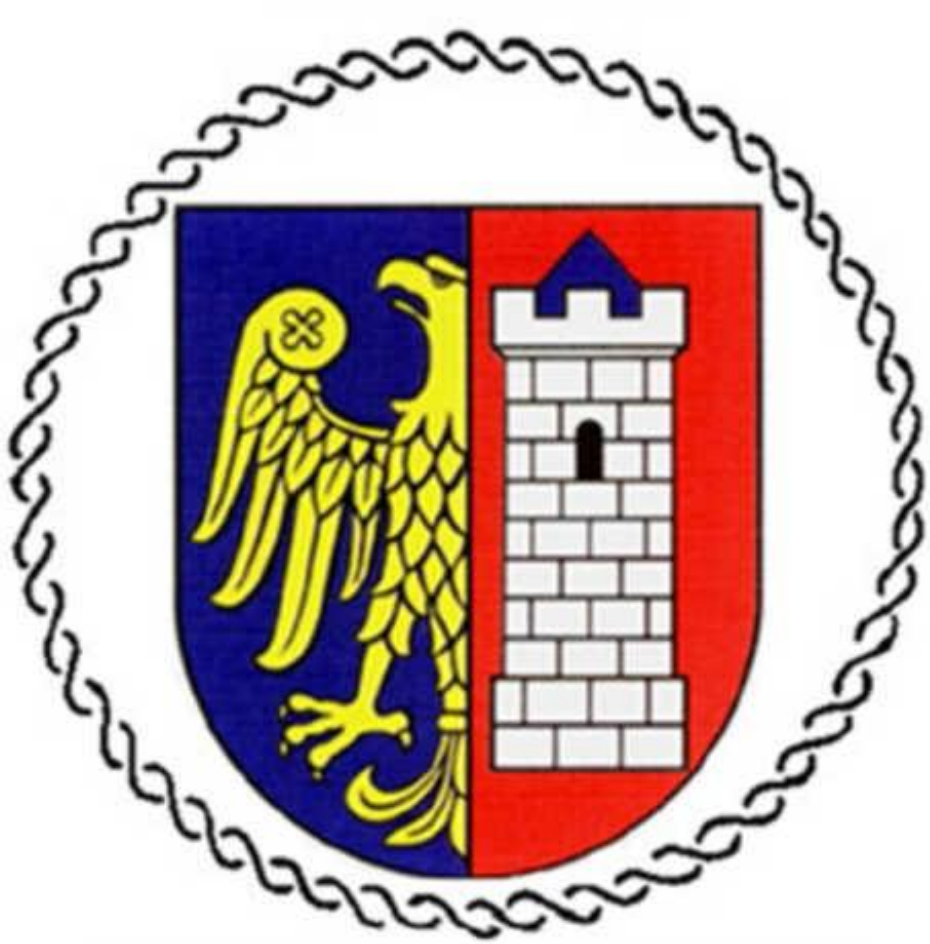
XXII[nd] Gliwice Scientific Meetings 2018



Gliwice, November 16-17, 2018

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Association for the Support of Cancer Research

Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch

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Gliwice Scientific Meetings 2018

Scientific Program

Friday 16th November 2018

09.00 – 09.15 Opening

09.15 – 11.15 Session I

Cancer Genetics and Genomics [chairperson: Ewa Grzybowska]

Asta Foersti (Heidelberg): Familial cancer variant prioritization pipeline applied to a Hodgkin lymphoma family

Pavel Vodicka (Praha): The role of DNA repair in response to treatment, disease progression and survival of sporadic colorectal cancer patients

Olga Haus (Bydgoszcz): Germinal mutations in myeloid neoplasms

Jan Lubiński (Szczecin): The latest advances in clinical genetics of hereditary breast cancer

Wojciech Fendler (Łódź/Boston): Detecting ovarian cancer with a data mining pipeline for circulating microRNAs

11.15 – 11.45 Coffee break

11.45 – 14.00 Session II

Immunity and Cancer [chairperson: Jolanta Jura]

Giulia Fontemaggi (Roma): Expression of ID4 protein in breast cancer cells determines reprogramming of tumor-associated macrophages

Claudine Kieda (Orleans/Warsaw): Angiogenesis-based normalisation of the inflammatory microenvironment in tumors

Theresa Whiteside (Pittsburgh): The potential diagnostic and prognostic role of plasma-derived exosomes in cancer

Marcin Majka (Kraków): SNAIL1 as a key player in a biology of rhabdomyosarcoma

Piotr Laidler (Kraków): Mechanisms of environmental signaling promoting epithelial-mesenchymal transition in cancer

