significant reduction in the expression of nearby gene(s) upon E-box disruption. Our results provide novel insights into MYC dependency in CML. Further functional studies for selected E-boxes and target genes as well as screens in other cancer cells are ongoing.

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TOWARDS AGRP NEURON SELECTIVE GENE MANIPULATION IN MICE USING AAV, CRE/LOXP AND CRISPR/CAS9

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Despite the fact that in recent years we have witnessed the rapid development of new methods of gene manipulation, still relatively old systems such as Cre/loxP or Tet system are most widely used for in vivo research. Recently, a new method – CRISPR/Cas9 – has joined the this group due to its high versatility. This method has significantly reduced the time to generate global knockout animals. However, a combination of these methods is required to obtain a deletion of the gene of interest restricted to a selected cell population and adult animals. We are interested in targeting a small population of AgRP neurons (several hundred cells) located in the hypothalamus of the mouse brain. These neurons are called "hunger neurons"' because their chemo- or optogenetic stimulation leads to voracious eating in well-fed mice. We have used a combination of Cre/loxP and CRISPR/Cas9 to precisely target the Dicer gene (a key molecule in microRNA biogenesis) in AgRP neurons only in adult animals. The latter is particularly important because the Dicer gene is crucial for cell differentiation. Moreover, to locally deliver components of the system we have used AAV viral vectors steraotaxically injected into the brain. Such manipulation of the Dicer gene induces hyperphagic obesity in mutated animals.

STATISTICAL INFERENCE OF GROWTH AND MUTATION PATTERNS OF TUMORS BASED ON GENOMIC DATA

Marek Kimmel

Rice University and Silesian University of Technology

We present a mathematical model of growth of secondary clones in cancer cell populations, anchored in the coalescence theory. Based on the model, we describe difficulties in reconstructing past demography of tumors. Considerations are illustrated by analysis of site frequency spectra of tumors from The Cancer Genome Atlas.

Collaboration of Khanh Dinh, Roman Jaksik, Amaury Lambert and Simon Tavaré is acknowledged'

THE IMPORTANCE OF PHYSIOLOGICALLY RELEVANT OXYGEN CONCENTRATION IN RESEARCH

Krista Rantanen

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Theoretically obvious truth: happy cells = good and relevant results, but what does it mean happy? Is our normoxia really the same thing for our cells? What conditions are normal for them to "feel relaxed at home"? We will try to briefly discuss what does it mean physiologically relevant conditions on example of oxygen concentration.

Poster sessions

I: Regulation of cellular processes
II: New molecules and experimental therapies
III: Bioinformatics and mathematical modeling
IV: Biomarkers
V: Varia

Poster session I: Regulation of cellular processes

[I-1] ANALYSIS OF STK32B KINASE AND ITS INFLUENCE ON SENSITIVITY OF CANCER CELLS TO CHEMOTHERAPEUTICS

Małgorzata Krześniak[1], Barbara Łasut-Szyszka[1], Magdalena Głowala-Kosińska[2], Agnieszka Gdowicz-Kłosok[1], Marek Rusin[1]

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The STK32B kinase is a poorly studied protein. We observed that substances, which strongly activate p53 protein also cause strong downregulation of *STK32B* gene. In cancers many kinases are hyperactive and therefore drive cell proliferation. Hence, any hyperactive kinase is a potential target for anticancer drugs and many substances recently introduced into clinical use are specific inhibitors of kinases.

Aim of our study was examination the hypothesis that *STK32B* gene is directly and negatively regulated by p53 protein and that the activity of STK32B may modulate the sensitivity of cells to the commonly used anticancer drugs.

Using RT-PCR, we confirmed our preliminary data showing that strong activation of p53 by treatment with camptothecin or actinomycin D with nutlin-3a was associated with reduced expression of *STK32B*. However knock-down of p53 expression did not influence *STK32B* gene activity. This suggests that p53 is activated and STK32B is repressed by a common trigger, however the two proteins do not belong to common signaling pathway. Consistently, we observed that wild-type p53 was not able to downregulate activity of gene regulatory DNA sequence from *STK32B* gene. Surprisingly, we observed that mutant p53 was able to stimulate the gene regulatory elements from *STK32B*. Thus, the stimulation of *STK32B* gene may be a part of gain-of-function observed for some cancer-related mutants of p53. We engineered fusion genes coding for fusion proteins consisting of STK32B and EGFP fluorescent protein attached either to amino or to carboxyl terminus of STK32B. We observed that fluorescently tagged kinase localized either within whole cells or was associated with chromosomes during mitosis. We also generated two cancer cell lines (A549 and U-2 OS) with *STK32B* expression knocked-down by virus-delivered shRNA molecules. In these cell lines we determined the IC50 concentration of commonly used anticancer drugs: camptothecin, etoposide, cisplatin and paclitaxel. We compared the sensitivity of knocked-down cells and the controls for knock-down to the treatment with aforementioned drugs at IC50 concentrations. We used the following endpoints: cell survival (MTS test), apoptosis (Annexin V staining), cell cycle distribution (PI staining) and the induction of executioner caspase for apoptosis. We found that cells with knocked-down activity of STK32B were more resistant to death induced by cisplatin. Consistently, A549 cells with STK32B knock-down showed lower caspase 3 activity following cisplatin treatment when compared with controls for knock-down. Thus, it appears that inhibition of STK32B would not increase sensitivity of cancer cells to chemotherapeutic agents.

We conclude, that STK32B protein may be worth studying due to its plausible role in chromatin physiology as judged by its localization.

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[I-2] IDENTIFICATION OF IRRADIATION-INDUCED ATM-DEPENDENT LNCRNAS

Marta Podralska[1], Marcin Sajek[1], Magdalena Żurawek[1], Iwona Ziółkowska-Suchanek[1], Marta Kazimierska[1], Marta Kasprzyk[1], Barbara Pietrucha[2], Bożena Cukrowska[2], Tomasz Kolenda[3], Agnieszka Dzikiewicz-Krawczyk[1]

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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.

7 mRNAs and 13 lncRNAs were significantly induced 1h after IR in healthy donors, whereas none in AT patients. 1059 mRNAs and 215 lncRNAs were induced 8h after IR in control group, while only 152 mRNAs and 22 lncRNAs in AT patients. Of these, 9 lncRNAs and 132mRNAs were common for both groups. Among IR-induced mRNAs were several genes with well-known function 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. ATM-dependent induction of 10 selected lncRNAs was confirmed by RT-qPCR.

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

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[I-3] ANALYSIS OF HCT116, K562 AND ME45 CELL TRANSCRIPTOMES TO COMPARE THE MECHANISMS REGULATING CELLULAR REDOX STATES

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Redox reactions maintain cellular homeostasis. Even a slight shift in redox balance can change the cell status from survival to death. Different cell types are characterized by different levels of reactive oxygen and nitrogen species (ROS and RNS) and can respond differently to the same stimulus. Cells may use distinct pathways to keep optimal ROS and RNS levels and sustain redox homeostasis. Different radicals can be converted and neutralized by different mechanisms, such as interaction of superoxide with superoxide dismutase which converts it to H_2O_2 or with NO with formation of ONOO-. H_2O_2 can be converted to H_2O by catalase, peroxiredoxins, and glutathione peroxidase. These neutralization pathways are not equally used by various cell types and some pathways may influence cell proliferation or be a specific response to oxidative stress.

To compare the main pathways responsible for redox regulation, we analyzed the transcriptomes of HCT116, K562 and Me45 cells assayed by the microarray method. Using GO terms such as oxide, oxidant, oxidative stress, superoxide, nitric oxide, hydrogen peroxide, ROS and reactive oxygen species 574 genes were found which are directly or indirectly engaged in redox processes. Comparison of the expression of these genes in K562, HCT116 and Me45 cell lines revealed possible differences in ROS neutralization pathways. For quick neutralization of superoxide to H_2O_2, HCT116 cells seem to use mainly the path engaging dismutase, and further peroxiredoxins and thioredoxins. K562 cells use catalase, and Me45 cells use NO with production of ONOO-, by inhibition of glutathione peroxidase and peroxiredoxins by inhibition of thioredoxins (reducing agent of peroxiredoxins) to cope with redox stress. This shows that depending on the cell line and the starting level of ROS/RNS and antioxidants, cells can differently cope with oxidative stress. Identification of the dominant antioxidant pathway/system in cancer cells might help in their suppression, which could help in improvement of some anticancer therapies.

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[I-4] ACSL4 EXPRESSION, A MARKER OF IRON DEPENDENT CELL DEATH – FERROPTOSIS, IS REGULATED BY MIRNA LET-7 IN COLORECTAL CANCER CELL LINE, HCT 116

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Ferroptosis known as a regulated cell death (RCD) plays an important role in a homeostasis in the body, both in physiological and pathological states, and could be nowadays a novel challenge in cancer studies[1]. Similar to other death forms, like apoptosis or autophagy, ferroptosis was also found as regulated by a specific non-coding RNAs (ncRNAs)[1,2]. Some from the miRNAs (miRNA let-7 family or miRNA 21) seemed to be especially focused in mediating the ferroptotic death, with the strong relation to apoptosis and autophagy[1,3]. Ferroptosis was characterized by the accumulation of lipid peroxidation products and ACSL4 activation with parallel down-regulated apoptosis *via* miRNA 21.

The aim of the research was to investigate the contribution of miRNA let-7 in regulation of gene ACSL4 expression (long-chain-fatty-acidCoA ligase 4), a ferrptosis marker under oxidative stress.

Materials and methods: HCT116 cells were transfected with a plasmid psiCHECK-2 (Promega) for reporter gene with miRNA let-7 binding regulatory site in the 3-UTR region. Cells were irradiated for reactive oxygen species (ROS) production, with ionizing radiation at dose of 4 Gy. At a different time points (1, 6, 12 and 24 hours after irradiation) levels of Renilla reporter gene for let-7, as well as the level of ACSL4 were determined by qRT-PCR reaction (BioRad). miRNA let-7 family levels were estimate using microarrays (Agilent). Finally, ROS were measured by flow cytometry (Aria III, DB).

Results and conclusion: We suppose, that lack or lowering cellular level of miRNA let-7 family positive regulates the ACSL4 time-dependent expression and stimulated ferroptosis pathway. However, irradiation enhanced miRNA let-7 production, what resulted with opposite - ACSL-4 silencing. Cells transfection with psiCHECK-2 plasmids decreased ROS level in comparison to the un-trasfected controls, what explain overall decreasing in other types of death, like apoptosis.

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[I-5] ROLE OF NITRIC OXIDE SYNTHASES IN PRODUCTION OF CELLULAR ROS AND RNS IN RESPONSE TO IONIZING RADIATION

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Introduction: Nitric Oxide Synthases (NOS) are group of enzymes that catalyses production of nitric oxide from L-arginine, with involvement of NADPH and in the presence of specific cofactors. One of these cofactors is tetrahydrobiopterin (BH4), a molecule essential for production of nitric oxide by NOS. The oxidised form of BH4, dihydrobiopterin (BH2), competes with BH4 for a binding site on the surface of NOS, and if BH2 is bounding the superoxide radical is produced instead of nitric oxide. We hypothesize that this switch mechanism is an essential part of the response of cells to oxidative stress and that its better understanding could provide vital information about regulation of cellular ROS and RNS levels in response to ionising radiation.

Materials and methods: A human melanoma cell line (Me45) was used for experiments. Superoxide radical and NO were detected by the fluorescent dyes MitoSOX Red and DAF-FM diacetate, respectively. Cells were irradiated with a 4Gy dose of ionizing radiation from a laboratory irradiator. Observations were performed at different times after irradiation on living cells in time-lapse fluorescence microscopy experiments. Computational analyses were performed with ImageJ and Matlab software.

Results and Conclusions: Control cells survived well and proliferated throughout the whole, 70h of microscopy observation. Irradiated cells showed a different behaviour; half the population died about 30h after irradiation and the other cells multiplied similarly to the control. To assess what are the sources of superoxide and nitric oxide in control and irradiated cells, the levels of MitoSOX and DAF-FM fluorescence in the same intracellular localisations were assayed. A short time after irradiation, a positive correlation between superoxide and nitric oxide levels in particular localisations was observed suggesting a very close distribution of their sources. The correlation efficient decreased with time, suggesting a change in the distribution of NO and O-. sources. The results suggest that the influence of radiation may cause reversible switching of the NOS enzyme from NO production to superoxide, which disappears with time.

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[I-6] MELANOMA-DERIVED SMALL EXTRACELLULAR VESICLES POTENTIALLY INDUCE ANTIGEN-SPECIFIC TOLERANCE

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Introduction: Cancer-induced immunosuppression is antigen-specific rather than systemic. We explored the option that tumor-associated antigens (TAAs) are transferred to antigen-presenting cells (APCs) through cancer-derived small extracellular vesicles (sEVs), such as exosomes. These vesicles might also carry immunosuppressive molecules to stimulate a suppressive APC phenotype concurrent with TAA uptake to yield antigen-specific tolerance.

Methods: sEVs isolated from melanoma cell cultures were used to demonstrate the transfer of major histocompatibility complex I (MHC I) molecules to the surface of APCs. The immunosuppressive influence of sEVs was assessed by flow cytometry analysis of activation markers on APCs, by quantifying their cytokine expression, and in mixed lymphocyte reactions.

Results: MHC class I molecules were transferred from melanoma cells to the cell surface of dendritic cells by melanoma cell-derived sEVs. Consequently, CD86 and CD40 co-stimulatory molecules were down-regulated and IL-6 production was strongly induced. TGF- transported by sEVs contributed to the observed effects on APCs. Furthermore, T cell proliferation in mixed lymphocyte reactions was inhibited in the presence of sEVs. Co-culturing sEV-treated APCs with autologous T cells increased the number of regulatory T cells.

Conclusion: The results support the existence of a mechanisms that explains antigen-specific tolerance induction to be mediated by cancer-derived sEVs that transfer MHC molecules to antigen presenting cells together with immunosuppressive molecules.

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[I-7] SENSITIVITIES OR RESISTANCE? A DIFFERENT POTENTIAL OF LEUKEMIA HL60 AND K562 CELL LINES TO FERROPTOSIS DEATH PATHWAY INDUCTION

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Regulated cell death (RCD) could be presented by mixed types of cell death associated with autophagy, apoptosis, necroptosis and ferroptosis in acute myeloid leukemia (AML) [1]. Ferroptosis, unlike to apoptosis or autophagy, is an iron-dependent cell death pathway and is characterized by the accumulation of lipid peroxidation products [2]. Some of the cell lines displayed more sensitive, like HL60, whereas the other one, K652 more resistant to ferroptosis induction profile [3].

The aim of the research was to characterize two cell lines HL60 and K562 after their potential to develop sensitivities or resistance to ferroptosis induction. Ferroptosis pathway, followed by aa specific indictor addition - Erastin, was examining by the ACSL4 (long-chain-fatty-acidCoA ligase 4) ferroptosis marker gene expression changes.

Materials and methods: Leukemia (K562 and HL60) cell lines were treated with two doses of Erastin (Sigma), 5 and 10 M. Tested cell lines were cultured under standard conditions in DMEM-F12 medium (PAA). After 24h of incubation cell lines were tested cytometricaly (Aria III, BD) for apoptosis (Annexin-V apoptosis assay) and ROS production (DCFDA/H2DCFDA, MitoSOX, DAF-FM). Cells proliferation and viability was evaluated by MTT test (Sigma). The ferroptosis marker ACSL4 were determined by qRT-PCR reaction (BioRAD).

Results and conclusion: Followed by Erastin addition the level of overall ROS increased in sensitive to ferroptosis HL60 cell line, whereas in K562 cells ROS level was similar to the untreated control. Pool of NO and superoxide anion did not correlate to the Erastin addition at higher doses, either. Without ROS waves the apoptotic death werent induced by Erastin. However, viability of Hl60 and K562 cells was reduced in presence of ferroptosis inductor at tested doses. Followed by MTT assay, a 40% of viability reduction for HL60 and about 20% for K562 cells were observed. Annexin-V apoptosis assay and cell cycle analysis confirmed with necrotic and sub-G1 fraction a higher sensitivities of Hl60 cells to ferroptosis induction. Finally, expression of ferroptosis marker, ACSL4 was down-regulated more in resistant K562, than in sensitive HL60 cell line.

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[I-8] HEAT SHOCK TRANSCRIPTION FACTOR 1 (HSF1) SUPPORTS ESTROGEN-INDUCED PROLIFERATION AND MIGRATION OF MCF7 CELLS

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Heat Shock transcription Factor 1 (HSF1) is ubiquitously expressed regulator of Heat Shock Protein (HSPs) expression in response to elevated temperature. Several studies on Hsf1-null organisms pointed on the HSF1 role in development and growth. A high level of HSF1 expression has also been found in a broad range of tumors and tumor cell lines. Its activity could be implicated in signaling pathways associated with growth and proliferation, apoptosis, glucose metabolism, angiogenesis, and cell motility.

We have found that 17b-estradiol (E2), the most potent estrogen, stimulates HSF1 phosphorylation in ER-positive (ER+) breast adenocarcinoma cells (e.g. MCF7). HSF1 gains transcriptional competence under E2 treatment and regulates the expression of several genes associated with E2 signaling. Therefore, we aimed to study whether HSF1 could contribute to E2-induced proliferation and migration. We down-regulated HSF1 expression in MCF7 by lentiviral transduction of shRNA specific for HSF1 or using the CRISPR/Cas9 editing system. In the case of HSF1 down-regulation, the remaining HSF1 (about 10-20%) was still able to effectively induce HSP gene expression under heat shock. Only complete HSF1 knock-out (obtained by CRISPR/Cas9 method) caused an inability to induce HSP gene expression in response to elevated temperature. Complete HSF1 knockout led also to decreased proliferation of MCF7 cells, although the luminal phenotype (EpCAM high/CD49f low) did not change. Interestingly, we noticed an increased population of CD44+ (cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration) cells after HSF1 knockout. To study the HSF1 role in MCF7 response to E2, wild-type and modified cells were treated with E2 for 12 days, and the migration ability was analyzed by Boyden chamber assay or live imaging microscopy. We found that the E2 increased the number of migrating wild-type cells, but the number of migrating cells was lower in population with HSF1 knockout. Live imaging microscopy revealed that E2 treatment stimulated the formation of membrane protrusions in wild-type cells and enhanced cell-cell dissociation. Amoeboid membrane protrusions were also observed in E2-stimulated cells without HSF1, but cell-cell dissociation was inhibited, thus these cells stayed in contact for a longer time.

Our results indicate that HSF1 could support E2-induced proliferation and migration of ER-positive breast cancer cells.

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[I-9] MORPHOLIDE AND METHYL ESTER OLEANOLIC ACID OXIME DERIVATIVES AND THEIR CONJUGATES WITH ASPIRIN MODULATE THE CELL CYCLE, PROLIFERATION AND APOPTOSIS IN HEPG2 HEPATOMA CELLS

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Hepatocellular carcinoma (HCC) is one of the leading causes of cancer related deaths and the sixth most prevalent cancer. Nonsteroidal anti-inflammatory drugs (NSAIDs) were suggested for liver cancer prophylaxis. Plant-derived oleanolic acid (OA) and synthetic triterpenoids possess anti-tumorigenic activities and ability to inhibit proliferation of human hepatocellular carcinoma cells (HepG2). Coupling OA conjugates with NSAIDs, such as aspirin (ASP) may enhance this effect.

The aim of this study was to evaluate the effect of oleanolic acid oxime (OAO) derivatives and their conjugates with ASP on the cell cycle distribution, level of proliferation marker Ki67 and induction of apoptosis in HepG2 cells.