14.00 – 15.00 Lunch

15.00 – 16.00 **Poster Session**

16.00 – 18.20 Session III

Metabolomics [chairperson: Piotr Widłak]

Malcolm Clench (Sheffield): Mass Spectrometry Imaging of drugs, metabolites and response in 3D cell models

Tone Bathen (Trondheim): In vivo molecular imaging of prostate cancer by MRSI and PET/MRI

Guro Giskeodegard (Trondheim): Metabolomics of breast cancer

Maciej Stobiecki (Poznań): Mass spectrometric techniques for structural characterization of natural products from biological material, applications in metabolomics

Ryszard T. Smoleński (Gdańsk): Analysis of cardiac and skeletal muscle metabolism with stable isotopomers and mass spectrometry

Karol Jelonek (Gliwice): Serum lipid profile can discriminate between early lung cancer patients and healthy participants of a lung cancer screening program

20.00 – 23.00 Conference Dinner

Saturday 17th November 2018

08.30 – 10.00 Session IV

Radiation Oncology [chairperson: Krzysztof Składowski and Tomasz Rutkowski]

Wilko Verbakel (Amsterdam): Developments in radiotherapy, from history to future

Jacek Jassem (Gdańsk): My vision for cancer treatment

Barbara Bobek-Billewicz (Gliwice): Oncology without imaging – mission impossible?

10.00 – 10.20 Coffee Break

10.20 – 12.30 Session V

Chromatin and Gene Regulation [chairperson Ronald Hancock and Joanna Rzeszowska]

Marion Cremer (Munich): Effects of selective degradation of the cohesin complex on higher order chromatin structures studied with live cell and super-resolved fluorescence microscopy

Thomas Cremer (Munich): Nuclear organization and function studied in space and time

Anastas Gospodinov (Sofia): The mammalian INO80 class chromatin remodelers in the maintenance of genome integrity during S-phase

Tomasz Sarnowski (Warszawa): Targeting of chromatin remodeling complexes as a basis for new anticancer therapies

Ryszard Rzepecki (Wrocław): Remodeling of chromatin during stress - a lesson from nuclear lamina proteins

12.30 – 12.50 Coffee Break

12.50 – 14.40 Session VI

Evolution of Cancer [chairperson: Marek Kimmel]

David Wheeler (Houston): Metabolic and developmental reprogramming in hepatocellular carcinoma

Simon Tavaré (New York/Cambridge): Studying tumours in 3.5D

Maciej Wiznerowicz (Poznań): Cancer stemness as hallmark of oncogenic progression

Marek Kimmel (Houston/Gliwice): Extracting tumor growth and mutation histories from sequencing data

14.40 – 15.00 Presentation of awarded posters, closing

15.00 – 15.30 Lunch

Lecture Abstracts

Session I:
Cancer Genetics and Genomics

chairperson: Ewa Grzybowska

FAMILIAL CANCER VARIANT PRIORITIZATION PIPELINE APPLIED TO A HODGKIN LYMPHOMA FAMILY

Asta Försti[1]

[1]*German Cancer Research Center (DKFZ), Molecular Genetic Epidemiology, Heidelberg, Germany*

Hodgkin lymphoma (HL) originates from germinal center B-cells and accounts for about 10% of newly diagnosed lymphomas and 1% of all *de novo* neoplasms worldwide. Though familial risk for HL is among the highest of all cancers, not many genetic risk factors have been identified with the exception of associations with the human leukocyte antigen (HLA) complex, 18 low-penetrance variants and some suggested germline mutations in specific subtypes of HL. We carried out whole genome sequencing of affected and unaffected members of a family with documented diagnoses of HL in an attempt to identify high/moderate-penetrance genes for HL. Mapping, variant calling, annotation and filtering were conducted using the familial cancer variant prioritization pipeline (FCVPP) recently developed by us. We identified a novel missense germline variant in exon24: c.T5133G: p.I1711M of the tumor suppressor gene *DICER1*. Down-regulation of tumor-suppressing miRNAs let-7b, let-7g, miR-100 and miR-204 in family members carrying the *DICER1* mutation compared to those without mutation provided functional rationale to the findings. The fact that *LIN28A* and *LIN28B* levels were not significantly different between the family members excluded *LIN28* as the factor responsible for the down-regulation of the *Let-7* family members analyzed. These results suggest that *DICER1* deregulation may be a possible mechanism for susceptibility to HL in this family.