HepG2 cells were incubated with OAO derivatives and OAO-ASP conjugates at the doses of 20 and 30 M for 24h. Muse Cell Analyzer was used to flow cytometric assessment of cells proliferation based on Ki67 expression, induction of apoptosis by Annexin V binding to cells externalizing phosphatidylserine and cell cycle progression by propidium iodide staining.

The most pronounced anti-proliferative and pro-apoptotic effect among OAO derivatives was observed as a result of treatment with compounds: **12**, 3-hydroxy-iminoolean-12-en-28-oic acid methyl ester and **18**, 3-hydroxy-iminoolean-12-en-28-oic acid morpholide differing, respectively, with methyl ester and morpholide groups at the C-17 position of OA molecule. Among the hybrids with ASP, compound **19,** 3-(2-acetoxy)benzoyloxyiminoolean-12-en-28-oic acid morpholide was the most efficient.

The highest percentage of resting cells (least proliferating cells exhibiting the lowest Ki67 expression) was found after treatment with OAO-morpholide and OAO-ASP carboxylic derivatives. The induced cell cycle arrest correlated with inhibition of cell proliferation and the highest accumulation of cells in S/G2 phases incubated with OAO-morpholide derivative. As result of treatment with this conjugate along with OAO-benzyl ester and OAO-ASP morpholide conjugates also the induction of apoptosis was observed.

These results indicate that OAO and OAO-ASP conjugates are more effective inhibitors of cell proliferation, cell cycle arrest and more effective apoptosis inducers than the parent compounds: OA and ASP. OAO derivatives showed a stronger effect against HepG2 cells compared with their conjugates with ASP. We can state that those compounds, especially morpholides, might be considered as a potential HCC chemopreventive and/or therapeutic agents.

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[I-10] TRANSFORMATION OF DMBA/TPA-INDUCED PAPILLOMAS IN THE SKIN OF MICE LACKING EPIDERMAL ZC3H12A GENE

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Skin cancer is the most common form of cancer among white populations, with basal cell carcinomas and squamous cell carcinomas being the most frequent subtypes. There is a growing list of evidence showing that one of the main factor that induces skin tumor initiation and progression is inflammation. The key role of Monocyte Chemoattractant Protein-Induced Protein 1 (MCPIP1) is the control of physiological and pathological processes of inflammation through the degradation of pro-inflammatory cytokine transcripts. Recent studies show that MCPIP1 also acts as a modulator of processes related to tumorigenesis and cancer progression, particularly by regulating the rate of metabolism and angiogenesis.

To investigate whether MCPIP1 is significant for the initiation and development of skin cancer, we generated conditional knockout mice lacking gene encoding MCPIP1 (*Zc3h12a*) in the epidermis (Mcpip1EKO). Furthermore, we induced a multi-stage chemical carcinogenesis in mouse skin using DMBA/TPA factors.

Our preliminary studies show that Mcpip1EKO mice developed a large number of papillomas as early as 7 weeks following DMBA treatment, while the control mice developed only one or two lesions after 9 weeks of DMBA application. Moreover, we observed that the growth of the Mcpip1EKO papillomas was suppressed a few weeks after the initial onset, while in the control mice, the tumor growth was continued. In the control mice, we observed well-differentiated papillomas indicating their exophytic growth and the presence of keratin pearls. In contrast, the Mcpip1EKO mice showed a reduced exophytic growth of papillomas and the appearance of clusters of pigment-filled melanocytes. Moreover, RNA-sequencing analysis of genes expressed in control and Mcpip1EKO mice tumors indicated that 447 transcripts were up- or downregulated. Functional analysis by Gene Ontology enrichment annotation revealed that the genes upregulated in Mcpip1EKO were mainly assigned to the groups related to lipid/glucose metabolic processes and melanin biosynthetic process, while the most strongly downregulated genes were related to epidermis development and lipid metabolic processes.

In conclusion, our studies indicate that MCPIP1 plays a significant role in the kinetics of both, the initiation and progression of chemically induced skin tumors.

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[I-11] ASSESSMENT OF THE EFFECT OF DOXYCYCLINE-INDUCIBLE IDH1R132H EXPRESSION ON INDUCED NEURAL STEM CELLS DIFFERENTIATION TOWARDS NEURONS

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Heterozygous point mutation in *IDH1* codon 132 leading to the substitution of arginine to histidine (R132H) is a frequent phenomenon in gliomas. Mutated IDH1 has been suggested as an early event contributing towards tumorigenesis, but the exact mechanism underlying such function remains unclear. Altered protein is thought to induce deregulation of gene expression, including oncogenes, tumor suppressors, and genes involved in differentiation process. Inhibition of differentiation is correlated with tumor development. Research on the mutant IDH1 proteins mechanism of action requires appropriate experimental model. Taking into consideration the hypothesis regarding the potential role of neural stem cells in gliomagenesis, we propose a model of human induced neural stem cells with inducible expression of IDH1R132H for the assessment of mutant protein effect on neuronal differentiation.

Stable iNS cell line expressing IDH1R132H in response to doxycycline was generated. Cells were subjected to a 14-day neuronal differentiation in the presence or absence of doxycycline (1 g/mL; medium was changed every 48 hours with fresh doxycycline). The effect of IDH1R132H expression on differentiation was assessed by the means of immunocytochemical detection of neuronal-specific marker Map2. We did not observe Map2-positive cells with the elongated morphology in iNSc expressing IDH1R132H. Our observation is consistent with data reported in the literature and demonstrates negative impact of mutant IDH1 protein on differentiation.

Development of appropriate *in vitro* experimental models will enable broadening of the knowledge concerning phenomena crucial for cancer initiation. Established inducible model provides an opportunity to control the level of mutant protein and analyze its impact on cell physiology as well as its role in gliomas.

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[I-12] HOMODIMERIZATION AS THE MAIN MECHANISM OF EGFRvIII ACTIVATION

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Introduction: Mutations in gene encoding epidermal growth factor receptor (EGFR) and its overexpression are observed in many cancer types, e.g. glioblastoma multiforme, breast, prostate, and colorectal cancer. One of the most common mutations of this receptor is EGFRvIII and is typically found in cancer cells. EGFRvIII is unable to bind ligand since it results from the deletion of exons 2-7 encoding the extracellular ligand binding domain of normal EGFR (EGFRwt). The mechanism of EGFRvIII activation is the subject of scientific disputes. It has been reported that EGFRvIII may be present both in monomeric and homodimeric form (covalent and non-covalent). Our studies, already published in Oncotarget in 2018, showed that dimerization of EGFRvIII is induced by bond formation between free cysteine residues at position 16 of the amino acid chain of its monomers. The results were found controversial, since some experts alleged that we obtained an artificial model in which we had to add NaOVa (sodium orthovanadate, a protein tyrosine phosphatase inhibitor) to observe the phenomenon of receptor dimerization. Due to the fact that EGFRvIII dimerization mechanism may be of great importance in development of anticancer therapies, we proposed a new model exhibiting increased expression of EGFRvIII DK-MG super high cell subline.

Methods: Mechanism of EGFRvIII activation was investigated using DKMG super high cell subline. Proteins isolated from cells grown in monolayer or suspension were analyzed. Analyzes were performed using Western Blot. The effect of EGF on phosphorylation of receptor-critical tyrosine residues was determined and comparison of EGFRvIII dimer formation in cells grown in suspension or monolayer was made.

Results: Formation of EGFRvIII covalent homodimers was observed in both monolayer and suspension cultured cells. The outcome suggest that phosphorylation of Tyr1045 in EGFRvIII upon EGF stimulation was weaker when compared to EGFRwt. In addition, despite very high EGFRvIII expression and phosphorylation of tyrosine residues, there was no increased activation of downstream pathways observed in DKMG super high cells compared to sublines with lower EGFRvIII expression.

Conclusion: Using another research model, we proved that EGFRvIII forms covalent homodimers. We also observed that increased EGFRvIII expression and proper receptor phosphorylation do not cause excessive activation of effector pathways.

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This work is continuation of analyses published in Oncotarget Stec WJ, Rosiak K, Treda C, Smolarz M, Peciak J, Pacholczyk M, Lenart A, Grzela D, Stoczynska-Fidelus E, Rieske P. Cyclic trans-phosphorylation in a homodimer as the predominant mechanism of EGFRvIII action and regulation. Oncotarget. 2018; Jan 6;9(9):8560-8572.

[I-13] IDENTIFICATION OF MIRNAS WITH REGULATED BIOGENESIS IN B CELLS AND B CELL LYMPHOMA

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MiRNAs are small non-coding RNAs that are involved in the regulation of gene expression. The canonical miRNA biogenesis pathway includes 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. In the cytoplasm pre-miRNAs are further processed by Dicer to mature miRNAs. miRNA biogenesis is strictly regulated resulting in cellular-specific miRNA expression profiles. Differential miRNA expression is often observed in many types of cancer including B-cell lymphoma. In this study, we aim to identify miRNAs that undergo regulated processing in B cells and B-cell lymphoma cells. Identification of regulated miRNAs was based on the analysis of pri-miRNA and mature miRNA levels in 12 samples of normal B cells, Hodgkin lymphoma (HL) and Burkitt lymphoma (BL). We analyzed 662 miRNAs that were present on both the mature and the pri-miRNA microarrays and showed that for 190 miRNAs both pri-miRNAs and mature miRNAs were detected. Additionally, for 47 miRNA, we detected only mature miRNAs and for 144 we detected the pri-miRNA transcripts and not the mature miRNAs. Next, we compared the group of pri-miRNAs with no miRNAs between B cells, HL and BL samples and showed that 40% of them was shared by all samples and nearly 70% by six HL and BL cell lines. We also determined which miRNAs are present at the level of primary miRNA in both analyzed lymphoma types, but at the level of mature miRNA only in one of them. We indicated 22 miRNAs detected only in HL and 4 that were detected only in BL despite similar pri-miRNA levels in HL and BL cells. This implied differential processing of the 26 miRNAs between BL and HL cells. Together, this analysis suggest that approximately half of the pri-miRNAs are expressed, but may not be processed to mature miRNAs in B cells or B-cell lymphoma. These miRNAs may regulate biogenesis.

[I-14] MODULATION OF NF-KB SIGNALING PATHWAY BY JAPANESE QUINCE (CHAENOMELES JAPONICA) LEAF PHENOL EXTRACT LEADS TO ITS ANTI-INFLAMMATORY EFFECTS IN LPS-INDUCED RAW264.7 CELLS

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Introduction: Japanese quince is an ornamental plant, rich in many biologically active compounds that have positive effects on human health. Our research presents the regulation of anti-inflammatory activity through the NF-B signaling pathway in mice macrophage (RAW264.7) cells stimulated with lipopolysaccharide (LPS), treated with Japanese quince leaf phenol extract (JQLPE).

Materials and methods: Phenolic compounds from Japanese quince leaves were extracted with methanol and then purified by SPE method on Sep-Pak C-18 cartridge. Phenolic profiles were analysed by UPLC-MS-MS and individual compounds were identified based on their retention times, Uv-vis spectra (200-600 nm), in comparison with standard reference compounds. The quantitative composition of phenolic compounds was determined by the UPLC-DAD analysis.

Next, *in vitro* research was conducted on RAW264.7 cells stimulated with the LPS (1 ug/ml), which then were treated with JQLPE for 1h, 6h or 24h at the concentrations of 10, 25, 50 ug/mL. The regulation of anti-inflammatory activity through the NF-B signaling pathway was determined *via* the measurement of the expression of NF-B at the mRNA level using qPCR. Furthermore, we examined the expression of NF-B p65, p-NF-B p65, IB- and p-IB- at the protein level by Western Blot. The statistical analysis of obtained results was performed by using one-way ANOVA test.

Results: The chemical characterization of JQLPE components showed a total of 33 phenolic compounds, including mostly flavonols and phenolic acids. The main constituents of the extract were chlorogenic acid and naringenin hexoside. Their percentages among total phenolic (303.67 mg/g) were 36% and 10%, respectively. The treatment with JQLPE markedly reduced the mRNA expression of NF-B p65 by 79.19%, when compared to the LPS-induced RAW264.7 cells. Also, NF-B p65 expression at the protein level was downregulated by JQLPE, both after 1h and 24h of treatment (by 62.08%and 51.83%, respectively). Further analysis revealed that p-NF-B p65 expression was increased by the extract by 299.56% after 1h. Similarly, the protein level of p-IB- expression was up-regulated by 131.99%, and IB- protein expression was also upregulated after 1h of JQLPE treatment by 71.74%, when compared to the LPS-induced RAW264.7.

Conclusions: The results suggest that JQLPE can modify the NF-B signaling pathway to resist inflammation in LPS-stimulated RAW264.7 cells. Chlorogenic acid is the main compound of the extract that is responsible for observed effects.

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[I-15] MCPIP MEMBERS IN REGULATION OF KERATINOCYTES-RELATED PROCESSES

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Introduction: The MCPIP family consists of four members: MCPIP1/2/3/4, encoded by the ZC3H12A/B/C/D genes. All MCPIP proteins contain a single CCCH-type zinc finger domain and a highly conserved PIN domain with RNase activity. The most studied member of this family is MCPIP1, which regulates the stability of many transcripts, including proinflammatory cytokines such as IL-6, IL-8, and IL-12p40 and also its own transcript. MCPIP1 protein functions as a negative regulator of inflammation. Previous studies indicated that MCPIP1 expression positively correlates with the level of keratinocyte differentiation. Our recent studies on mice with keratinocyte ablation of MCPIP1 indicated that it plays an important role in the physiology and pathophysiology of the epidermis. However, the role of the other members of the MCPIP family in regulating keratinocyte-related processes remains largely unknown. It was reported that ZC3H12C is, similarly to ZC3H12A, upregulated in human psoriatic skin, suggesting potential involvement of MCPIP3 in modulating epidermal physiology. Recent studies indicated that MCPIP3, like MCPIP1, is an RNase, which can degrade mRNAs, such as Zc3h12a. The aim of this study was to evaluate expression of MCPIP3 in human keratinocytes in vitro and in human skin in vivo.

Methods: Transcriptional expression of MCPIP1 and MCPIP3 was compared in different keratinocyte cells: human primary keratinocytes (NHEK) and HaCaT cell line. Localization of MCPIP1 and MCPIP3 protein in human skin was evaluated by immunohistological staining of samples from healthy skin and of basal or squamous cell carcinoma biopsies. To silence MCPIP3 gene expression, keratinocyte cells were transfected with siRNA against MCPIP3 or no-sense siRNA (negative control). Keratinocytes differentiation was promoted using medium supplemented with Ca++. The mRNA and protein levels were studied by real-time PCR and Western Blot.

Results: In line with previous reports, immunohistological staining showed that MCPIP1 is mainly localized in suprabasal layers of the epidermis. In contrast, MCPIP3 is expressed predominantly in the basal layer of both healthy and cancerous human skin. Transcriptional expression of MCPIP1 and MCPIP3 is at similar level in both keratinocytes cells. In addition, the mRNA level of MCPIP3 is downregulated during keratinocytes differentiation in contrast to MCPIP1, which is upregulated. Treatment of keratinocytes with siRNA against MCPIP3 increases mRNA levels of transcripts related to skin inflammation, such as IL36, s100a7/a8/a9 and decreases level of some keratinocytes differentiation markers, such as Krt1, Krt10, Flg. Furthermore, silencing of MCPIP3 in keratinocytes leads to increased mRNA, but not protein level, of MCPIP1.

Conclusions: Our results suggest a potential novel role of MCPIP3 in the regulation of skin inflammation and keratinocyte differentiation.

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[I-16] HIGH-FAT DIET INDUCE PHENOTYPE CHANGE IN LIVER CELLS

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A high-fat diet is a big problem in our society, because it can lead to the development of many diseases, for example non-alcoholic fatty liver disease (NAFLD). The NAFLD is diagnosed when steatosis develop in a minimum of 5% hepatocytes and is currently considered as the most common chronic liver disease. The next stage of NAFLD is non-alcoholic steatohepatitis (NASH) which can develop into fibrosis or hepatocellular carcinoma. An inherent element of tumor progression is a change in cells phenotype. They break out of their ordered structure by switching the phenotype into a more invasive one. Thus in our study we check whether liver cells acquire mesenchymal features during NAFLD development.

To check whether high-fat diet can induce phenotype change, C57BL/6J mice were fed for 20 weeks with control and high-fat diet, then RNA and protein from liver were isolated and the expression of mesenchymal markers were checked. In mice fed a high-fat diet, the level of mesenchymal markers such as Slug, Snail or -katenin increases significantly. During *in vitro* studies hepatocarcinoma cells, Huh7, were stimulated with sodium oleate for various time points and the level of mesenchymal markers were measured. We have shown that the level of mesenchymal markers is higher in cells stimulated with sodium oleate compared to control cells. It can therefore be concluded that a high-fat diet leads to changes in cells phenotype into more invasive.

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[I-17] CELL SPECIFIC REGULATION BY MIRNA – A STUDY ON TWO HUMAN CANCER CELL LINES AND LET-7 FAMILY MIRNAS

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Introduction: micro-RNAs (miRNAs) are small 19-22 nucleotides-long RNAs that regulate more than 60% of messenger RNAs (mRNAs). One miRNA can influence many mRNAs, and one mRNA can be affected by many miRNAs. MiRNAs do not act as separate molecules, but interact with Argonaute (AGO) proteins forming the core of RNA-induced silencing complexes (RISCs). In human cells there are four types of AGO proteins (AGO1-4) forming a RISC complex which recognizes the target mRNA, normally in the 3UTR, by complementary base pairing. MiRNAs cause downregulation of mRNA expression by inhibition of translation or by mRNA degradation. In human and animal cells, most miRNAs only require a seed region of perfect complementarity with their mRNA target to inhibit translation or accelerate mRNA decay, and this property enables different miRNA interactions with the same seed. Let-7, the first discovered miRNA, is now known as a family containing 12 members encoded by genes on different chromosomes.

The aim of our study was to examine how members of the Let-7 family influence the expression of a reporter gene in different cell types.

Methods: the study was performed on two human tumor cell lines, HCT116 and Me45, which were transfected with a psiCHECK-2 vector containing a luciferase gene with Let-7 targeted sequences in its 3UTR or with a nonregulated control vector. To check the influence of particular Let-7 members, inhibitory oligomers complementary to Let-7a, Let7d, Let-7f, Let7g or Let-7i were co-transfected. The levels of luciferase mRNA and protein were assayed by RT-qPCR and a luciferase activity measurement kit.

Results: a non-regulated luciferase gene was expressed differently in both cell lines, showing a higher translation efficiency in Me45 cells. Transfection with a plasmid bearing a gene containing a Let-7 targeted sequence caused a decrease of luciferase mRNA in both cell lines, and a slight increase of protein level in HCT116 cells. Calculation of translation efficiency showed an increase in HCT116 cells and a decrease in Me45 cells due to Let-7 action. The cell lines differed in their expression of Let-7 family members, with highest expression of Let-7i in Me45 cells and of Let-7a and Let-7f in HCT116 cells. Experiments with inhibitors suggest that the two cell lines have different regulatory mechanisms, in Me45 cells mainly focused on mRNA stability and in HCT116 cells on regulation of translation.

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[I-18] THE EFFECT OF CELL CULTURE CONDITIONS ON THE NUMBER OF CD34+ PROGENITOR CELLS ISOLATED FROM ADIPOSE AND HEART TISSUES

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Mesenchymal stromal cells (MSC) are one of the most intensively studied cells in recent years. MSC have the therapeutic potential to repair damaged tissues and they exert an immunomodulatory effect on cells of the immune system. Despite substantial progress in research, the origin of these cells are still poorly known. Increasing data indicate that CD34+ cells are MSC entity *in situ*. The CD34 antigen is specific for many types of progenitor cells. It is possible that the disappearance of CD34 antigen occurs due to changes in the cells microenvironment.

The aim of the study was to investigate how culture conditions affect the number of CD34+ cells in MSC population.

MSC were isolated from two different tissues. The first tissue, the right ventricle fragments, were obtained from human hearts removed during heart transplant surgery performed at the Silesian Center for Heart Diseases in Zabrze, Poland. The second tissue, the subcutaneous adipose tissue fragments, were collected during planned surgery in Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Gliwice, Poland. The tissue fragments were minced and digested with collagenase NB4 solution. The suspension of hMSC (heart mesenchymal stromal cells) was placed in IMDM medium, aMSC (adipose mesenchymal stromal cells) in DMEM medium with high glucose concentration. The medium was supplemented with 20% or 2% concentrations of FBS. The cells were incubated at 37C in 21% (normoxia) or 5% (physoxia) concentration of oxygen. From the second to the ninth day of cell culture MSC phenotype was analyzed using a flow cytometry technique.

The presence of CD105 and CD34 antigens was analyzed within a population of cells negative for leukocytes and endothelial cells antigens (CD45-CD31-). On the second day after isolation, an average of 70% or 80% of hMSCs cultured in medium supplemented with 20% or 2% FBS respectively expressed CD34 antigen. A higher percentage of CD105+CD34+ cells was observed in the following days among hMSC cultured in medium supplemented with 2% FBS compared to hMSC cultured in medium supplemented with 20% FBS. At the same time, in both hMSC cultures decrease and ultimate disappearance of CD105-CD34+ cells took about 5-6 days. The same trend of changes in cell phenotype was observed among aMSC cells growing in medium with two FBS concentrations. Also higher oxygen concentration (21%) caused a faster decrease in the percentage of CD105+CD34+ cells compared to the cells cultured at 5% oxygen concentration.

We suggest that MSC could derive from CD34+ progenitor cells. In the early days of culture MSC lost CD34 antigen. *In vitro* culture conditions are important for the presence of CD34 antigen on the cell surface. MSC grown in medium with 20% FBS or incubated in 21% oxygen lost CD34 antigen faster than MSC grown in medium with 2% FBS or in 5% oxygen.

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[I-19] MCPIP1 THROUGH ITS RNASE ACTIVITY INHIBITS EPITHELIAL-MESENCHYMAL TRANSITION IN CLEAR CELL RENAL CELL CARCINOMA AND NORMAL KIDNEY CELLS

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Introduction: The negative regulator of inflammation is the MCPIP1 (Monocyte Chemoattractant Protein-1 Induced Protein), induced by many pro-inflammatory factors. The role of MCPIP1 focuses primarily on regulating the stability of transcripts coding for proinflammatory cytokines (IL-1, IL-2, IL-6) and suppresses microRNA biosynthesis via cleavage of the terminal loops of precursor miRNAs.

One of the factors that stimulate tumor development is inflammation. Inflammation is one of the inducers of epithelial to mesenchymal transition (EMT). During EMT clearly polarized epithelial cells with high expression of E-cadherin change their morphology and gain mesenchymal features enabling their invasion. An important step in the EMT process is an increased expression of Snai1/2, ZEB1/2 and Twist in order to reprogram gene expression in a cell. RNase activity of the MCPIP1 might be a connection between inflammation and EMT process during tumor progression.

Material and method: The level and distribution of genes and proteins were studied by real-time PCR, western blot and immunofluorescence staining. Microarray analysis was conducted on samples from tumors of patients suffering from ccRCC. Motility assays were performed to check migration and invasion. The level of miRNA was conducted by Next Generation Sequencing and real-time PCR. We used 6-week-old female NOD-SCID (Charles River Laboratory) mice injected subcutaneously as a suspension of Caki-1 cells with stable overexpression and mutant form of the MCPIP1. Tumor growth was monitored for 6 weeks then resection of tumors and lungs and analysis were performed.

Results and discussion: Our study showed that low expression of the MCPIP1 in ccRCC cells induces the acquisition of mesenchymal phenotype. In contrast, overexpression of MCPIP1 causes a decrease in Snai2, vimentin and -catenin and an increase in E-cadherin. The lack of RNase activity of the MCPIP1 causes an increase in mesenchymal markers, including -catenin, which is overexpressed in many tumors. We have observed that the loss of RNase activity increases migration activity. We have also shown a reverse correlation between the level of MCPIP1, IL-1, focal adhesion kinase (FAK) and Src kinase, which are associated with the activation of migration in tumor cells, both *in vitro* and *in vivo*. In addition, ccRCC tumor microarray analysis showed high levels of mesenchymal markers, that correlates with tumor progression. Based on the NGS results, we identified several miRNAs regulated by MCPIP1, that may be responsible for the EMT process by a reduction of inhibitors of the canonical WNT pathway dependent on -catenin.

Conclusion: The results indicate that the RNase activity of MCPIP1 is critical in the regulation of EMT and tumor progression. Our results may contribute to a better understanding of ccRCC progression, which may help in the development of more effective therapy in the future.

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[I-20] THE ROLE HSPA2 IN MAINTENANCE OF KERATINOCYTES HOMEOSTASIS

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HSPA2 (Heat Shock Protein A2) belongs to a highly conservative HSPA (HSP70) family and acts as a molecular chaperone. The main functions of HSPs in the cell are stabilizing of newly synthesized proteins and binding to misfolded proteins to prevent their aggregation in stress conditions. Nowadays, there is very little information about an expression pattern and role of HSPA2 in somatic cells. HSPA2 is detected in adrenal gland, bronchus, cerebellum, cerebrum, colon, esophagus, kidney, skin, small intestine, and stomach. Interestingly, in epithelial tissues, HSPA2 is expressed in a cell-type-specific manner. Namely, HSPA2 positive cells localized in the basal layer of the esophagus, small intestine epithelia and in skin epithelium the epidermis. Undifferentiated basal keratinocytes are characterized by high expression of HSPA2. Furthermore, we have recently shown that reducing the level of HSPA2 in spontaneously immortalized epidermal keratinocyte HaCaT cell line may commit them to terminal differentiation.

This work was aimed at extending the current understanding of the HSPA2 role in epidermal keratinocytes. In this work, we used HaCaT cells and immortalized human foreskin keratinocyte *Ker-CT* cell line. Using lentiviral vectors we established stable cell lines characterized by up- and down-regulation of HSPA2 expression. Taking into account that calcium, via impact on keratinocyte proliferation, cell-to-cell adhesion and epidermal barrier homeostasis is considered the main regulator of keratinocyte differentiation we searched for a potential relationship between HSPA2 and calcium homeostasis. We found that any change in extracellular calcium concentration leads to alterations in HSPA2 level in keratinocytes. An increase in extracellular calcium concentration in culture medium led to an elevation in HSPA2 level in unmodified *HaCaT* and *Ker-CT* cells. We also found that the level of intracellular calcium in *Ker-CT* was sensitive to changes in HSPA2 expression. The level of intracellular calcium was decreased in cells deficient in HSPA2 in comparison to control cells. Additionally, we found that HSPA2-deficient Ker-CT cells showed reduced membranous expression of integrin 1, an adhesion protein involved in the formation of focal adhesions, as well as integrin v5, a receptor for vitronectin. Of note integrin-dependent adhesion of keratinocytes is calcium-dependent.

Our results suggest that HSPA2 via regulating calcium homeostasis in keratinocytes may have a direct impact on keratinocyte adhesiveness to extracellular matrix components. However, further research is needed to explain the mechanism of HSPA2 impact on keratinocyte biology.

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Poster session II:
New molecules
and experimental therapies

[II-1] BIOACTIVE POTENTIAL OF DICLOFENAC DERIVATIVES IN LONG-TERM ANTIMICROBE AND ANTICANCER STUDIES AGAINST E.COLI SP. AND HCT116 CELL LINE

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Phthalocyanines (Pc) are a group of chemical compounds which are structurally analogous to naturally occurring porphyrins [1]. Due to a chemical structure of these compounds they can be applied in many research fields. They are used as dyes, photosensitizers in medicine (PDT) [2], as chemical sensors [3] and catalysts [4]. The latter plays an increasingly significant role in the use of phthalocyanines in the pro-ecological aspect e.g. when it comes to detection and degradation of environmental pollution. Diclofenac (DNF) is a widely used nonsteroidal anti-inflammatory drug. Diclofenac in the presence of iron octacarboxyphthalocyanine and hydroxyl radicals (HO) (from H2O2) undergoes a transformation into diclofenac-2,5-iminoquinone (DNF-2,5-IQ) causing distinct changes in the UV-Vis absorption spectrum.

Material and methods: Evaluation *in vitro* of DFN derivatives against prokaryotic *E. coli* species and eukaryotic cell lines HCT116 w.t. and p53-knock down were estimated by viabilities assays (24 h optical density and 7 days MTT assay, respectively). The dose-dependent activity of acid- and dimer-product of DNF were confirmed with long-term live microscopic observations using JuliStage system (NanoEnTec).

Results and conclusions: Dose-dependent activity against bacterial Gram-negative streams, *E. coli* and cancer cells HCT116 (w.t. and p53-/-) were obtained for DNF derivatives, after 24 h and in long-term observations. Biological activities seemed to be promising and further analysis of anticancer potential will be discussed.

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[II-2] THE ROLE OF NEW MELPHALAN ANALOGUES IN THE INDUCTION OF CELL DEATH (APOPTOSIS OR AUTOPHAGY) IN LEUKEMIA CELLS

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Chemical modification of known, effective drugs is one method to improve the chemotherapy. Thus, the object of our experiment was to provide a melphalan derivatives having an improved cytotoxic activity in human cancer cells (HL-60 and THP-1). For this purpose, we have synthesized several melphalan derivatives modified in its two important functional groups. Group of tested new compounds consist of 4 analogs that included: melphalan compounds modified only at carboxyl group: methyl and ethyl esters of melphalan (EM-MEL, EE-MEL) and melphalan compounds modified at both functional groups (EM-MOR-MEL, EE-MOR-MEL).

We checked what mechanisms of cell death are activated by the studied analogues. For this purpose we evaluated the abilities of melphalan derivatives to induce autophagy (measurement of autophagic vacuoles) and apoptosis (Annexin V/Propidium iodide double staining method, activity of caspase-8 involved in the external pathway of apoptosis, caspase- 9 involved in the internal pathway of apoptosis and activity of caspase -3). We compared the results with their parent drug.

Our investigations clearly revealed that all tested new forms of melphalan demonstrated high apoptosis-inducing ability in cells of acute monocytic and promyelocytic leukemia. In THP-1 and HL-60 cells, mainly an increase in caspase-9 activity was observed. This suggests the dominance of the internal apoptosis pathway. We also examined another cell death mechanism-autophagy. However, neither melphalan and the derivatives tested dont induced autophagy in leukemia cells.

To sum up, our study showed that all the melphalan derivatives demonstrated high apoptosis-inducing ability. This is the preferred type of cell death, as it is a physiological process that does not cause inflammation. All investigated derivatives caused significant changes compared to melphalan treated cells, which indicates that proposed modifications may serve as a potent drug therapeutic system.

[II-3] GLYCOCONJUGATION AS A STRATEGY TO IMPROVE THE CYTOTOXICITY PROPERTIES OF QUINOLINE AND URIDINE DERIVATIVES

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Glycoconjugation is a strategy for coupling sugar derivatives and aglycons (usually with biological activity) by creating a covalent bond. By this treatment, a molecule with better bioavailability, selectivity and solubility can be obtained. Therefore this strategy is widely used in research on the development of new drugs.

The aim of our work is the synthesis of glycoconjugates derivatives of 8-hydroxyquinoline or uridine as new potential anti-cancer drugs. Their therapeutic potential is due to the ability to coordinate divalent metal cations, such as Cu(II) ions, which act as a growth factor for many types of cancers. In addition, these compounds can act as inhibitors of glycosyltransferases metal-dependent enzymes involved in the formation of a glycosidic bond in the living system. Disorders of the glycosylation process generate incorrect exchange of information between cells, which leads to pathogenesis. For this reason, their activity should be controlled.

We have developed various methods of connection suitably functionalized derivatives of 8-hydroxyquinoline or uridine with derivatives of D-glucose and D-galactose. Some of the obtained combinations have shown significant cytotoxicity against the tested cell lines at the micromolar level, compared to their parent compounds. The structural modification of glycoconjugates used in our research is the replacement of the oxygen or nitrogen atom in the sugar anomeric position by a sulfur atom. Compounds with *S*-glycosidic bond are less susceptible to enzymatic degradation, especially under the action of glycosylhydrolases. Thiosugars due to their enzymatic stability may exhibit great therapeutic potential. Structural modifications of glycoconjugates have been further enriched by introducing into the linker structure a 1,2,3-triazole ring, which acts as both a link between molecules important for obtaining biological activity, as well as an additional element capable of coordinating divalent metal ions.

Here, we present the results of the preliminary cytotoxicity tests of the new group of glycoconjugates against selected cancer cell lines (HCT 116, MCF-7) and normal human dermal fibroblasts (NHDF-Neo). Some of the new compounds appeared to be active on the tested cell lines, being at the same time less toxic for healthy cells. The effect of various protecting groups for the activity of glycoconjugates was also investigated, which allowed determining the effect of compound lipophilicity on their biological activity. The most active compounds will be subject to further detailed biological studies to determine the mechanism of their action.

[II-4] DEVELOPMENT OF GENETIC DRUG FOR HUTCHINSON–GILFORD PROGERIA SYNDROME THERAPY USING A NEW CELLULAR MODEL

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Hutchinson-Gilford progeria syndrome (HGPS, OMIM #176670) is a rare genetic disorder, that causes a wide range of symptoms connected with premature aging. HGPS is caused by *de novo* point mutation (C1824T) in the *LMNA* gene, in the region coding for exon 11. *LMNA* codes for lamin A and C proteins, which are structural components of the nuclear envelope. Lamin A and C are responsible for nuclear shape maintaining and take part in chromatin organization. The mutation leads to cryptic splice site creation in lamin A pre-mRNA, incorrect mRNA splicing, deletion in mature mRNA and synthesized protein. The product of synthesis is then called progerin, its lack of signaling sequence important for appropriate post-translational maturation. In lamin A, post-translational modification includes farnesylation of the C-terminus, and its cleavage after transport into the nucleus. Because of the deletion, progerin C-terminus remains permanently farnesylated and attached to the nuclear membrane. Accumulation of progerin disturbs the nuclear lamina organization, its structure, and function. Accordingly, nuclear shape change, abnormal chromatin organization, and gene expression are observed.

We decided to develop gene therapy based on the long-term silencing of progerin expression at the mRNA level. Up to now, strategies to treat HGPS were based on decreasing the progerin protein level, farnesylation inhibition, or both. A cellular model of HGPS was created by transduction of HeLa cells with retroviruses encoding GFP, GFP-lamin A or GFP-progerin. The designed cellular model allows us to perform fast and easy genetic drug screening by measurement changes of fluorescence intensity in living cells, as well as by other typical biochemical analysis.

We designed a set of siRNA sequences able to recognize progerin mRNA at the joint of exon 11 and 12, all of them were tested in our model system. Specificity and efficiency of siRNAs were evaluated by changes in cells fluorescence intensity and protein level measured with flow cytometry and western blotting respectively. siRNA treatment was effective to decrease GFP-progerin level up to 25% in comparison to non-treated cells, measured by flow cytometry. Moreover, the level of endogenous lamin A and C was not affected in HeLa cells after siRNA treatment, as western blotting analysis has shown. Drugs previously shown in the literature to be effective in the improvement of the phenotype of HGPS fibroblasts were also analyzed in our model cells.

Selected siRNAs will be overexpressed in HeLa sublines and HGPS patients fibroblasts as miRNA under promoter recognized by RNA polymerase II to achieve a stable decrease of progerin mRNA level. We would also test gene therapy with drugs to estimate the efficiency of combination treatment. The last step will be the production of AAVs coding selected miRNA sequences to test them in the mouse model.

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[II-5] THE COMPARISON OF HADSCS ADMINISTRATION METHOD IN MURINE MODEL OF HINDLIMB ISCHEMIA

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Mesenchymal stromal cells (MSCs) are multipotent cells, which modulate immune responses and stimulate tissue regeneration. Our previous study indicated that MSC isolated from human adipose tissue (hADSCs) administrated locally into ischemic limb, through secreted IL-6 stimulate the M2 macrophages responsible for repairing damaged muscle and forming new blood vessels. The aim of present work was the comparison administration of human ADSCs in murine model of hindlimb ischemia into ligated and contralateral limb.

Unilateral femoral artery ligation was performed in quadriceps muscles on males of the C57BL/6NCrl mouse strain. hADSCs were administered into the gastrocnemius muscles: a) locally into hindlimb where unilateral femoral artery ligation was performed, b) of contralateral limb, where unilateral femoral artery ligation was absent. Muscles were collected and homogenized 48 hours after hADSCs administration. To identify the cytokines secreted by hADSCs in mouse muscles Human XL Cytokine Array Kit was used. Additionally, at 7th and 14th days of the experiment the muscles were obtained, fixed in liquid nitrogen and stained using immunofluorescent antibodies.

Human ADSCs injected into muscle, both locally and into contralateral limb, secrete mainly the same cytokines and growth factors, including: macrophage migration inhibitory factor, adiponectin, extracelluar matrix metalloproteinase inducer, nexin and urokinase receptor. Immunofluorescent assessment of the quadriceps muscles extracted at 7th and 14th days showed that capillary density was significantly increased in both hADSCs groups compared to control (PBS). No significantly difference in the number of vessels was observed between contralateral and locally administration of hADSCs. However, in the gastrocnemius muscles after locally administration of hADSCs in 7th and 14th days, more new blood vessels were formed in comparison to contralateral administration. There was no differences in the influx of F4/80+CD206+ macrophages in the muscles where unilateral femoral artery ligation was performed (quadriceps) in both hADSCs groups. However, the influx of F4/80+CD206+ macrophages in gastrocnemius muscles was significantly higher in locally administration compared to contralateral.

In conclusion, our data show that administration of hADSCs into contralateral limb show a similar therapeutic effect as locally administration in muscle where unilateral femoral artery ligation was performed (quadriceps), but there are differences in gastrocnemius muscles. This observation might potentially lead in the future to an attractive novel strategy of administration of the mesenchymal stromal cells.

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[II-6] THE ROLE OF MACROPHAGES IN NEW BLOOD VESSELS FORMATION IN WOUND HEALING PROCESSES

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During wound healing, the formation of new blood vessels plays a major role. In the first phase of wound healing, an increase in the number of blood vessels is observed (angiogenic phase). In the next phase, the number of blood vessels is reduced (regression phase). In the last stage, the architecture and functionality of the vessels are restored (remodeling phase). The density of blood vessels returns to its original state. M2 (immunosuppressive and proangiogenic) macrophages seem to play an important role in the formation of new blood vessels.

The induced mouse hind limb ischemia was an experimental model of wound healing. Unilateral femoral artery was ligated. C57BL/6NCrl mice were divided into two groups: the first one represent a group were physiological processes of wound healing were observed. The second group received 1x106 adipose derived stromal cells (ADSC) injected into injured muscle. After 24h, 3, 7, 14,21 days post injury (dpi) the mice were sacrificed. The obtained muscles were subjected to either immunofluorescent or flow cytometric analysis.The number of blood vessels, (isolectin B4+), was higher in a group of mice which received ADSC at each time point of the experiment. In injured muscle not subjected to any treatment, the physiological processes of revascularization occurred (the angiogenic and regression phase). However, in muscles after ADSC administration, at the 7th dpi the peak of IB4+ cells occurred and lasted until 21 dpi, without visible regression phase. The number of proliferating endothelial cells (Ki67+IB4+) was the highest at the 3rd and 7th dpi in both groups, but still at the elevated level at the 14th and 21th dpi in muscles injected with ADSC.The influx of the inflammatory cells (macrophages and neutrophils) to the site of the injury was diminished in the muscles injected with ADSC. However, ADSC at the injury site directed macrophages into anti-inflammatory, pro-angiogenic M2 phenotype (F480+CD206+). Further, macrophages (CD68+) in the injured muscles changed their morphology over time. Initially, after the wound induction, the macrophages were large with irregular shape, scattered over tissue. Subsequently, they became more infiltrating, forming a clusters. At the end of the healing processes macrophages began to acquire the shape of pericytes or cells which stabilize blood vessels.