THE ROLE OF DNA REPAIR IN RESPONSE TO TREATMENT, DISEASE PROGRESSION AND SURVIVAL OF SPORADIC COLORECTAL CANCER PATIENTS

Pavel Vodicka[1,2,4], Sona Vodenkova[1,2,3], Katerina Jiraskova[1,2], Veronika Vymetalkova[1,2], Alena Opattova[1,2], Michal Kroupa[1,4], Ludmila Vodickova[1,2,4]

[1]*Institute of Experimental Medicine Czech Academy of Sciences, Prague, Czech Republic*
[2]*First Medical Faculty, Charles University Prague, Czech Republic*
[3]*Third Medical Faculty, Charles University Prague, Czech Republic*
[4]*Medical Faculty in Pilsen, Charles University Prague and Biomedical Center in Pilsen, Czech Republic*

State of the art: DNA repair and DNA damage response maintain universal genomic stability and preserve cellular functions in healthy cells, whereas the suppression of DNA repair capacity (DRC) in malignant cells would enhance the effectiveness of chemotherapy through DNA damage accumulation and consequent apoptosis. Alternatively, the patients with tumor cells with high DRC may contend with poor response, resistance to treatment and decreased survival. We investigated DRC of excision repair in target tissue as a predictive marker for a treatment strategy and long-term survival in patients with newly diagnosed colon cancer. Additionally, we explored links between functional genetic polymorphisms (SNPs) in DNA repair genes covering the main DNA repair pathways, the risk of colorectal cancer (CRC) and clinical outcomes.

Methodology: Our set of patients (with data on microsatellite stability and 5-FU treatment) was followed-up at least for 30 months. Tumor tissue and adjacent mucosa samples were obtained at surgical resection. Protein extracts from tissues were isolated both for protein expression (Western Blot) analysis and for measurement of DRC. Functional DRC was performed by comet assay-based *in vitro* DNA repair assay. Functional and genomic databases enabled identification of DNA repair gene variants affecting protein coding.

Findings: DNA repair gene variants were proven to modulate clinical outcome of colorectal cancer. In CRC patients, interestingly, the DNA repair capacity, significantly lower at the time of diagnosis, increased to the levels observed in healthy control subjects following the completion of chemotherapy. There are interesting associations between DRC measured in tumor tissue, adjacent mucosa and peripheral blood lymphocytes.

Conclusions: Present results identify plausible candidate DNA repair gene variants affecting survival of CRC patients and clinical outcome of the disease. DRC may constitute predictive biomarker in colorectal cancer therapy and targeting DNA repair processes may pose clinical benefit in cancer treatment.

GERMINAL MUTATIONS IN MYELOID NEOPLASMS

Olga Haus, Hanna Janiszewska, Aneta Bąk, Anna Junkiert-Czarnecka, Marta Heise, Maria Pilarska-Deltow, Katarzyna Skonieczka

Department of Clinical Genetics, Faculty of Medicine, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland

Hematopoietic system malignancies were for many years seen as somatic mutations-dependent cancers. However, some data indicated familial incidence of leukemias, especially of lymphoid lineage. It was only some years ago, that molecular studies in leukemia started to focus on germline mutations as the basis for development of hematopoietic cancer. These mutations may concern widely known tumor supressor genes, as *BRCA1,2*, *CHEK2*, and many others. On the other hand, the key genes involved in normal and malignant hematopoiesis, as *AML1*(*RUNX1*), *CEBPA*, and others may also bear germinal mutations which become a basis for abnormal hematopoiesis. Current (2016) WHO classification of hematopoietic neoplasms distinguishes already a new entity: Myeloid neoplasms with germline predisposition.

At the beginning, our investigations focused on germline *CHEK2* mutations in myeloproliferative neoplasms (MPN) – essential thrombocytemia (ET) and polycythemia vera (PV). We found that the most frequent *CHEK2* germline mutations were associated with increased risk of ET and PV (OR=3.8, p=0.002 and OR=3.0, p=0.004, respectively). It was also associated with the younger age at the onset of ET, and the older of PV. The median age at ET diagnosis among *CHEK2+*/*JAK2+* patients was 7 years lower that among *CHEK2+*/*JAK2-* patients (p=0.04). On the contrary, the median age at diagnosis of PV was 7 years higher among *CHEK2+* patients, which may be associated with a protective role of mutated *CHEK2* against the development of PV.

Recently, we focused our work on myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Analysis of the impact of germline *CHEK2* mutations on MDS and AML development showed a strong association of these mutations with the risk of MDS ($p<0.0001$) but not with the risk of *de novo* AML. *CHEK2* inherited mutations were in MDS associated with aberrant karyotypes and unfavorable disease types.