The recovery of the muscle following femoral artery ligation is accelerated when ADSC are injected into the injury site. The new blood vessels appear faster and their density is higher. It might be due to a reduction of inflammatory cells infiltration and polarization of the macrophages into M2 phenotype. It causes the environment more favorable and the healing processes are accelerated. It seems also that recruited macrophages may take over the role of pericytes or even endothelial cells and stabilizes the new blood vessel formation.

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[II-7] ENHANCING THE CYTOTOXIC EFFECT OF OLAPARIB WITH ATR AND CHK1 KINASE INHIBITORS IN HR DEFICIENT CELL LINE

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Ovarian cancer is considered as one of the most lethal gynecologic malignancies worldwide. It is the seventh most common cancer and the fifth leading cause of cancer-related deaths. As a result of no formal screening and still lacking early detection methods, the majority of patients are diagnosed at an advanced stage (III/IV). The 5-year survival rate of high grade serous ovarian carcinomas (HGSOCs) still ranges between 35 and 40%.

The initial, standard-of-care, adjuvant chemotherapy in epithelial ovarian cancer is usually a platinum drug, such as cisplatin or carboplatin, combined with a taxane. However, despite surgical removal of tumour and initially, high response rates to first-line chemotherapy, around 80% of women will develop cancer recurrence. Effective strategies are necessary to improve prognosis. Replication stress response (RSR) is characteristic for tumours development, including ovarian cancer. Hence, RSR pathway and DNA repair proteins have emerged as a new area for anticancer drug discovery. Although clinical trials have shown that PARPi is responsible for tumour suppression, but not for complete tumour regression. Recent reports suggest that cells with impaired HR activities due to TP53 or specific DNA repair proteins mutations are specifically sensitive to ATR inhibitors. Moreover, replication stress activates DNA repair checkpoint proteins (ATR, CHK1), which prevent further DNA damage. Inhibition of ATR or CHK1 may, therefore, amplify DNA replication fork instability and promote cell death on the path of synthetic lethality.

The aim of the study was to determine the cytotoxic effect of the ATR kinase inhibitor (AZD6738; ATRi) and CHK1 inhibitor (MK8776; CHK1i) in combination with PARP inhibitor (AZD2281; PARPi; olaparib) in HR deficient ovarian cancer cell line PEO1 (grade 3, HGSOC). PEO1 carry a mutation in the BRCA2 gene. The cytotoxicity was determined with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and clonogenic assay.

PARPi, ATRi and CHK1i monotherapy decreased cell viability however, ATRi had the most cytotoxic effect. PARPi in combination with ATRi and CHK1i decreased the cell viability by around 30%, compared to PARPi monotherapy. The clonogenic assay confirmed the results of the MTT assay.

Preliminary studies suggest that simultaneous delivery of PARPi with ATRi or CHK1i can have a high cytotoxic effect, decreasing the cell viability of BRCAmut ovarian cancer cell line. The drug synergism in all tested combinations was observed.

[II-8] INFLUENCE OF RADIATION SHIELD PLACED IN TISSUE CELL CULTURE ON THE EFFECTIVENESS OF CONTACT DOSE IN RADIATION THERAPY

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The aim of the project is to investigate the effect of the shield placed on the way of ionizing radiation on the effectiveness of radiation therapy and the type of induced cell death. Materials such as titanium implants, amalgam fillings or other implants seem to have an impact on the effectiveness of radiation therapy. These materials scatter ionizing radiation and may increase the effectiveness of contact dose in radiation therapy. Therefore, when calculating the radiation dose, the presence of such materials should be taken into account.

Our previous study indicated that the number of living cells after irradiation depended on the dose. The most cells after irradiation were in G2/M blockade. Some of them underwent apoptosis. Dose dependent senescence/autophagy occurrence was observed and all of the irradiated cells following G2/M blockade start to proliferate (repopulation) in a dose dependent manner.

The present research is trying to explain, on a cellular model, the effect of the shield on the effectiveness of inducing cell death.

We examined the effect of lead sheath on the effectiveness of radiation therapy. The shield was placed under and over the irradiated cells. It has been shown that a lead shield especially placed under irradiated cells decreased the number of cancer cells. It increased the double-stranded DNA damages and increased the number and size of lysosomes (autophagy) in treated cells. In addition, placement of the shield delays the process of tumor cell repopulation.

It therefore seems important to consider the impact of different barriers in the radiation path in the calculation of the proper dosage of patients irradiation.

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[II-9] METHACRYLATE COPOLYMERS AS CONTRAST AGENTS IN 19F MRI

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Magnetic resonance imaging (MRI) is one of the newest methods of medical imaging. It allows to easily diagnose pathological changes without the need to interfere in the connective tissue.

The classic 1H MRI imaging method needs help in the form of contrast agents (CA) due to low signal intensity and background signals[1,2]. However, despite the CA application, there is the problem of a long time of imaging and the lack of complete removal of background signals[3].

19F is the most similar nuclei to the normally used 1H so that makes this promising way to replace the conventional method. Thanks to the use of fluoride in MRI, it is possible to completely eliminate ambient noise. CAs must meet several requirements such as water solubility, high signal intensity and high fluorine content. Examples of the CAs contemplated are perfluorinated hydrocarbons, paramagnetic complexes or polymers containing organofluorine groups. Polymer CAs contain in their structure a large number of fluorine nuclei per one molecule, but there are problems with their solubility in water. Another important problem is the possibility of expelling the given CA. Due to the limited capacity of the kidneys, polymer contrast agents with the lowest possible molecular weight should be prepared. The ATRP reaction turns out to be useful here, which, due to the possibility of regulating the rate of radical generation, allows to obtain low-molecular polymers.

The copolymers containing DEAEMA groups and tFEMA with variable proportions (66%, 34%, 20%, 14%, 12%, 11% and 1%) were synthesized and characterized by the 1H NMR and 19F NMR. It was noted that with the increase in tFEMA content, the intensity of the received signal increases and the relaxation times T1 and T2 significantly decrease. The effect of pH on the solubility of molecules in water was also investigated. As the tFEMA content increases, the solubility in aqueous solutions decreases. In the case of high levels of fluorine in the polymer, an acidic pH must be used. Due to the very large differences in solubility between pH differing by only one, it is possible to use the obtained polymers as pH sensitive probes.

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[II-10] APOPTOSIS AND AUTOPHAGY IN T98G CELLS WITH KNOCKDOWN OF MIR-210 GENE EXPOSED TO EPIGALLOCATECHIN GALLATE

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Introduction: Glioblastoma multiforme (GBM) is the most aggressive of the gliomas, a tumors arising from glia or their precursors within the central nervous system. Treatment with chemotherapy, radiotherapy or surgical resection often turns out to be a failure and most patients with GBMs die of their disease in less than a year. Searching for other methods and substances which may prolong patients lives and improve the quality of the persons life, is a difficult task, but highly desirable. It was proved that over 60% of human genes, which are involved in carcinogenesis are regulated by microRNA (miR). One of them, *miR-210*, has been identified as regulated by hypoxia and thereby driving the expansion and growth of the tumor. It has been noted that the knockdown of the *miR-210* in the cell line of the glioma induces cell death processes. A lot of research is focused on both searching for other microRNA or connecting *miR-210* with other substances, which would sensitize the glioma to chemo/radiotherapy. Epigallocatechin gallate has been suggested as a substance which causes cell death processes in glioblastoma cell line T98G. In the following study the influence of epigallocatechin gallate on the processes of apoptosis and autophagy in glioblastoma cell line T98G with the knockdown of *miR-210* has been described.

Methods: In order to analyze cell death processes in glioma cells, the *miR-210* gene was silenced and cells were treated with Epigallocatechin gallate with a concentration of 10 M for 24 hours. The analysis of apoptosis was performed by the flow cytometer using Vybrant DyeCycle Violet/SYTOX AADvanced Apoptosis Kit (Thermofisher) Analysis of the process of autophagy was done using LysoTracker Red marker. After isolating RNA from the cells, the quantitative PCR reaction was performed. The conducted reaction was based on specific, designed primers KiCqStart SYBR Green Primers (Sigma Aldrich) and GoTaq 1-Step RT-qPCR System (Promega) set.

Results: The results have shown that apoptosis and autophagy occurs in exposed to epigallocatechin gallate cells with *miR-210* knockdown. The intensity of fluorescence median in exposed to epigallocatechin gallate cells with *miR-210* knockdown was 2 times more intense than in control cells. Additionally, the analysis has demonstrated that cells exposed to epigallocatechin gallate with *miR-210* knockdown halved the necrotic cell population, which is a desirable effect of cancer treatment. The high expression of mRNA related to autophagy (e.g. *BECN1* control cells 105,5 vs. *BECN1* analysis cells - 109) proves that the presence of this phenomenon is a response to *miR-210* gene knockdown treated with epigallocatechin gallate.

Conclusion: Concluding, epigallocatechin gallate may have potential in significance in glioblastoma multiforme treatment.

[II-11] INHIBITOR OF GLYCOGEN SYNTHASE KINASE 3 (CHIR-98014) INCREASES PRO-APOPTOTIC POTENTIAL OF ANTICANCER DRUGS

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Introduction: Apoptosis is a cellular process that occurs in the physiological and pathological conditions. It is also a form of cell death, which is one of the best studied topics among cancer cell biologists due to its role in cancer therapy. Generally, apoptosis can proceed in several pathways: intrinsic pathway (mitochondrial), extrinsic pathway (receptor-initiated), pathway using perforin and granzyme B and pathway using the endoplasmic reticulum. Glycogen synthase kinase-3 (GSK3) is one of enzymes participating in regulation of apoptosis although its role in this process is not clear because it has the capacity to either increase or decrease the apoptotic threshold. GSK3 belongs to the serine/threonine protein kinases, which has an important role in numerous biological processes including: glucose regulation, cell survival, Wnt signaling and a lot of regulatory mechanisms, which are probably unknown yet. Generally it is considered that GSK3 promotes cell death caused by the intrinsic apoptotic pathway, but also inhibits the extrinsic apoptotic signaling pathway. Currently, GSK3 inhibitors are in clinical trials as potential drugs for neurodegenerative diseases.

We have observed that two substances: actinomycin D and nutlin-3a induce a synergistic activation of p53 in different cancer cell lines and in normal human fibroblasts. We have detected that the specific inhibitor of GSK3 - CHIR-98014 in combination with A+N may sensitize cells to apoptotic cell death in cancer cells line of the different origin. We have measured the level of gene expression by transcriptome sequencing (RNA-seq) in nonstressed A549 cells and in cells exposed to A+N, A+N in the presence of CHIR-98014 and in cells treated only with the inhibitor. The analysis has shown that a CHIR-98014 prevented upregulation of several antiapoptotic genes, which were strongly stimulated by treatment with A+N. Based on this observation we have developed hypothesis according to which CHIR-98014 can sensitize cancer cells to apoptotic death induced by chemotherapeutic agents.

Methods: The cells in culture U-2 OS (osteosarcoma), A549, NCI-H292 and NCI-H1299 (lung cancers) have been treated with: actinomycin D, nutlin-3a and CHIR-98014. We have measured apoptosis by flow cytometry and Western-blotting (WB). The results of RNA-seq have been validated by Real-Time PCR. The experiments on the combination of chemotherapeutic drugs and CHIR-98014 for the induction of apoptosis has been studied by WB. Additionally, we have tested the role of GSK3 in apoptosis of A549 cells using versions with knocked-out expression of either GSK3 or GSK3.

Results: We have confirmed our hypothesis. The co treatment of cells with CHIR-98014 sensitized them to the proapoptotic activity of various chemotherapeutic agents. The observed effect could be associated with the increased phosphorylation of GSK3.

Conclusions: This recently identified substance CHIR-98014 can promote proapoptotic activity of anti-cancer drugs.

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[II-12] VARIABLE POSITION OF DISULFIDE BRIDGES IN EGFRvIII MONOMERS AND COVALENT DIMERS CAN NOT ADVERSELY AFFECT L8A4 BASED CAR-T THERAPY

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Introduction: Epidermal Growth Factor Receptor variant III mutation (EGFRvIII) arises as a result of amino acids deletion encoded by exons 2-7. This type of deletion involve an appearance a free cysteine in monomer. It was assumed to be a cysteine at position 16 (C16). Moreover C16 seems to be crucial in development of covalent homodimers occurs in EGFRvIII, an essential step in receptor activation. However, cysteines in other positions than C16 may also play an important role in the creation of EGFRvIII dimers. The variability in occurance of different covalent dimers arrangment, dependent on the engaged cysteine in particular position, may complicate the development the EGFRvIII dimerization blockers.

Methods: To investigate the disulfide bridge creation between particular cysteines the EGFRvIII mutants were created and overexpressed in AD293 cell line by lentiviral vectors. The semi-native Western Blot techniques with anti-total EGFR A10 and anti-EGFRvIII L8A4 antibodies was used to verification if changes in dimers arise may disturb the binding between antibodies and future potential blockers with EGFRvIII protein.

Results: Analizes of semi-native Western Blot results indicate that both A10 and L8A4 antibodies identify dimers and monomers emerging in all investigated cysteine mutants. Moreover there were no differences in the binding between antibodies according to the densitometric analysis.

Conclusions: Obtained results indicate that A10 and L8A4 antibodies recognized covalent dimers and monomers of EGFRvIII independently of cysteine bond and configuration. Flexibility in disulfide bond creation in monomers and dimers should not affect the CAR-T therapy against EGFRvIII positive cells.

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[II-13] SUGAR DERIVATIVES OF 3,4-DICHLORO-5-HYDROXY-2(5H)-FURANONE EXHIBITING ANTIPROLIFERATIVE PROPERTIES AGAINST BREAST CANCER CELLS MCF-7 IN VITRO

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Background: Carbohydrates (sugars) are organic compounds that occur naturally in nature. Sugars fulfill many functions in animal and plant organisms, but their most important feature is providing them with energy necessary to function correctly. Literature on the subject mentions many biologically active compounds containing sugar fragments in their structure. One of them is doxorubicin, an anthracycline antibiotic that exhibits anticancer properties. This compound is made of naphtacenchinone linked with an aminosugar daunosamine by an *O*-glicosidic bond. Research shows that this sugar has a significant influence on the intercalating properties of doxorubicin.

Our earlier research has confirmed that the derivatives of 3,4-dichloro-5-hydroxy-2(5*H*)-furanone (MCA) show selective anticancer activity towards non-small cell lung cancer A549. In connection with that we have synthesized a number of MCA derivatives containing a sugar unit (glucose or rhamnose) in their structure. Most of the obtained compounds have better antiproliferative properties against breast cancer cell line MCF-7 than the basic compound not conjugated with sugar.

Materials and methods: The cytotoxicity of derivatives of 2(5*H*)-furanone was marked through the MTT assay, and the impact of the derivatives on the cell cycle has been determined by flow cytometry. Long-term proliferative properties were tested through clonogenicity.

Results: The compounds with silyl group in C-5 position of the 2(5*H*)-furanone ring arrests the cell cycle in G2/M phase but when we added additional propalgyl group cells were blocked in G1 phases.

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[II-14] ANTICANCER ACTIVITY OF DENDROBEANA VENETA CELL-LESS CELOMIC FLUID

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Introduction: Recent years discovered an effective anti-cancer substances from the natural animals sources. One of the directions is use of earthworm celomic fluid (CF). World literature suggests, that cell-less celomic fluid possesses an anti-tumor potential [1]. We decided to check those scientific reports, using a *Dendrobaena veneta* celomic fluid against cancer HCT116, A549 and normal BEAS-2B cell lines. Biological activities of CF was additionally tested after 70 C of heating to enhance/activate the components with anticancer potential.

Material and methods: Commercially obtained, adult (marked as size no. 4) specimens of the earthworm *D. veneta* were used. The earthworms were kept in the original boxes in laboratory conditions. Three days before CF harvesting, earthworms were placed on clean, moisturized paper towels to clean their digestive system. After this, earthworm (one by one) was placed in falcon tube with 0,5ml of 0,9% NaCl and was electro stimulated with 9V, that caused the release of CF from distal and proximal pores. Obtained CF was centrifuged at 4629 g for 10 min at 4 C, to separate CF from cells and any other contaminations. One portion of CF was heated up to 70oC (HtCF). Cell-free celomic fluid was sterilized by filtration through 0.22 m Millipore filters, we also estimated protein concentration with the Bradford assay. Following protein concentration, for both control and heat treated CF, were used: 500, 250, 125, 62,5 and 31 g/ml. Anticancer activities were tested against cancer HCT116, A549 and normal BEAS-2B cells (ATTC) using 1-72 h MTT assay (Sigma), cytometric cell cycle and apoptosis analysis with ROS level estimation (Aria III, BD). Additionally, long-term single-cell live microscopic observations for morphological changes and ROS production were made using JuliStage apparatus (NanoEnTec).

Results: 72h MTT assay has revealed that all cell lines were affected by CF. The highest cell mortality was observed after incubation with CF, heating changed activity of CF with lowering the selectivity against cancer. Over 90% viability reduction, at doses 500, 250, 125 and 62,5 g/ml, and about 85% at 31g/ml was observed. Heating of CF caused selective, but rather protective action against HCT116 and A549 cells, than normal BEAS-2B. ROS production 1 h after CF addition was higher, than in untreated control HCT116 cells, what could be a good prognostic for anticancer usage of celomic fluid with pro-oxidative potential. Further microscopic ROS visualization with cytometric pro-apoptotic measurements confirmed biological activities on cell cultures *in vitro*.

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[II-15] ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS AS CARRIER OF ONCOLYTIC MYXOMA VIRUS FOR THERAPY OF MURINE PANCREATIC CANCER

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Introduction: Protective carriers are beneficial for the effective administration and delivery of oncolytic viruses, which are a great promise of anti-cancer therapy as they are able to trigger a oncolysis and elict antitumor immune response. Thanks to the easy procurement, tropism to inflammatory sites like tumor beds, mesenchymal stem cells derived from adipose tissue (ADSCs) are prospective cell carriers for oncolytic viruses, such as myxoma virus (vMyx).

Aim: Assessment of ADSCs usefulness for therapy of experimental murine pancreatic cancer using a myxoma virus construct (vMyxEGFP)

Methods: Permissiveness of isolated ADSCs and two pancreatic carcinoma cell lines (human Panc-1 and murine Pan02-luc) for vMyxEGFP was examined using fluorescence microscopy. Cytotoxicity of vMyxEGFP for the tested cells (ADSCs, Panc-1 and Pan02-luc) was determined using MTS viability test. Spread of vMyxEGFP infection from ADSCs to co-cultured Panc-1 or Pan02-luc was observed under fluorescence microscope at 4, 24 and 48 h post infection. Possible inhibition of tumor growth in pancreas head of C57BL/6 mice was monitored with bioluminescence imaging (BLI) following orthotopic implantation of Pan02-luc cells that had been previously co-cultured with vMyxEGFP-infected ADSCs.