We extended our study on *BRCA1* and *TP53* suppressor genes as well as *RUNX1* and *CEBPA* hematopoietic genes. In the group of 100 patients, only *RUNX1* mutations showed an association with the risk of AML (OR=6.8, p=0.22), as well as with the presence of chromosome 7 prognostically poor aberrations.

In each study we examined a group of at least 100 patients and 100 control persons from general populations. The mutations were searched in peripheral blood cells DNA, and their congenital nature was confirmed by analysing DNA from buccal swabs.

The search for germline mutations in leukemia and lymphoma is a new very promising approach for discovering new treatment options. Taking into account the inborn genome changes may be important in various conditioning and treatment procedures, and germline changes may as well become a good molecular goal of therapy.

THE LATEST ADVANCES IN CLINICAL GENETICS OF HEREDITARY BREAST CANCER. SELINA – CLINICAL TRIAL ON LOWERING THE RISK OF MALIGNANCIES BY OPTIMIZING SELENIUM LEVELS IN FEMALES FROM FAMILIES WITH HEREDITARY BREAST CANCER*

Jan Lubiński[1,2]

[1]*Department of Genetics and Pathology, Pomeranian MedicUniversity in Szczecin*
[2]*Read-Gene SA Poland*

Aim: Prospective observational studies showed that blood selenium (Se) levels associated with significantly lower risk of cancers can be identified in Polish females from families with hereditary breast cancers (HBC). For BRCA1 mutation carriers it is: 70-89 g/l at age 50 yrs (OR~12) and 95-120 g/l at age 50 yrs (OR~4). For females without detected BRCA1 mutation but from families with pedigree/clinical features of HBC it is 98-108 g/l (OR~5).

The main goal of SELINA is validation of hypothesis that optimization of Se level by supplementation or diet changes can decrease the risk of malignancies in groups described above.

Method: 7000 females (including 1200 BRCA1 carriers) from families with HBC and deficiency or excess of Se will be recruited and randomly qualified to one of the following arms: placebo, prospective observational, supplement (Sodium Selenite) or diet modification. Blood Se level will be systematically measured using ICP-MS and appropriately optimized. Follow-up will take 5 yrs.

Results: At present we are performing recruitment. It is planned to close it at the end of 2018.

Conclusion: SELINA is the first clinical trial aimed to decrease the risk of cancers by active control of blood selenium levels . All interested scientists/institutions are welcome for collaboration.

**Project - INNOMED/I/16NCBR/2014 sponsored by National Ctr of Research Development and Read-Gene SA.*

DETECTING OVARIAN CANCER WITH A DATA MINING PIPELINE FOR CIRCULATING MICRORNAS

Wojciech Fendler[1]

[1]*Department of Biostatistics and Translational Medicine, Medical University of Lodz Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, USA*

Recent studies have identified a role for circulating miRNAs as cancer biomarkers due to their good stability, accessibility, ease of quantification and good dynamic range of expression values. While small RNA sequencing has the highest sensitivity for detecting miRNA in bodily fluids, translating these data into a diagnostic test has not been possible due to uncertainty surrounding the most appropriate data mining approaches. We present a study in which we developed a framework to create, robustly test and calibrate neural network-based classification tools for clinical use. After optimization of variable selection methods and classification techniques, the neural network analysis yielded the best results. Through this approach we identified a serum miRNA signature with high predictive accuracy for a diagnosis of EOC (AUC 0.90; 95% CI: 0.81-0.99). The model significantly outperformed CA125 (AUC 0.74; 95%CI 0.65-0.83; p=0.001) and functioned well regardless of patient age, tumor histology, or clinical stage. Afterwards, the network was recalibrated to accept qRT-PCR data and subsequently validated the locked-down 7-miRNA signature using an independent dataset, yielding a positive predictive value of 91.3% (95% CI: 73.3% - 97.6%) and a negative predictive value of 78.6% (95% CI: 64.2% - 88.2%). A direct connection of the serum miRNAs in the signature with the carcinomas was substantiated as serum levels of a subset of the miRNAs decreased within 72 hours of tumor removal. Finally, the biologic relevance of this assay was tested using *in situ* hybridization, which demonstrated that several miRNAs in the signature were highly expressed in lesional cells in 15 serous tubal intraepithelial carcinomas (STICs) and 15 Stage I high grade EOC. The presented study demonstrates how to efficiently process small RNA sequencing data through an analytical pipeline, streamlining the transition from high-throughput data to a 7-miRNA signature. These results suggest that, after additional validation, circulating miRNAs might have the potential to be developed into a diagnostic test for ovarian cancer.