Results: vMyxEGFP replicates in ADSCs, Panc-1 and Pan02-luc cell lines. At 24-hour time point post infection the survival of ADSCs was 87% (MOI=10) and 90% (MOI=5), respectively, whereas viability of pancreatic carcinoma cell lines exposed to vMyxEGFP diminished significantly. vMyxEGFP-infected ADSCs were shown to transmit the infection to pancreatic carcinoma cells in vitro thereby confirming their suitability for oncolytic in vivo virotherapy studies. Orthotopic injection of co-cultures of Pan02-luc cells with vMyxEGFP-infected ADSCs has shown a potent reduction effect upon induction of pancreatic tumor growth.

Conclusion: vMyxEGFP-infected ADSCs confirmed potential usefulness as a Trojan horse system for experimental therapy of pancreatic cancer.

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[II-16] MYXOMA VIRUS-LOADED MESENCHYMAL STEM CELLS IN EXPERIMENTAL ONCOLYTIC THERAPY OF MURINE PULMONARY MELANOMA

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Introduction: Oncolytic viruses can target neoplasms triggering oncolytic and immune effects. Their delivery to tumors remains challenging. Oncolytic myxoma virus (MYXV) was transferred to melanoma cells using bone marrow-derived mesenchymal stem cells (MSCs).

Methods: MSCs were infected with MYXV recombinant construct encoding fluorescent proteins to investigate *in vitro* transfer of virus to co-cultured murine or human melanoma cell lines. Cytarabine (Ara-C) was used to determine involvement of input and/or viral progeny in such transfer. Inhibition of melanoma foci formation in lungs of C57BL/6 mice was studied following intravenous injection of B16-F10 cells previously co-cultured with MYXV-infected MSCs. Bioluminescence imaging (BLI) in tissues was performed in mice bearing melanoma pulmonary foci using MSCs loaded with MYXV encoding firefly luciferase. Treatment of experimental melanoma lesions in lungs was studied using MSCs loaded with IL-15-encoding MYXV construct. After three weeks lungs were dissected into individual lobes, and visible tumor foci counted. Innate and adaptive immune response to treatment was assessed by examining NK, CD4+ and CD8+ cells whole blood and single cells suspensions from lungs.

Results: MSCs were sufficiently permissive to MYXV yet transferred input and progeny viruses to melanoma cells leading to rapid killing of cancer cells. Inhibitory effect on melanoma foci formation in murine lungs was revealed using melanoma cells previously co-cultured with MYXV-infected MSCs. Virus accumulation and persistence in lungs of lesion-bearing mice was shown in mice administered intravenously with MSCs preinfected with luciferase-encoding MYXV. Therapy of experimentally-induced melanoma lung lesions with IL-15-carrying MYXV construct delivered by MSCs led to their marked regression. Increased NK cell percentages in blood indicated robust innate responses only against unshielded virus. Lung infiltration by NK cells was followed by inflow of CD8+ T lymphocytes into melanoma lesions.

Conclusion: MSCs allow for efficient ferrying of therapeutic MYXV to pulmonary melanoma foci.

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[II-17] THE EFFECT OF A DIFFERENT DOSES OF BRACHYTHERAPY ON INHIBITION OF MURINE MELANOMA TUMORS GROWTH

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Radiotherapy (RT) is one of the main treatments for cancer patients. It is applied for approximately 60% of all newly diagnosed patients as a frontline therapy. Radiotherapy uses high energy radiation for local cancer treatment. It destroys cancer cells. Despite many studies conducted, optimal dose and RT regimen are not clearly determined. More knowledge is needed about the effects of irradiation doses in anticancer therapy. It may help in the optimization of RT in patients treatment.

In this study we examined the effect of various doses of brachytherapy (contact radiotherapy): 2Gy, 5Gy, 10Gy and 15Gy on the inhibition of the growth of B16-F10 murine melanoma tumors. Brachytherapy were performed in the shielded therapeutic room with high-dose-rate afterloader equipped with iridium-192 radioactive source (Microselectron, Nucletron) in the Brachytherapy Unit. We checked how selected doses of RT affect the immune cells in tumors of treated mice compared to control mice. Using immunofluorescence (IHC, FACS) analysis we examined the levels and phenotypes of tumor-infiltrating immune cells.

We observed that brachytherapy in a single dose of 2Gy slightly inhibits the growth of B16-F10 murine melanoma tumors compared to the control group. Furthermore, in a single dose of 5Gy treatment, the tumors growth were more reduced compared to the 2Gy and untreated group of mice. There were no significant differences in the level of tumor-associated macrophages (TAMs) and CD8+ T lymphocytes infiltrating tumors after these doses. The most effective tumor growth inhibition was observed in the group of mice that received a brachytherapy in a single dose of 10Gy or 15Gy. In tumors of mice treated with these doses, the lowest levels of TAMs with pro-tumor M2 phenotype were observed. A single dose of 10Gy also significantly increased the level of tumor-infiltrating CD8+ T lymphocytes.

In summary, our results indicate that brachytherapy inhibits the growth of murine melanoma tumors. Tumor growth inhibition was dependent on the dose of radiation. It seems that a single dose of 10Gy most effectively increases the level of CD8+ T lymphocytes and reduces the level of M2 TAMs. This leads to a significant inhibition of tumor growth. The obtained preliminary data requires further research and analysis.

This work was performed within the framework of the project no. UMO-2018/31/D/NZ5/01754 financed by the National Science Centre (Poland).

[II-18] DUAL CHEMOTHERAPEUTIC DRUGS AND IRE1-A INHIBITOR THERAPY IN RH30 AND A204 RHABDOMYOSARCOMA

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Introduction: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma amongst children and adolescents. Standard anti-RMS therapy comprises of vincristine, dactinomycin, irinotecan, and cyclophosphamide. Negative effects on the functioning of the endoplasmic reticulum, brought by chemotherapy regimen, are eliminated by the unfolded protein response (UPR), which initially compensates for damage, allowing for prolonged cancer cell survival. In this study, we investigated the role and molecular mechanisms of IRE1- inhibitor and chemotherapeutic drugs in RH30 and A204 Rhabdomyosarcoma cell lines.

Methods: Anti-proliferative effects of MKC886 (as an IRE1- inhibitor) in the presence and absence of antineoplastic drugs on two RMS cell lines - RH30 and A204 - were evaluated by MTT assay. The effect of MKC8866 and chemotherapeutic drugs on cell cycle progression was examined using flow cytometry. The expression levels of ER stress and apoptosis regulatory proteins were measured by western blotting.

Results: Significant differences in cell viability were observed between cell treated with standard RMS chemotherapy, and also those treated with MKC8866. The combination of chemotherapeutic drugs and MKC8866 induced apoptosis in the cells. Furthermore, western blotting results presented accumulation of proteins participating in the UPR pathway upstream of IRE1- and decrease of expression of proteins downstream of IRE1-.

Conclusions: Our results are in line with novel drugs on RMS chemotherapy. Inhibiting UPR interferes with the RMS defense mechanism and sensitizes cells to chemotherapy. Further studies need to verify the possibility of IRE1- inhibition on an *in vivo* model.

[II-19] PREPARATION OF SELECTED QUINOLINE DERIVATIVES USING N-OXIDES

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This work focused on refining the syntheses of selected quinoline derivatives using N-oxides and comparing them with syntheses of quinoline derivatives that do not contain an oxygen atom associated with a nitrogen atom. Oxidation reactions of the starting substrates led to corresponding N-oxides. Then, the obtained quinoline basis was subjected in turn to nitration and to reduction reactions. Aluminum TLC plates with silica gel and F254 indicator were used to monitor the progress of the reaction, and they were visualized using a UV lamp (254 nm). ^{1}H NMR spectra were recorded using a Bruker AM-500 spectrometer (500 MHz). Chemical shifts are expressed in ppm. As part of this study, quinoline, quinaldine and 4-methylquinoline N-oxides were obtained. Nitro derivatives were obtained, which were then reduced to amines. Based on theoretical and experimental data the obtained compounds might play a potential role as anticancer or anti-tuberculosis drugs and could serve as precursors for the synthesis of more complex structures of interest to pharmacology.

Poster session III:
Bioinformatics
and mathematical modeling

[III-1] THE PDBRT DATABASE: A COMPREHENSIVE COLLECTION OF DRUG-TARGET STRUCTURES WITH RESIDENCE TIME DATA

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The residence time of a drug in its molecular target is becoming a key parameter in the design and optimization of new drugs, as it can reliably predict drug efficacy in vivo. In 2006 Copeland introduced the drug-target residence time concept which dictates a significant proportion of pharmacological activity in vivo [1]. The traditional in vitro methods perceive drug-target interactions only in terms of the affinity in equilibrium; the residence time concept takes into account the conformational dynamics of the target molecules that affect the binding and dissociation of the drug [2]. Experimental approaches to binding kinetics and target ligand complex solutions are currently available, but known bioinformatics databases do not usually store information about the drug residence time in its molecular target. The Protein Data Bank Residence Time (PDBrt) is a free, non-commercial repository for 3D protein-ligand complex data with measured drug residence time inside binding pocket of the specific biological macromolecules deposited in the Protein Data Bank [3]. The database is implemented using Python/DRF/HTML/CSS and contains information about both the protein and the drug separately as well as the entire complex and time of drug residence inside the protein. Collected dataset consists of ca. 150 crystallographic structures of protein-ligand complexes with known drug-target residence time (measured using experimental methods) and can be crucial for many computational or machine learning studies on drug binding/unbinding in biological systems.

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[III-2] MOLECULAR HETEROGENEITY OF PAPILLARY THYROID CANCER: COMPARISON OF PRIMARY TUMORS AND SYNCHRONOUS METASTASES IN REGIONAL LYMPH NODES BY MASS SPECTROMETRY IMAGING

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Introduction/Rationale: Mass spectrometry imaging (MSI) characterizes and visualizes molecular profile of tissue samples. A key advantage of MSI is its spatial registration, which allows to conduct comparative analyses between histologically-defined regions of tissue samples (region of interest, ROI) or analyze molecular heterogeneity within particular tissue region. Here we used a MSI technique to analyze phenotypic heterogeneity in papillary thyroid cancer (PTC) and compared molecular profiles of primary tumors located in the thyroid gland and synchronous metastases of cancer in regional lymph nodes.

Methods: MSI dataset of 11 tissue samples derived from postoperative material of patients with PTC was analyzed to assess molecular differences between four types of ROIs (primary tumor, metastasis and two types of normal tissue: thyroid gland and lymph node). Standard spectra preprocessing procedure was applied and the Gaussian mixture model approach was used for peak detection. Normalized dot-product of two mass spectra (pairwise similarity index) was calculated to assess spectra similarity. Pairwise similarity index was calculated in three different manners: (i) within the same type of ROI and within the same specimen, (ii) between different types of ROI within the same specimen, (iii) within the same type of ROI among different specimens. Populations of computed similarity values were plotted as cumulative distribution functions (CDFs) to visualize similarities between spectra among and between analyzed ROIs. An effect size analysis was applied to indicate discriminatory molecular components and unsupervised spectra clustering was performed. In parallel, tryptic peptides were identified using a shotgun LC-MS/MS approach in lysates from corresponding tissue samples to enable hypothetical annotation of components detected by MALDI MSI.

Results: Separate clusters generated by unsupervised segmentation of cancer ROIs from all patients dominated in tumor ROIs and metastasis ROIs, indicating molecular differences between both types of cancer regions. The graphs of CDFs evaluated at similarity values revealed the highest inter-patient heterogeneity for normal thyroid tissue and the lowest heterogeneity for normal lymph nodes. Furthermore, higher differences between primary tumor and its metastasis from the same patients in comparison to differences among tumors and among metastases from different patients were disclosed. The largest number of components with significantly different abundances were detected between normal thyroid and lymph node ROIs.

Conclusions/Novel aspects: Biostatistical approaches were applied to assess intra-tumor heterogeneity and molecular differences between primary thyroid cancer and its lymph node metastases in tissue samples imaged by MALDI-MSI. We concluded that phenotypical inter-tumor heterogeneity between primary tumors from different patients was lower than intra-tumor heterogeneity between primary tumor and lymph node metastases from the same patient.

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[III-3] PIECE-WISE LINEAR MODELS AS A TOOL FOR ANALYSIS OF HETEROGENEITY IN THE CELL RESPONSE TO EXTERNAL STIMULI

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Biological research is not trivial due to the high heterogeneity of biological systems, which can be observed on all levels of complexity. Heterogeneity of cell population means that cells deriving from the same cell line, living in the same environmental conditions, behave in different ways, especially activate different responses for the same external stimuli. For example, the same stress can activate programmed cell death (apoptosis), cell cycle blockade and DNA repair or in some cells no response. Process activation and deactivation are usually regulated by a variety of organic and inorganic molecules (enzymes, regulatory chemicals). Between cells occurs differences of the molecules concentrations, which induces differences in processes activation, that results in different cell response.

Mathematical modeling can serve as a support for biological experiments, by examinations of theoretical cell response and the possible influence of the altered system parameters. For a better understanding of the influence of change of protein concentration for enzymatic process activation, we propose application of systems with switchings with different threshold localizations. The system with switchings is a mathematical framework to model system, where its behavior abruptly changes with the state change. The threshold reflects the protein concentration at which the process is activated/deactivated. By changing the threshold values we can easily create the differentiated population, in which each cell activates process with slightly change protein level, which can be used to model differentiation in cell response to given stimuli.

Our approach we apply to the p53 regulatory module to investigate influence the differences in cell sensitivity to given regulatory protein levels on the cell response. The p53 model is quite simple, contains protein p53, PTEN and cytoplasmic and nuclear MDM2, which are linked by 2 feedback loops: positive and negative. The model predicts 3 different cell fates: normal (no response activation), cell cycle blockade and DNA repair (increased p53 level) and apoptosis (high p53 level). Decreased functionality of p53 (caused for example by mutation) results in increased p53 level needed for gene activation, which is modeled by an increased threshold value. Results show that such increase extends protein p53 oscillation and can prevent apoptosis activation. Similarly, we check how reduced PTEN functionality will influence on the protein response. If in cell high PTEN concentration is needed to activate positive feedback, the apoptotic response cannot be achieved. On the contrary, low activity of the MDM2, which is part of negative feedback, enables apoptosis activation. The higher MDM2 level is needed to activate degradation of p53, the easier high p53 level is achieved.

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[III-4] COMPREHENSIVE SURVIVAL ANALYSIS OF THE PATIENTS SUFFERING FROM THE MOST COMMON LUNG CANCERS – LUAD AND LUSC

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Survival analysis is nowadays one of the most common approaches in examining tumour risk factors and comparison of different cancer types. This work focuses on the survival analysis of the patients diagnosed with one of the two most common types of lung cancer – adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC). These are classified as environmentally driven tumours – their development is mostly a result of environmental exposures leading to DNA damage. The study of the factors affecting patient survival among specified cancer types has been originally supported by The Cancer Genome Atlas (TCGA) now continued as Genomic Data Commons (GDC) database.

In the presented study, six features, highly dispersed between those tumours, that could have an impact on the patient survival time were analysed. The feature list includes clinical data as patient's gender and age; and additionally the clone number, CNV abundance, MKI67 gene expression, and percentage of lymphocytes in a tissue sample. We used Kaplan-Meier survivor function estimator and Cox proportional hazards models. The preliminary analysis revealed that majority of the analysed features were differentially distributed in LUAD and LUSC. To exclude the possible impact of distribution differences, we performed features standardisation using standard and robust approaches. Then, we utilised the nonparametric method to estimate survivor function. For gathered data, using Kaplan-Meier survival function estimates and the Talone-Ware test we checked if there were any differences between survival curves. We found out that there were significant differences in the survival times between the analyzed patient populations. Therefore, we used Cox univariate and multivariate proportional hazard regression models, a semi-parametric method not assuming specific survival data distribution, to search for statistically significant predictors affecting the survival time. We identified MKI67 gene expression and clone number as essential factors, affecting survival time for both LUAD and LUSC. MKI67 gene expression level has not only a significant impact on survival time but also has great potential to predict the type of lung cancer. Our results show that there is a strong association between MKI67 gene expression and clone number, which is consistent with literature reports.

Keywords: Survival analysis, LUAD, LUSC, Lung cancer

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[III-5] SINGLE CELL AND CLASSIC RNA SEQUENCING – ANALYSIS AND OPTIONS FOR DATA INTEGRATION

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Single cell RNA-sequencing (scRNA-Seq) allows for separation and quantitative analysis of gene expression from separated cells. The method allows for unprecedented resolution while compared to classic RNA sequencing. Unfortunately, computational analysis of scRNA-Seq is not standardized and requires further development.

First, we used Seurat normalization and cell clustering package to identify cell types and expression levels in the 10X Genomics dataset consisting of 5527 peripheral blood mononuclear cells. Next we compared the results to classic GTEx bulk RNA-Seq dataset, which includes expression patterns in whole blood cells from healthy individuals.

We clustered cells from 10x Genomics dataset into 7 groups and precisely identified type of 4 of them, based on a list of canonical markers specific to particular cell types, eg. CD14 and LYZ for CD14+ monocytes. Cell types of 3 clusters remained ambiguous and further biological interpretation is needed to interrogate cell clusters. We observed that 12360 genes were expressed in GTEx whole blood dataset. 5814 of them displayed expression in a cluster which contained NK or CD8+ T cells. B cells expressed 3438 genes and DC cells expressed 5814 genes in common with GTEx. CD14+ cells cluster displayed expression of 4403 genes.

Two ambiguous clusters, which contained naive CD4+ T or memory CD4+ cells, expressed 2952 and 4670 of genes expressed in GTEx, respectively.

ScRNA-Seq allows for precise and credible analysis of gene expression in single cells. Integration of single cell data with classical RNA-Seq datasets is challenging but feasible, which provides the possibility to reuse data from prior experiments for new research.

[III-6] PETRI NETS VS ODE - COMPETITION OR COOPERATION? SYSTEMS APPROACH TO MODELING AND ANALYSIS OF THE CROSSTALK BETWEEN ATM, P53, AND NF-KB

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Until now, Petri nets and ordinary differential equations (ODEs) have been treated as an alternative approaches to modeling and analysis of biological processes. In this study, these methods are presented as complementary tools, which make it possible to perform a comprehensive structural and parametric analysis of a complex biological system. To demonstrate the benefits of such an approach a mathematical model combining ATM, p53 and NF-B signaling pathways was analyzed.

NF-B signaling pathway is responsible for early immune response while the p53 signaling pathway main role is to maintain genome integrity. After the DNA damage, detection module called ATM activates both p53 and NF-B. When activated, NF-B and p53 cooperate to determine the cells response to the threat and ultimately the fate of the cell.

The chosen biological system has been described using two methods: (i) Petri nets, and (ii) ODEs. In the case of the Petri net-based model two types of analysis can be distinguished: a structural analysis and simulation analysis. Structural analysis is mainly based on the analysis of t-invariants, the following methods can be distinguished: MCT set analysis, significance analysis (allows to distinguish which subprocesses are more crucial for the functioning of the modeled system, based on attendance frequency for each transition in all supports of t-invariants) and knockout analysis. Simulation analysis is related to a dynamics of the model (as a result an average number of firing of a transition can be obtained). Moreover, knockout simulation can be performed. In the case of the models described by ODEs one of the standard tools used to analyze them is sensitivity analysis (SA). SA methods can be used to identify critical regions in the parameter space and subsequently biological processes related to these parameters. Here we used a method of sensitivity analysis based on the frequency distribution of a model transient response that can be classified as an OAT SA (changing one factor at a time).

Based on the significance and knockout analysis for the Petri net model, we distinguished the most important elementary subprocesses. Similar result was obtained through sensitivity analysis for ODEs model. One of the most important elementary subprocesses in both analyzes are those related to DNA damage.

It turned out that while some of the results are similar for both methods, others are different. These differences arise from the limitations of both methods and thus, the use of a dual modeling approach allows for comprehensive analysis and enables a broader view of the modeled biological system.

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[III-7] STUDY OF THE SYNERGISTIC EFFECT IN MODELS OF COMBINED ANTICANCER THERAPY USING SECOND-ORDER SENSITIVITY ANALYSIS

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The synergy effect plays important role in a combined anticancer therapies, and denotes that the combined effect of two therapies is greater than sum of the effect of each therapy alone. For example, in combined radio-chemotherapy this effect usually means a radiosensilisation of tumor cells through by action of concurrent chemotherapy. Mechanism of radiosensilisation is quite complex, and therefore it is often omitted in mathematical models of radio-chemotherapy. But from the perspective of therapy planning, lack of the effect of radiosensilisation in these models may impair their predictive capability [1]. That why it is important to know which model shows the synergistic behaviour.

In this work we propose a numerical method to check the existence of the synergistic effect in models of combined therapy described by ordinary differential equations (ODE). Proposed method is based on the concept of second-order sensitivity analysis, and assumes numerical calculation of objective functions second derivatives with respect to two input signals representing different therapies. Sign of these derivatives calculated in particular times inform us about the existence of the synergistic effect in the analysed model.

We used proposed method to analyse different ODE models of combined radio-chemotherapy. Results shows that for models assuming pharmacokinetics, the synergy effect only occurs when the chemotherapy is delivered before radiotherapy.

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[III-8] SEARCHING FOR THE ALPHA-1,6-FUCOSYLTRANSFERASE INHIBITORS

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Fucosyltransferases (FUT) catalyze the transfer of L-fucose from GDP-fucose to various oligosaccharide-acceptor substrates. There is increasing evidence that the activity of this enzyme is important from the point of view of the physiology of the body, both in normal conditions and in pathological conditions. Many studies documented the prevalence and diagnostic value of FUT enzymes in human cancer. However, the molecular basis of these processes are not been fully understood yet. Therefore, it is necessary to obtain properly designed, active and selective compounds capable of modulating the activity of fucosyltransferases.

The research objective of this project is to discover and develop small-molecules for bacterial NodZ -1,6-fucosyltransferase inhibition. Although sequence homology of entire NodZto human FUT8 -1,6-fucosyltransferase is relatively low, key residues in GDP binding sites are generally the same, and thus we believe that NodZ can serve as a simple model system for inhibitors pre-selection and screening. Using a combination of advanced synthetic methods supported by computational chemistry, biochemistry, and cell biology techniques, it is planned to obtain a set of compounds that binds selectively to NodZ. Molecular modeling and *in silico* ligand optimization proceduresupport experimental part of the project and run in parallel according to project requirements.

The ligands structuresderivatives of L-Fucose were obtained according to synthetic sugar and chemistry methods and procedures developed in our laboratory.

For *in silico* part of the study we selected structures of *Bradyrhizobium sp.* NodZ-1,6-fucosyltransferase (PDB ID: 3SIX). Two missing loops in the vicinity of protein binding were rebuilt with Modeler software and AMBER suite was used to perform MD simulation. Next, every 10th frame of the simulation was used as receptor structure in docking of inhibitors molecules with AutoDock Vina software. Additionally the AQUA-DUCT was used to search for hotspots - places where water molecules spent most of the simulation time and the hotspots were compared with docked ligands to provide feedback on dynamic changes occurring during MD simulations.

Designed molecules have great promise as chemical probes for better understanding the biology and drugability of FUTs and they can be used in basic research on fucosylation and may serve as the basis for the development of new therapeutic strategies for diseases in which the fucosylation processes play a pivotal role.

The computational part of the work is supported bythe National Science Centre Poland (www.ncn.gov.pl) grant no DEC-2013/10/E/NZ1/00649.

[III-9] PAN-CANCER COMPARATIVE ANALYSIS OF MOLECULAR SIGNATURE BASED ON THE MALDI-MSI DATA

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Introduction: Mass spectrometry imaging is an analytical technique which allows measuring the spatial distribution of different molecules across different tissue samples. However, the comprehensive analysis examining the molecular structure of various cancer types has not been performed so far for MALDI MSI data. In this study, we present the preliminary results obtained in the comparative study of molecular images for tissue samples of six cancer types. An integrated picture of commonalities and differences among different tumour types (head and neck, testicle, intestine, thyroid, prostate, stomach) was constructed covering for both, inter-cancer and inter-patient heterogeneity.

Methods: The data came from the MALDI-TOF-MSI experiments using biological material from biopsies of different types of tissue, including cancer and healthy control. Spatially distributed spectra were collected in a mass range of 700-3500 Da. Preprocessing pipeline included: (1) data curation and spectra resampling, (2) adaptive baseline correction, (3) PAFFT alignment, (4) TIC normalisation, and (5) Gaussian mixture peak extraction and redundant components filtration [1]. Spectra clustering was carried out using hierarchical deglomerative clustering with Spearman distance and the k-means algorithm [2]. An analysis of similarities between tissues was carried out both within healthy and cancer tissues independently as well as for relative fold-change estimates.

Results: The preprocessed data set included 60,617 spectra and 2,439 peptides. The implemented algorithms allowed to analyse various substructures within cancer and healthy tissues and to find the molecular signatures for the observed biological processes. The results of k-means unsupervised clustering showed that both healthy tissue and cancer tissue is characterised by high molecular heterogeneity. Hierarchical clustering on the calculated fold-change values for each patient allowed to find groups of cancers for which the cancerogenesis causes similar molecular changes in the tissue. It was noticed that prostate and intestine cancer showed similar molecular characteristics. On the other hand, the stomach, thyroid, testis and head and neck cancers group together, too. Clustering performed across tumour tissues only showed that head and neck cancer, probably due to its very large internal variations, differs the most from the rest of the tumours.

Conclusions: The proposed approach allows identification and analysis of the molecular image heterogeneity of different hormone-dependent and environmental cancers. It also supports the discovery of molecular response to neoplasia despite a different biological origin.

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[III-10] INFLUENCE OF SAMPLE IMPURITY ON THYROID CANCER DIAGNOSIS BASED ON MALDI MASS SPECTROMETRY IMAGING DATA AND SIMULATED BIOPSY

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Rationale: Fine needle aspiration biopsy, while being the routine diagnostic procedure for thyroid cancer recognition, is difficult to analyze. However, enhancing it efficiently with a molecular classifier is hindered by impurity of the sample, which, due to the nature of specimen acquisition, is contaminated by non-malignant cells, in addition to other tissues. We use MALDI mass spectrometry data, containing information about spatial distribution of proteins and peptides, to assess *in silico* the influence of biopsy technique on diagnosis. We hypothesize that utilizing an impure dataset might be beneficial in terms of stable feature selection and classifier training.

Methods: We propose a biopsy model to generate simulated samples based on MALDI-MS imaging. We compare several methods of predictor selection and dataset construction to perform in-depth feature ranking stability analysis and obtain markers valid for the majority of sample composition spectrum. We estimate the optimal set dimensionality and use it to train some of the popular machine learning models in order to evaluate their performance depending on sample purity.

Results: Subsetting biopsy dataset according to cancer cell content proves to be an effective way to select features whose discriminative value is consistent even for samples with low purity. Consequently, models trained on those subsets outperform classifiers trained on a purified set (where cancerous and non-malignant tissue are perfectly distinguished) for contaminated samples, while retaining their accuracy for non-contaminated ones. In addition, we found that samples containing from 40 to 60 percent of cancer cells make for the most stable marker selection and model training. Both the observed feature rankings and classification quality show unequivocally that a training set containing cancer samples contaminated with normal tissue allows for building a model better suited for diagnosing biopsy specimens.

Conclusions: We show that the currently preferred tactics of maximal sample purification might be ineffective (and even superfluous) for constructing diagnostic classifiers targeted at fine needle biopsy specimens. Furthermore, we believe that our findings for mass spectrometry may be extrapolated to other types of molecular assays, for which obtaining complete spatial distributions is beyond the reach of contemporary technology.

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[III-11] IMAGE PROCESSING TOOLS FOR THE CEREBROVASCULAR MAGNETIC RESONANCE ANGIOGRAPHY IMAGES

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An extremely important part of the brains circulatory system is the circle of Willis. It forms arterial circulatory anastomosis which protects the brain from the ischemia. There are many anatomical variants of the circle. Changes in the normal morphology of the circle may condition the appearance and severity of symptoms of cerebrovascular disorders, such as aneurysms, infarctions and other vascular anomalies. Cerebral aneurysm often occurs in specific regions of the circle of Willis.

The aim of this work was to segment the artery cross sections from the magnetic resonance angiography images, process them in order to achiere the centerlines ot the arteries and their futher analysis and characterization. The second research aim was to perform the blood flow simulations for the circle of Willis part of the cerebral vascular system.

We analysed several cases of Time-of-Flight (TOF) MRI angiography data with selection of region of interest. In the process of image processing we use enhancing filters for tubular structures and a segmentation process. On the basis of successive centroids of the vessels its skeleton is being formed. Appropriate parts of circle of Willis ware selected manually and quantified.

Developed algorithm allows the creation of three-dimensional model of cerebral vascular network and is potentially useful for the diagnosis of various vascular system pathologies of the brain (e.g. cerebral aneurysm) as well as for the scientific simulations for blood flow or prediction of the drug distribution in the brain.

Developed tools of the angiographic images have a potent application in analysis of any kind of tissue vasculature, e.g. abdominal aortic aneurysm (AAA) which is characterized by a very high mortality rate.

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Poster session IV: Biomarkers

[IV-1] DDR SIGNALING PATHWAY GENE POLYMORPHISM – THE EFFECT ON PROGNOSIS IN RADIO(CHEMO)THERAPY-TREATED HEAD AND NECK CANCER PATIENTS

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The DNA-damage response (DDR) pathway is a system that monitors genome integrity and constitutes the first barrier against damage to the genetic material. In response to DNA damage, the DDR signaling cascade is activated which stops the cell cycle and triggers DNA repair. The DDR is controlled by ATM, ATR and DNA-PKcs protein kinases. The DNA-PKcs containing complex acts as a sensor for double strand breaks (DSBs) and its major role is promoting non-homologous end joining (NHEJ). ATM is responsible for global orchestration of the response to DSBs, i.e. repair, checkpoint activation, apoptosis, senescence, chromatin structure alterations, transcription and splicing. It is recruited by the MRN complex and responds mainly to DSBs, while ATR is activated by broad spectrum of DNA lesions and protects the integrity of replicating chromosomes. The most important DDR effector is tumor suppressor p53, which is stabilized and activated by ATM that leads to cell cycle arrest, senescence or apoptosis. BRCA1 is implicated in checkpoint activation and both NHEJ and homologous repair. Inhibition of the DDR increases sensitivity of tumor cells to radiotherapy and cisplatin-based chemotherapy. Single nucleotide polymorphism (SNPs) in key DDR genes may affect levels and activity of the encoded proteins and thus be of great importance in the response to the DNA damage-inducing treatment.

This work aimed to investigate the association between common variants in five DDR genes and therapy results in 422 head and neck cancer (HNC) patients treated with radiotherapy alone (RT) and cisplatin-based radiochemotherapy (RTCHT). Two SNPs in ATM gene were associated with the outcome in multivariate models. Carriers of ATM 1853A and -111A alleles had higher risk of loco-regional recurrence and metastasis. Using combined genetic predictors, we have shown that the presence of 0 unfavorable genotypes for DNA-PKcs 6721, intron 31 and TP53 72 conferred over 2-fold increase in risk of death. Patients with 3 adverse genotypes for ATM 1853,-111 and TP53 72 were at significantly elevated risk of local and loco-regional failure, while those with 2 risk genotypes for TP53 72 and BRCA1 871 had almost 1.5-fold higher risk of disease. When confronted with clinical factors, DNA-PKcs 6721 AA and intron 31 CC were independent indicators of inferior OS, while ATM 1853A and TP53 72G were independently associated with increased risk of loco-regional recurrence. Moreover, the ATM -111A/ATM 1853 A/TP53 72G combination was found to be the strongest independent risk factor for loco-regional failure.

Our data suggest that some polymorphic variants in DDR genes, especially in DNA-PKcs, ATM and TP53 may be significant factors influencing treatment outcome in HNC patients given RT and RTCHT. Our findings may in the future contribute to the modification of treatment guidelines in selected cases.

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[IV-2] INHERITED VARIATION IN MRN COMPLEX GENES CONTRIBUTES TO PROGRESSION IN UNRESECTED HEAD AND NECK CANCER TREATED WITH RADIOTHERAPY

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Radiation therapy and cisplatin-based chemotherapy are the mainstay in the treatment of head and neck cancer (HNC). Their mechanism of action is based on the ability to induce DNA damage, the most serious of which are double strand breaks (DSBs). DSBs are substrates for DNA repair proteins belonging to non-homologous end joining (NHEJ) and homologous recombination (HR) mechanisms. The MRE11-RAD50-NBS1/NBN (MRN) protein complex plays an essential role in detection and signaling of DSBs, in HR and NHEJ repair pathways, cell cycle checkpoint regulation, telomere maintenance, meiosis and immunoglobulin class switching. Inherited rare mutations in *NBS1/NBN* gene predispose to Nijmegen breakage syndrome a genetic instability disorder characterized by an increased radiation sensitivity and cancer risk. Numerous data indicate that common single nucleotide polymorphisms (SNPs) in various repair genes may modulate DNA repair capacity, cancer susceptibility and anticancer therapy response. Therefore, we assume that the MRN complex SNPs, by altering the activity and levels of the encoded proteins, may influence individual sensitivity to ionizing radiation, which translates into effectiveness of treatment, progression and prognosis in HNC patients.

The study aim was to assess whether functional polymorphisms in MRN complex genes affected treatment results and prognosis in 422 HNC patients receiving radiotherapy with or without cisplatin-based chemotherapy. The possible impact of the SNPs on overall (OS), local (LRFS), nodal (NRFS) and loco-regional (LRRFS) recurrence-free, disease-free (DFS) and metastasis-free (MFS) survival was analyzed individually and in combination. The TaqMan-MGB technology was applied for genotyping. The Kaplan-Meier method and Cox regression were used in survival data analysis. Out of 6 SNPs studied, three of them rs1805794, rs2735383 and rs1805787 were identified as independent risk factors for LRFS, LRRFS and MFS. Furthermore, from among all factors remained in the model, the rs1805794, rs2735383, rs180578 and rs21552787, as well as rs1805794, rs2735383, rs1805787 and rs2240032 combinations of unfavorable genotypes revealed to be the strongest independent risk predictors of local and loco-regional recurrence, respectively.

Presented data show for the first time that MRN complex genetic variation significantly affects therapy results in HNC patients treated with radiotherapy. The impact of the *NBS1* gene variants on risk of local and loco-regional failure was particularly pronounced in our dataset. These pilot observations imply that particular functional SNPs in DSB repair pathway may have the potential to constitute helpful predictive biomarkers in HNC standard therapies.

This work was supported by the National Science Centre (NCN), Poland, grant no. 2016/23/B/NZ5/03470.

[IV-3] DETECTION OF ITGBL1 MRNA ISOFORMS IN OVARIAN CANCER CELLS

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Previously, we have identified two molecular subgroups of high-grade serous ovarian cancer (OC) with distinct gene expression and survival [1,2]. Among differentially expressed genes was *ITGBL1* (Integrin beta-like1). We constructed two OC cell lines with complete coding sequence (cds) of *ITGBL1* (OAW42-ITGBL1 and SKOV3-ITGBL1); these cells had increased invasiveness [3] and migration rate, decreased adhesion [4] and no change in proliferation [5]. Later it appeared, based on computational data that 4 mRNA variants of *ITGBL1* may exist. Our aim was to evaluate these variants in OC cells.

RNA was isolated from 5 commercially available OC cell lines (OAW42, SKOV3, ES2, OVP10 and OVCAR3) and one cell line (OVPA8) established by our group [6]. We also analyzed OAW42-ITGBL1 and SKOV3-ITGBL1 lines, as well as OAW42-PLNCX2 and SKOV3-PLNCX2 control cells (empty vector). Primers for each mRNA isoform were designed based on its reference sequence from Nucleotide NCBI using Primer3 and Primer-BLAST. cDNA was synthesized and used for semi-quantitative PCR.

Variant 4 mRNA was present in low quantity only in ES2 cells. Variant 2 was absent or very low in all cell lines. Variant 3 was absent or very low in all cell lines except ES2 and OAW42-ITGBL1. Variant 1, containing all exons was prevalent in ES2 and OVPA8 cells. In OAW42, SKOV3 and OVCAR3 it was very low. It was extremely high in SKOV3-ITGBL1 and high in OAW42-ITGBL1. Very low level of variant 1 was observed in OAW42-PLNCX2 and SKOV3-PLNCX2.

Our previous results show that insertion of a construct with complete *ITGBL1* cds under control of CMV promoter influences phenotype of OC cells. These cells overexpress either transcript variant 1 or variants 1 and 3. As variant 1 is prevalent and variant 3 is second detectable in wild-type OC cells, our experimental model is well suited to study ITGBL1 function in OC.

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[IV-4] GERMLINE GENETIC POLYMORPHISMS IN CORRELATION WITH GASTROINTESTINAL SIDE EFFECTS IN BREAST CANCER CHEMOTHERAPY

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Introduction: Breast cancer (BC) is one of the most common malignancies in women. Chemotherapy is currently the standard treatment for patients with breast cancer. Unfortunately, the observed improvement in survival increases the incidence of toxicity and side effects in patients with breast cancer. Pharmacogenomic studies have elucidated the inherited nature of these differences in drug effects and showed that functional single nucleotide polymorphisms (SNPs) are one of the possible causes of the interindividual differences in clinical outcomes between breast cancer patients. Moreover, SNPs in genes encoding enzymes of the absorption, distribution, metabolism, and excretion (ADME processes) correlated with the treatment toxicity. The aim of this study was to establish the prevalence and spectrum of common germline genetic variants in genes modifying the ADME-related genes expression that may play a role in the toxicity and side effects of FAC chemotherapy. This study presents associations of 3UTR of ADME genes with gastrointestinal side effects and toxicity.

Material and methods: We analyzed genetic variations within 3UTRs of ADME-related genes in breast cancer women treated with FAC regime (5FU, doxorubicin, cyclophosphamide). The impact of the polymorphic variants on therapeutic toxicity was based on multivariate models based on 12 symptoms of toxicity or side effects. Blood form 305 breast cancer patients treated with FAC regime was collected for testing. Carriers of germinal mutations in BRCA genes characteristic of the Caucasus population were excluded from the study. Genotypes of 23 ADME-related genes were analyzed using RFLP-PCR and sequencing.

Results: The accumulation of adverse clinical and genetic factors increased the risk of gastrointestinal side effects. The pre-menopausal carriers of *UGT2B15* rs3100 had a higher risk of severe nausea (OR 5.76; 1.53-21.67; p=0.009). In the cumulative analysis, we observed risk gradation of nausea recurrent from OR 1.78; 0.66-4.80; p=0,25 in 2s factors carriers to OR 15.86; 0.87-289.61; p=0.06 (no statistically significant results) for carriers four unfavorable factors *ERCC4* rs2276464, *SULTA4A*1 rs138057, *DPYD* rs291593, *NOS3* rs2566508, *ALDH5A1* rs1054899.

In addition, the presence of *ABCC5* rs3805114 in pre-menopausal women increases the risk of vomiting (OR 4.01; 1.43-11.22; p=0.008). The simultaneous presence of two or three adverse factors *ABCB1* rs17064, *SULT4A1* rs138057, and premenopausal age increased the risk of severe vomiting (OR 15.97; 5.12-49.87; p 0.00001).

Conclusion: Our findings demonstrated that detection of germline alternations and clinical factors might be helpful as a complementary tool for the identification of increased risk of chemotherapy toxic and side effects.

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[IV-5] THE DEVIL IS IN THE DETAILS – ANALYSIS OF MUTATION IN A MODERATE-PENETRANCE GENES: CHEK2, PALB2, NBS1 OR NOD2 IN PATIENTS WITH BREAST CANCER

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Introduction: Breast cancer is one of the most common cancers in women. This cancer is a multifactorial disease caused by a combination of environmental and genetic predisposition. A hereditary predisposition to breast cancer significantly influences screening and follow-up recommendations for high-risk women. However, in patients with a suggestive personal and/or family history, a specific predisposing gene is identified in 30% of cases. Up to 25% of hereditary cases are due to a mutation in one of the highly penetrant genes (especially BRCA1 and BRCA2), which confer up to an 80% lifetime risk of breast cancer. An additional 2%3% of cases breast cancer are due to a mutation in a rare, moderate-penetrance gene, each associated with a twofold increase in risk, such as CHEK2, PALB2, NBS1. Although, many of works highlights the fact that only a small proportion of breast cancer is due to mutations in these genes, we think that the devil is located in the details and therefore our work is focused on this problem.

Materials and methods: 200 breast cancer patients aged 39 took part in the study. Each patient provided informed consent prior to venous blood collection for a genetic test. DNA was isolated from peripheral blood leucocytes. Carriers of germinal mutations in BRCA1 and BRCA2 genes were excluded from the analysis. The status of CHEK2 (1100delC, c.470C/T, c.444+1GA, del5395), PALB2 (c.509_510delGA, c.172_175delTTGT), NBS1 (c.657_661delACAAA,c. 643CT) and NOD2 (c.3016_3017insC) mutations was assessed by ASA-PCR, RFLP-PCR or multiplex PCR - techniques, respectively.

Results: Preliminary results showed that the frequency of mutations in the modulatory genes of breast cancer in young women with breast cancer is similar to frequency of high risk genes. In the examined group of women carriers of the modulator gene mutations constituted nearly 20% of the analyzed group. We identified 38 mutation carriers (19%) in exanimated young breast cancer women. The most common detected mutations were: 3020insC in NOD2 gene (6.8%), c.470C / T (5%) and del5395 (2%) in CHEK2 gene. In addition, we identified four carriers of mutations in the PALB2 gene and two in the NBS1 gene.

Conclusion: Analysis of mutations modulating the risk of breast cancer may result in better screening, prevention and therapeutic strategies for patients and their families. A better understanding of the genetic susceptibility spectrum and clarification of the risks associated with the breast cancer gene will enable further research project.

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[IV-6] SYNERGIC EFFECT OF CHEMO- AND RADIOTHERAPY COMBINATION OBSERVED AT THE SERUM METABOLOME LEVEL

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Objectives: Radiotherapy and chemotherapy induce systemic molecular changes that could be detected at the level of bio-fluids. Understanding how human metabolism is influenced by these treatments is crucial to predict the individual response and adjust personalized therapies. Here we aimed to compare profiles of metabolites in serum of head and neck cancer patients treated with concomitant chemo-radiotherapy, radiotherapy alone or induction chemotherapy.

Materials and methods: Serum samples were analyzed by a targeted quantitative approach using combined direct flow injection and liquid chromatography coupled to tandem mass spectrometry. This strategy allowed simultaneous quantification of 149 metabolites including 37 amino acids and biogenic amines, 18 acylcarnitines, 80 glycerophospholipids, and 14 sphingomyelins.

Results: Concomitant chemo-radiotherapy induced faster and deeper changes observed in the serum metabolome during and after the end of treatment than radiotherapy alone. However, chemotherapy alone did not induce significant changes, which suggested that the difference between chemo-radiotherapy and radiotherapy is not a simple additive effect. Moreover, the systemic response to treatment measured at the level of serum metabolome was observed faster than acute radiation toxicity estimated based on morphological and functional parameters.

Conclusions: Combination of chemo- and radiotherapy induced synergic effect observed at the serum metabolome level.

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[IV-7] miRNAs AS CANDIDATE PROGNOSTIC MARKERS IN PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: T cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy arising by accumulation of genetic/epigenetic aberrations, activating oncogenes, inactivating tumor suppressors, triggering oncogenic pathways. miRNAs are extensively studied for prognostic potential in various malignancies. When aberrantly expressed, miRNAs may have oncogenic or suppressive role by silencing tumor suppressors or oncogenes, respectively. High stability of miRNAs and accessibility of bone marrow samples used for T-ALL diagnostics, make miRNAs attractive candidates for prognostic markers in T-ALL. Investigating global miRNA expression by next-generation sequencing (NGS) is currently the best way to select such candidates. T-ALL therapy is directed by the levels of residual leukemic cells (minimal residual disease, MRD) assessed during treatment. MRD provides rapid evaluation of treatment response and might be used as substitute of long-term survival analysis. The aim of the study was to assess prognostic potential of miRNAs in pediatric T-ALL by identification of miRNAs differentially expressed in patients with high, intermediate and low MRD levels.

Methods: miRNA-seq (NextSeq500 Illumina) was done in 63 T-ALL samples at diagnosis. Data preprocessing was done with miRge2 based on GRCh38 human genome and latest miRNA database (miRBase v22). Read normalization and identification of differentially expressed miRNAs was done using edgeR. Pairwise comparisons and one way ANOVA with Benjamini and Hochberg correction for multiple testing was used with .05 significance level. MRD was assessed in bone marrow samples by flow cytometry on day 15, 33, 72 of therapy. MRD-based risk groups were classified based on levels of residual T-ALL cells: high risk (10%), intermediate (0.1 to 10%), standard risk (0,1%).

Results: Out of 89 miRNAs differentially expressed between MRD-based risk groups, we selected most promising candidates based on average expression (40 normalized reads) and validated target genes of established/putative oncogenic, suppressive role. We identified 4 miRNAs (miR-151a-3p; miR-199a-5p; miR-340-5p; miR-224-5p) showing higher expression in patients with persistent high MRD-levels, targeting known tumor suppressors. These miRNAs are potential markers of poor treatment response. We selected 2 miRNAs (miR-1246; miR-203a-3p) showing higher expression in patients with low/absent MRD, targeting oncogenes. These miRNAs are candidate markers of good treatment response.

Conclusions/Novelty: NGS-based studies aimed to identify candidate prognostic miRNAs in T-ALL are lacking thus far. Our results are the first steps towards a comprehensive assessment of candidate miRNAs prognostic potential, including qRT-PCR validation, long-term survival analysis in an independent cohort and machine learning methods.

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Poster session V:
Varia

[V-1] COMBINATION OF ATRP METHOD AND "CLICK" CHEMISTRY REACTION FOR SYNTHESIS OF POLYMER CONJUGATES AS NEW CARRIERS OF ACTIVE SUBSTANCE IN COSMETOLOGY

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The progress of medicine and cosmetology corresponds to the increase in the need for new drug design and delivery methods, due to the already common cancer risk, as well as other diseases, which are still difficult for curing. Regardless of the type of active substance and its final application, the selection and synthesis of the appropriate carrier is crucial to provide the pharmaceutics to the target with the controlled release for a set period of time, including the intelligent activity. Depending on the way the bioactive substance is bound to carriers in the drug delivery systems (DDS) they are classified onto: carriers that physically encapsulate them inside i.e. nanostructural lipid carriers, solid lipid nanoparticles, liposomes, nanoparticles (nanospheres, nanocapsules), micelles, and carriers that chemically bind the active substance, i.e. conjugates.

We focused on developing conjugates (carrier-active substance) by combining atom transfer radical polymerization (ATRP) and *click* reaction. The ATRP reaction ensures that the well-defined copolymers with assumed topology and molecular weight are obtained while *click chemistry* is often used in DDS due to high chemical yields, possibility to obtain no toxic and physiologically stable products at mild conditions. A number of advantages for DDS application has prompted us to use *click* strategy in designing carriers of cosmetic substances.

In our work, an alkyne modified 2-hydroxyethyl methacrylate (HEMA-Al) was copolymerized with methyl methacrylate (MMA) [1,2]. Importantly, the newly obtained bromoester derivatives of retinol (vitamin A) or 4-n-butylresorcinol were used as initiators of the ATRP reaction. The introduction of a biologically active compound into the copolymer as a starting unit has significantly improved the biocompatibility of the system, and in addition may have a positive effect on the skin condition (brightening and anti-wrinkle effect). In the next stage, a *click* reaction was carried out between HEMA-Al units in the copolymer and azide-functionalized active substances such as ferulic acid or lipoic acid. The synthesized polymers were characterized by NMR (1H, 13C) and GPC.

The obtained conjugates are potential candidates for carriers with adjustable characteristics of active substance delivery in cosmetology. In the next stage the studies on release of active substance and permeability through artificial skin are planned. Results will verify the possibility of application of proposed systems in cosmetic products.

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[V-2] APPLICATION OF TOPOLOGICAL INDICES FOR THE ESTIMATION THE LIPOPHILICITY OF SELECTED BIOACTIVE BENZIMIDAZOLE DERIVATIVES AND STEROIDS

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Lipophilicity descriptor is one of the most important compound properties influences on metabolism as well as on pharmacokinetics, pharmacodynamics and toxicological profile of a proper bioactive molecule. It can be useful to predict its potential biological task and transport in biological systems. Need for rapid and cost-effective method for the determination of lipophilic properties of bioactive compounds, especially of new drug candidates, resulted in the development of many chromatographic modes and various computational methods i.e. *in silico* for the estimation of lipophilicity descriptor. The relationships between physiochemical properties like, for example, lipophilicity and biological activity of molecules is known as QSAR study (Quantitative Structure Relationships Activity). The aim of this work was estimation the utility of selected topological indices, i.e. molecular topological index (T), Wiener and Balaban index, thus some computed descriptors to predict the lipophilicity parameter of two chemically different bioactive compounds, namely four benzimidazole derivatives (pantoprazole, omeprazole, rabeprazole, lansoprazole) and five steroid compounds such as exemestane, formestane, dutasteride, finasteride and canrenone. The relationships observed between the chromatographic parameters of lipophilicity (RMW) of all examined compounds under various chromatographic conditions, i.e. by using different binary mixtures as mobile phases and two chromatographic plates precoated with silica gel RP-18F254 and RP-2F254 and logarithm of partition coefficient (logP) coming from different software packages and topological indices show the potential utility of proposed topological indices for the rapid and economical prediction of lipophilicity of all tested bioactive substances. On the basis of obtained results it can be concluded that the proposed topological indices can be a cheap and good tool in lipophilicity study of examined groups of drugs.

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[V-3] RISK OF PRESENCE OF BLEOMYCIN IN AQUATIC ENVIRONMENT

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Due to the growing problem of cancer diseases, cytostatic drugs have become a great environmental threat. As these compounds are not removed during wastewater treatment with sufficient efficiency, they are found in the surface, ground and drinking water. In case of bleomycin, glycopeptide antibiotic used in lymphoma and testicular cancer, its quantities come up to 124,000 ng/L in hospital effluent and 17 ng/L in surface waters.

The aim of this work was to establish possible negative effects of chosen cytostatic drug - bleomycin on the representatives of three trophic levels of surface waters. An acute toxicity test was conducted on the producer an aquatic freshwater plant *Lemna minor* in accordance with the OECD 221 (2006) standard, the consumer - crustaceans *Daphnia magna* following the OECD 211 (2012) and ISO 6341 (2012) guidelines, and the decomposer - bacteria *Pseudomonas putida* according to PN-EN ISO 10712 (2001). Results were expressed by half maximal effective concentration (EC50) values. Tests were conducted three times for each organism and each replicate covered a range of at least five different concentrations of the drug.

Bleomycin show acute toxicity in concentration 10 mg/L in all the tests. The highest toxicity was demonstrated toward the aquatic freshwater plant (EC50=0.2 mg/L). In addition the first symptoms of *L. minor* necrosis (water-soaked white dead tissue) were also observed, in concentrations close to 3 mg/L. According to obtain results this drug could be classified as very toxic water pollutant (class of toxicity under the EU-Directive 93/67/ EEC (EC 1996).

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[V-4] AMPHIPHILIC MIKTOARM STAR-SHAPED POLYMERS

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Miktoarm star-shaped polymers contain arms with different chemical composition, connected to one central core. So far, several strategies for the synthesis of miktopolymers have been developed, which can be divided in view of synthetic approach (e.g. arm-first, core-first or coupling-onto) through the use of various radical polymerization methods with reversible deactivation of active centers. [1][2]

In our research, we have focused on miktopolymers obtained by arm-first and core-first techniques combining atom transfer radical polymerization (ATRP) and ring-opening polymerization (ROP). The polymers were synthesized from *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA) and various cyclic esters such as: ε-caprolactone, lactide, glycolide [3]. The structures of the obtained macromolecular compounds were confirmed by spectroscopic analyzes, i.e., 1H NMR and ATR-IR. Due to the presence of PDMAEMA blocks with thermo-/pH-sensitive properties, the cloud point temperatures of polymer solutions in PBS and H2O were determined using UV-Vis spectroscopy.

The results showed that the molecular weight distributions were lower when polymers were obtained by core-first (1.14 - 1.26) than by the arm-first method (1.24 - 1.51). In addition, the cloud point temperature increased when the pH changed from neutral to pH 7.4.

The obtained miktopolymers can be used in the synthesis of the antipsoriatic prodrugs with improved pharmacokinetic properties[4].

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[V-5] NEW URETHANE-DIMETHACRYLATE MATRICES IN COMPOSITE DENTAL RESTORATIVE MATERIALS

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Introduction: The object of this study was to synthesize and characterize of new urethane-dimethacrylate monomers and their polymers. Due to, the estrogenic effect of bisphenol A, there is a need to search for alternatives to the Bis-GMA based resin.

Methods: Urethane-dimethacrylate monomer (HEMA/MEBDI) was obtained by the addition reaction of 2-hydroxyethyl methacrylate (HEMA) to 1,3-bis(1-isocyanate-1-methylethyl)benzene (MEBDI). The new monomer was homopolymerized and copolymerized with triethylene glycol dimethacrylate (TEGDMA) in a weight ratio of 80:20. The cured products of photopolymerization were examined by the degree of double bond conversion (DC), polymerization shrinkage, water sorption, modulus, and mechanical strength, hardness and impact strength. For comparative purposes, a Bis-GMA:TEGDMA (60:40) composite, which is used in commercial dental restorative materials, was prepared and characterized.

Results: The HEMA/MEBDI monomer is a liquid resin with high viscosity (18,8 Pas at 45˚C). For comparison, the UDMA resin has a viscosity of 1,38 Pas at 45˚C. The addition of diluting monomer resulted in a decrease in monomer viscosity to 1,21 Pa s at 45 ˚C. The DC in the homopolymer was 57,7%. The addition of TEGDMA caused its increase to 73,6%, which is comparable to the DC in the UDMA homopolymer (77,6%). Thus, the tested polymers met the conversion criterion, which should be higher than 50%, in dental restorative materials. HEMA/MEBDI and UDMA have similar polymerization shrinkage of about 3%. The HEMA/MEBDI homopolymer, compared to the UDMA homopolymer, was characterized by: higher flexural strength (87 and 79 MPa, respectively), higher modulus (4657 and 3352, respectively), lower hardness (151 and 173 MPa, respectively), lower impact strength (respectively, 3,7 and 5,4 MPa) and higher water sorption (13,34 and 11,34 g/mm^3, respectively). The introduction of 20% TEGDMA into HEMA/MEBDI resulted in the following changes of hardened material compared to the HEMA/MEBDI homopolymer: increase in strength by 12%, decrease in modulus by 11%, decrease in hardness by 19%, 3,5-fold increase in impact strength and increase in water sorption by 44%. This means that adding a diluting monomer, a significant increase in impact strength is obtained, at the expense of increased water absorption. However, water absorption is significantly lower than 50 g/mm3, which is the limit of applicability in dental restorative materials. All the properties of the cured HEMA/MEBDI:TEGDMA composition proved to be more favourable compared to the Bis-GMA:TEGDMA composition.

Conclusion: The HEMA/MEBDI monomer is an interesting alternative to the UDMA monomer. Its use in composition with comonomers, for example TEGDMA, gives a chance to obtain new dental composite reconstruction materials with comparable properties and even better than currently used materials, containing Bis-GMA.

[V-6] POLYANHYDRIDES BASED ON DICARBOXYLIC DERIVATIVES OF BETULIN FOR USE IN DRUG DELIVERY SYSTEMS

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Polyanhydrides are a class of surface-degradable polymers characterized by anhydride bonds in the main chain. They are obtained by polycondensation of compounds containing two carboxylic groups. Polyanhydrides undergo hydrolytic degradation to their respective diacids, which are completely eliminated from the body within a short period of time. Due to their properties, such as lack of toxicity and appropriate release kinetics of active substances, they are mainly used in medicine, both as drug carriers and as biomaterials. Betulin, a lupane derivatives, is a compound naturally occurring in nature. Betulin and its derivatives have a broad spectrum of biological activity, including anticancer activity. Betulin derivatives are promising as new, potential therapeutic agents. Dicarboxylic derivatives of betulin, such as disuccinate betulin (DBB) and diglutarate betulin (DGB), containing two carboxylic groups are excellent raw materials to obtain polyanhydrides.

The aim of this work was the synthesis and characterization of a new polyanhydrides based on two dicarboxylic derivatives of betulin: betulin disuccinate and betulin diglutarate. In order to provide betulin-based polymers in a form suitable for controlled drug release, attempts were made to obtain polymer microspheres.

Polyanhydrides were obtained by melt polycondensation with the use of acetic anhydride. Chemical structure of the polyanhydrides was characterized by NMR spectroscopy, FT-IR spectroscopy, DSC and GPC. Obtained polymers were next analyzed to determine their cytotoxic activity against selected cancer cell lines. In studies, cell lines representing cervix, breast, lung, liver, central nervous system and nasopharynx tumors were used to find concentrations causing inhibition of cell growth in culture by 50% (IC50). Polyanhydrides were used for the preparation of microspheres using emulsion (O/W) solvent evaporation technique. Poly(vinyl alcohol) was used as emulsion stabilizing agent. Obtained particles were characterized by scanning electron microscope.

Polyanhydrides obtained from dicarboxylic derivatives of betulin can be used as a polymeric prodrugs, because the active substance (DBB or DGB) is chemically bounded to the polymer chain and its therapeutic properties are obtained by polymer hydrolysis under physiological conditions. The release of DBB and DGB is controlled by the degradation rate of the polymers. Cytotoxicity tests indicated the effectiveness of dicarboxylic derivatives of betulin and obtained polyanhydrides in inhibiting the growth of cancer cells, with limited cytotoxicity towards normal cells. The obtained particles can be easily administered by injection or inhalation and can be used in the controlled drug delivery systems.

[V-7] *IN VITRO* ANALYSIS, MODELING AND TESTING OF LYMPHOCYTES FLOW IN MICROFLUIDIC DEVICE

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In the last few decades, progress in microfabrication technologies has attracted the attention of researchers in several areas. Microfluidic devices are a new tool in medicine and biomedical engineering [1]. Microchannels could also be useful to provide a better understanding of the biophysical behavior of the blood flow in microvessels and separate the suspending physiological fluid from whole *in-vitro* blood. Biomedical science found a favorable field in microfluidics to replace routine analysis and diagnosis tests, as well as to conduct fundamental biological studies in cells and diseases.

Microfluidic devices use constructions based on passive and active fluid flow through microscopic vessels. This leads to an increase in capacity i.e. the ability to perform multiple measurements at the same time and with the same efficiency, in the smallest possible volume.

Lymphocytes, flowing through the vessel have a tendency to undergo axial migration due to the parabolic velocity profile, which results in high shear stress around the wall that forces lymphocytes to move towards the center. As a result, there is a formation of a cell-free layer (CFL) with an extremely low concentration of cells. There are available publications in which authors show the dependence of the CFL thickness on the width of the contraction. Conducted experiments shown in [2] proved that a smaller width of the hyperbolic contraction results in the higher thickness of the CFL downstream of the contraction.

The CFL formation in microvessels reduces the viscosity of blood and increasing the CFL thickness implies lower blood viscosity. Therefore, the CFL thickness can be influenced by flow rates and rheological parameters.

The scope of this work was to design, fabricate and test microfluidic devices. Two microfluidic chip manufacturing techniques have been tested - xurography and photolithography. A microchip was produced using PDMS (polydimethylsiloxane). In the case of xurography PDMS was applied on a plastic mold that has been cut using a laser plotter. In a fabricated device biological experiment was carried out and CFL thickness was measured for selected geometries of microchannel.

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[V-8] CELLULOLYTIC POTENTIAL ESTIMATION FOR BACTERIA ISOLATED FROM SEWAGE SLUDGE

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Increasing amount of sewage sludge produced in wastewater treatment plants and restrictive regulations and high cost of their management encourage scientists to look for methods that allow to their maximum utilization. One of these methods is the usage of sludge for biogas production in the methane fermentation process, from which heat and electricity can be obtained. Sewage sludge after methane fermentation are stabilized, however a large part of sewage sludge contain difficult to degrade cellulose for microorganisms of fermentation process. The decomposition of cellulose prior to fermentation could significantly increase the production of biogas, while reducing the amount of sewage sludge remaining to be managed. In this process bacteria with cellulolytic potential can help due to their ability to break down cellulose into simpler compounds, which can be used by microorganisms in fermentation process to produce biogas. Ten (10) strains of cellulolytic bacteria were obtained from sewage sludge from Wastewater Treatment Plant Klimzowiec in Chorzw, Poland. The ability for decomposing cellulose by isolated microorganisms has been confirmed by testing them on CMC medium (containing carboxymethyl cellulose). The cellulolytic potential was estimated in liquid mineral medium with cellulose as a sole carbon source. The Gram staining were used for morphological characterization of isolated bacteria.

[V-9] ISOSORBIDE-BASED POLYSEBACATES AS POLYMERIC COMPONENTS FOR *IN SITU* FORMING PARENTERAL DRUG DELIVERY SYSTEMS

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In situ forming implants (ISFI) appear to be a satisfactory drug delivery systems, alternative to conventional preformed implants and microparticles for parenteral drug delivery applications. They offer several advantages including easy and minimally invasive application, potential for local/site specific drug delivery which allows reduction of side effects associated with systemic administration of a drug. In details, the drug is incorporated into the polymer solution immediately before the injection, and as a result an injectable solution or dispersion is formed. *In vivo*, upon injection of the formulation, the organic solvent, e.g. 1-methyl-2-pyrrolidone (NMP) diffuses out, while aqueous physiological fluids penetrate into the implant, resulting in a phase separation and precipitation of the polymer, and in consequence, the depot formation at the injection site. A few ISFI formulations based on poly(a-hydroxyacids) such as polylactide or poly(lactide-*co*-glicolide) are currently commercially available [1].

The goal of this research was to prepare a new type of ISFI formulation, in which isosorbide-based polysebacate is the basic polymeric component and evaluation of its selected properties, which are crucial regarding the possible usage as an injectable drug delivery system.

In this work, polyesters based on sebacic acid, isosorbide and optionally 1,2-propanediol were synthesized by polycondensation method in the presence of Novozyme 435 as catalysts, and characterized. Poly(isosorbide sebacate-*co*-1,2-propylene sebacate) (PISEBPG) was chosen as a principal constituent of new ISFI formulations dedicated to controlled release of doxycycline hyclate (DOXY). Basic characteristics of new ISFI formulations were investigated. In particular, the influence of addition of a relatively hydrophobic cosolvent (triacetin, TA) to a more hydrophilic NMP as well as the presence of calcium carbonate (CAC) on the morphology of resulted depots and DOXY release profile was evaluated. SEM analysis revealed that the presence of TA resulted in more porous morphology of the depots. DOXY has been releasing continuously from depots *in vitro* within 12 weeks depending on the composition.

The release profile of the PISEBPG-based formulation containing CAC indicates that it could be useful where short-term (up to 14 days), rapid release of the antibiotic is required, while formulation without CAC, where after 21 days about 50 % of the drug loaded may still be available for release, may be better for the long-term delivery of DOXY.

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[V-10] THE ISOFORMS OF THE P53 PROTEIN IN SELECTED HUMAN CELL LINES

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The human TP53 tumour-suppressor gene is expressed as several protein isoforms created by different mechanisms, including usage of splicing sites, alternative promoters, and translational initiation. Therefore, the human p53 gene can encode at least twelve different p53 protein isoforms: p53, p53, p53, 40p53, 40p53, 40p53, 133p53, 133p53, 133p53, 160p53, 160p53 and 160p53. Recent studies have shown the clinical significance of p53 isoforms because they have demonstrated the different expression levels of individual p53 protein isoforms in normal and tumor tissue.

The aim of the study was identifying p53 protein isoforms in five human cell lines (HCT116p53 +/+, HCT116p53 -/-, U2OS, RKO/NEO, RKO/E6), determining mRNA variants and investigating the relationship between the amount of p53 protein and the amount of mRNA transcript encoding the selected p53 isoforms.

Using the qPCR and Western Blot methods allowed showing which of the p53 isoforms exist in the selected human cell lines. Differences and similarities have been demonstrated in the presence of mRNA variants and the relative expression of selected isoforms.

Knowledge of p53 protein isoforms in selected cell lines will allow better understanding of the mechanisms occurring in cells and will be the basics for further research related to determining the role of individual p53 protein isoforms in the cellular response to oxidative stress.

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[V-11] IDENTIFICATION AND VERIFICATION OF THE ROLE OF DISTAL SURFACE AMINO ACIDS FOR LINB ENZYME ACTIVITY

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Haloalkane dehalogenases are enzymes that belong to the family of hydrolases. Their activity is based on cleaving the halide bond in the presence of water between a halogen and a carbon atom in halogenated aliphatic compounds. Due to the fact that their active site is buried inside the proteins structure, their activity is strongly regulated by the speed of migration of substrates and products through their tunnels. Previous *in silico* experiments have shown that the amino acids placed on the surface of the LinB protein may participate in substrates binding and influence enzymatic activity. These findings inspired us to perform analysis of the effects of substrate surface amino acids interactions in a LinB.

The present study is the *in vitro* continuation of the computational simulation analysis performed in order to examine the results provided by the prior *in silico* analysis. The previous simulations have identified spots on the surface of the protein with which substrates interact and where they are held before they can enter the active site of the enzyme. We have chosen several amino acids which according to the *in silico* analysis proved to be the most important in the process and proposed overall 10 mutations that could change the interactions between substrates and protein surface. To verify their role, we expressed the mutated proteins in the *Escherichia coli* expression system using pET21b(+) plasmid with T7lac operator, IPTG as the inducer and C-terminal 6xHis-tag as the Ni-NTA affinity chromatography purification tag. After the purification process, we assessed the specific activity of the mutants using the gas chromatography as the analytical method, compared their activity with the activity of the native LinB protein and with the results provided by the *in silico* analysis. We strongly believe that our results can aid in understanding the relationship between the activity of LinB haloalkane dehalogenase and the transportation of the substrate. Verification of the importance of the amino acids that are distal from tunnel entrance into substrates transportation supports our *in silico* findings and provides a new strategy that can be used for safe enzyme reengineering.

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[V-12] SYNTHESIS OF DRUG-CARRIER CONJUGATES FOR DRUG DELIVERY SYSTEMS BASED ON GRAFT COPOLYMERS

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Controlled drug delivery via nanocarriers is designed to improve bioavailability and increase therapeutic efficacy, and ensure optimal concentration of the therapeutic at the destination place. The polymeric carrier must be biocompatible and non-toxic to the healthy cells. For special attention deserve polymer brushes due to their specific topology with chains densely attached to a main chain. The grafting strategy gives the opportunity to regulate polymer dddproperties by obtaining the appropriate degree of grafting, or the selection of the type and length of the main and side chains. The well-defined graft copolymers can be obtained by grafting from reaction with the use of multifunctional macroinitiator. In this case, one of the possibilities is the attachment of polymeric ionic liquids (PIL) as side chains containing anions with pharmaceutical properties to form specific conjugates of polymer-drug [1,2].

In the first stage of research, the copolymers of 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) were obtained by Atom Transfer Radical Polymerization (ATRP), and then subsequently modified to introduce bromoester initiating groups. The prepared multifunctional macroinitiators were used in the polymerization of choline methacrylate containing trimethylammonium cation with chloride counterion (TMAMA) and MMA as a comonomer. The amphiphilic copolymers were obtained with different content of ionic units in the side chains P(TMAMA-*co*-MMA). In the next stage, the exchange process of the chloride anions in the side chains was carried out to introduce pharmaceutical anions like ascorbate, p-aminosalicylate ones.

The structures of obtained polymers and the ion exchange reactions were confirmed by 1H NMR method. The ability to self-assemble of amphiphilic grafted copolymers was confirmed by critical micelle concentration (CMC=0,025-0,059 mg/mL). The formed superstructures had sizes in the range of 87-693 nm.

The mentioned copolymers, obtained by ATRP via grafting from technique and the ion exchange process are potential candidates for conjugate nanocarriers, which are designed as the future systems containing two types of drugs for antibacterial combined therapy devoted to tuberculosis.

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[V-13] COMPARISON OF BIOPHYSICAL PROPERTIES OF SINTERED SCAFFOLDS BASED ON NATURAL- AND SYNTHETIC- HYDROXYAPATITE SUPPLEMENTED WITH SELECTED DOPANTS

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Introduction: Augmentation of alveolar bone, to counter its defects or atrophy is an increasingly common surgical procedure, which is why there is a growing interest in innovative solutions in this area. Recent publications draw attention to the use of hydroxyapatite from extracted teeth (NHAP). On the one hand, good treatment results are underlined, on the other, microbiological purity is considered. The goal of the presented work was to seek novel candidates for bone replacement biomaterials, based on hydroxyapatite – the main inorganic component of bone.

Methodology: According to the qualification criterions, 14 teeth were included in the study, which were subjected to the preparatory procedure according to proprietary standards (cleaning, rinsing, sintering) and divided into two research groups. We have compared the properties on newly developed composite materials, manufactured by sintering. The synthetic- (SHAP) or natural- (NHAP) hydroxyapatite serves as a matrix, and is doped with: (i) organic: multi-walled carbon nanotubes (MWCNT), fullerens C60 (ii) inorganic: Cu nanowires. The physico-mechanical properties of the developed composite biomaterias where tested by Scanning Electron Microscopy (SEM), Dielectric Spectroscopy (BSD), Nuclear Magnetic Resonance (NMR), Different Scanning Calorimetry (DSC) as well as for microhardness (Vickers Method). The bio-compability was tested by MTT assay.

Summary/conclusions: Our results indicate that NHAP-based biomaterials were most biocompatible (i.e. supported cell growth 20% better than the corresponding SHAP-based biomaterials). Doping the composites with MWCNT, did not alter their biologic properties, especially at lower concentrations. Fullerens markedly decreased biocompatibility, in a concentration-dependent manner. The effect was mostly observed in SHAP, where marginally in NHAP-based composites. Copper nanowires even at 1% exhibited marked cytotoxicity, hence much lower quantities of this dopant should be considered when seeking to boost antibacterial properties of biomaterial under consideration.

[V-14] SAR-MEDIATED SIMILARITY ASSESSMENT OF THE PROPERTY PROFILE FOR NEW, SILICON-BASED ACHE/BCHE INHIBITORS

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A set of 25 novel, silicon-based carbamate derivatives as potential acetyl- and butyrylcholinesterase (AChE/BChE) inhibitors was synthesized and characterized by their in vitro inhibition profiles and the selectivity indexes (SIs). The prepared compounds were also tested for their inhibition potential on photosynthetic electron transport (PET) in spinach (*Spinacia oleracea L.*) chloroplasts. In fact, some of the newly prepared molecules revealed comparable or even better inhibitory activities compared to the marketed drugs (rivastigmine or galanthamine) and commercially applied pesticide Diuron®, respectively. Generally, most compounds exhibited better inhibition potency towards AChE; however, a wider activity span was observed for BChE. Notably, benzyl *N*-[(*1S*)-2-[(*tert*-butyldimethylsilyl)oxy]-1-[(2-hydroxyphenyl)carbamoyl]ethyl]-carbamate (**2**) and benzyl *N*-[(*1S*)-2-[(*tert*-butyldimethylsilyl)oxy]-1-[(3-hydroxyphenyl)carbamoyl]ethyl]-carbamate (**3**) were characterized by fairly high selective indexes. Specifically, compound **2** was prescribed with the lowest IC_{50} value that corresponds quite well with galanthamine inhibition activity, while the inhibitory profiles of molecules **3** and benzyl-*N*-[(*1S*)-2-[(*tert*-butyldimethylsilyl)oxy]-1-[(4-hydroxyphenyl)carbamoyl]ethyl]carbamate (**4**) are in line with rivastigmine activity. Moreover, a structure–activity relationship (SAR)-driven similarity evaluation of the physicochemical properties for the carbamates examined appeared to have foreseen the activity cliffs using a similarity–activity landscape index for BChE inhibitory response values. The 'indirect' ligand-based and 'direct' protein-mediated in silico approaches were applied to specify electronic/steric/lipophilic factors that are potentially valid for quantitative (Q)SAR modeling of the carbamate analogues. The stochastic model validation was used to generate an 'average' 3D-QSAR pharmacophore pattern. Finally, the target-oriented molecular docking was employed to (re)arrange the spatial distribution of the ligand property space for BChE and photosystem II (PSII).

Keywords silicon-based carbamates; in vitro cholinesterase inhibition; CoMSA; IVE-PLS; molecular docking; similarity-activity landscape index

[V-15] REGULATION OF RNA METABOLISM IN HUMAN BY 3' URIDYLATION

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There is a growing experimental evidence of non-templated nucleotide additions to RNA 3' ends [1]. Among these uridylation by terminal uridyltransferases has come into focus as an important player in regulation of RNA stabilities and functionalities. The scope of RNAs regulated by uridylation is broad and includes cytoplasmic coding RNAs, non-coding structured RNAs and retrotransposon RNAs [2,3]. The latter are RNA intermediates in a life cycle of mobile genetic elements, retrotransposons, that can propagate in the human genome by a copy-and-paste mechanism called retrotransposition. Besides shaping human genome in an evolutionary time scale, also retrotransposonal intermediate RNAs impact cellular homeostasis with confirmed roles in autoimmunity and senescence. In this contribution a newly discovered uridylation-mediated LINE-1 regulatory mechanism will be presented.

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[V-16] COSMETIC DELIVERY SYSTEMS – NOVEL APPROACHES

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Antioxidants are compounds that can cause delay or inhibition of the lipids oxidation or other molecules by impeding the initiation or propagation of oxidizing chain reactions [1]. Most of the antioxidants and free radical neutralizers, which are currently applied in cosmetics, are absorbed quickly into deeper layers of the skin and are then carried away by the bloodstream. Temporary binding of antioxidants and free radical neutralizers to specific polymeric carriers is a novel approach resulting in their retard penetration into deeper layers of the skin and consequently promoting intracellular antioxidant and free radical neutralizing activity.

Herein, three methods of coupling specific bioactive compounds with antiaging and free radical neutralizing properties with non-toxic, biocompatible and biodegradable polymeric carriers will be presented and compared. Recently, we elaborated synthetic strategies for bioactive (homo) and (co)oligoesters, based on the anionic ring opening oligomerization of *β*-butyrolactone as well as *via* the ring opening copolymerization of *β*-butyrolactone with selected *β*-substituted-*β*-lactones initiated by sodium or potassium salts of bioactive compounds with antioxidant properties used in cosmetics. These novel bioactive polyesters have a larger loading of biologically active substances per polymer macromolecule in comparison to already reported conjugates of oligo(3-hydroxybutyrate) (OHB) with the several selected antioxidants [2-4]. Another approach to obtain novel potential cosmetic delivery system includes synthesis of amphiphilic triblock copolymers consisting of atactic poly[(R,S)-3-hydroxybutyrate] (PHB) and poly(ethyleneglycol) (PEG) as the side hydrophobic block and middle hydrophilic block via ring opening polymerization of (R,S)-*β*-butyrolactone from PEG macroinitiators, which were prepared using a convenient yet efficient TEMPO mediated oxidation. Conditions of the process were optimized to obtain amphiphilic triblock copolymers with different molecular weights.

The molecular architecture of the block copolymers was ascertained using SEC, ^{1}H NMR, ^{13}C NMR and FT-IR analyses. Molecular characterizations based on MALDI and ESI-MS^{n} analyses confirmed the well-defined structure of the obtained triblock copolymers.

The preliminary studies of micelle formation and antioxidant loading will be also presented. Micelles from triblock copolymers prepared by solvent-evaporation method were nicely formed with precise size and characterized by dynamic light scattering (DLS) and Zeta Potential.

In vitro studies demonstrated that two types of the conjugates studied were well tolerated by KB and HaCaT cell lines, as they had no marked cytotoxicity, while conjugates with a relatively short OHB carrier are optimal to support keratinocyte function. The preliminary study of the biological activity confirmed the protective effect of VA–OHB conjugates against H2O2-induced lipid peroxidation in human keratinocytes (HaCaT). It was also demonstrated that the selected bioconjugates can penetrate all layers of the skin, which shows their functionality and opens up their potential application in cosmetology.

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AZM

ACADEMIC MUSIC ENSAMBLE OF THE SILESIAN UNIVERSITY OF TECHNOLOGY

ABOUT AZM

Academic Music Ensamble of The Silesian University of Technology led from its beginnings by Professor Krystyna Krzyzanowska-Loboda, features student singers as well as instrument players.

The Ensemble repertoire is diverse, from classical to contemporary works, including gospels, sacral and spiritual music, jazz, and pop, to name a few, but it is not limited to any particular music category, style or era.

The Ensemble can perform numerous pieces of character and content that suit various types of ceremonies, making every performance an extraordinary and memorable experience.

AZM has won numerous performance awards, both in Poland and abroad.

OUR NEWEST ALBUM „AROUND THE WORLD WITH AZM"

After years of cooperation with composer Dariusz Janus, we have decided to release a joint album which will include pieces drawing upon from Poland and all over the world, even Africa. Music composed by Dariusz Janus and lyrics written by Anna Ruttar, will take the audience on an amazing tour around the globe. Releasing this album has been our biggest dream and undertaking !

DURING TODAY'S CONCERT YOU WILL LISTEN TO:

1. Locus iste, Anton Bruckner
2. When I fall in love, arr. Antonio Velasco Polonia
3. Yesterday, arr. Bob Chilcott
4. Wspomnienie, arr. Marcin Wawruk
5. Bytomski mosteczek, arr. Norbert Kroczek:
6. Jakem maszerował, arr. Norbert Kroczek:
7. Listeczku dębowy, arr. Norbert Kroczek:
8. Goodnight, well it's time to go, arr. Calvin Carter, James Hudson

Find us on Facebook

YouTube PL

CONTACT

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