Session II:
Immunity and Cancer

chairperson: Jolanta Jura

REPROGRAMMING OF TUMOR-ASSOCIATED MACROPHAGES THROUGH ID4-DEPENDENT PARACRINE ACTIVITY OF BREAST CANCER CELLS

Giulia Fontemaggi

IRCCS Regina Elena National Cancer Institute, Rome, Italy

Tumor Associated Macrophages (TAMs) represent one of the most prominent stromal components of tumor microenvironment. TAMs have been largely demonstrated to promote progression to malignancy in breast cancer (BC), supporting tumor-associated angiogenic switch, tumor cell invasion, migration and metastatization.
ID4 is a member of the ID (Inhibitors of Differentiation) family of proteins, dominant negative transcriptional regulators of basic Helix Loop Helix (bHLH) transcription factors that lack the basic DNA binding domain but have intact HLH domain. ID4 is associated with a stem-like phenotype and poor prognosis specifically in basal-like and triple-negative breast cancer. ID4 protein favors angiogenesis by enhancing post-transcriptionally the expression of pro-angiogenic cytokines IL-8 and CXCL1, and by modulating the balance of VEGF isoforms in breast cancer cells.
Here, we investigated whether ID4 protein exerts its pro-angiogenic function by modulating the activity of tumour-associated macrophages in breast cancer.
We determined that ID4 and macrophage marker CD68 protein expression are significantly associated in a series of triple-negative breast tumours. *In vitro* and *in vivo* migration assays demonstrated that expression of ID4 in breast cancer cells stimulates macrophage motility. Interestingly, high ID4 mRNA levels robustly predicted poor distant metastasis-free and overall survival, specifically in the subset of tumours showing high macrophage infiltration.
At the molecular level, ID4 protein expression in breast cancer cells controls, through paracrine signaling, the activation of an angiogenic program in macrophages. This program includes both the increase of a panel of angiogenesis-related mRNAs (including also HIF1A, GRN, EPHB2, NRP2) and the decrease of members of the anti-angiogenic miR-15b/107 group (miR-107, miR-15b and miR-195). By using siRNAs to inhibit VEGF expression in BC cells or blocking antibodies to VEGF, we assessed that the establishment of the ID4-dependent angiogenic program in macrophages is at least in part VEGF-dependent.
Of note, the majority of the identified ID4-dependent mRNAs present putative binding sites for miR-107 in their 3'-UTR or CDS. Indeed, inhibition of miR-107 expression by transfection of LNA oligonucleotides in macrophages leads to upregulation of HIF1A, GRN and EPHB2 proteins.
Basing on its role as soluble factor and on its capability to modulate cytokines production in macrophages, we further explored the possible involvement of GRN (Granulin) in conferring angiogenic potential to TAMs. By modulating GRN expression in macrophages, we demonstrated that its induction significantly increases the angiogenic potential of macrophages, both *in vivo* and *in vitro*.

This work was supported by the Italian Ministry of Health.

ANGIOGENESIS-BASED NORMALIZATION OF THE INFLAMMATORY MICROENVIRONMENT IN TUMORS

Claudine Kieda[1]

[1]*LOMIT, WIM, Warsaw, Poland CBM, CNRS, UPR 4301, Orleans, France*

Endothelial cells organospecificity and plasticity make them invaluable diagnostic and potential therapeutic tools for repair strategies. Endothelial damage in tumors is caused by hypoxia which results in the pathologic angiogenesis with key consequences on cells spreading and shaping the tumor permissive microenvironment, immune suppression and persistent inflammation

Hypoxia, the common parameter in tumor development turns on angiogenic switch, selects and maintains aggressive cells defined as cancer stem like cells in niches where pathologic angiogenesis controls the microenvironment properties.

Targeting cancer stem cells cannot be considered separately from microenvironment mainly through their selective interactions with endothelial cells populations New approaches of angiogenesis treatment aim to angiogenesis normalization as opposed to destruction. This causes the reversion of the immune suppression and controls the cellular inflammation mediators. Endothelial cells-mediated modulation of the microenvironment properties opens new means to target the tumor cells themselves.

The particular properties of normal endothelial cells which use the glycolytic pathway in normoxic conditions, as resistant cancer cells do, make them particularly able to survive in harsh hypoxic conditions in the tumor and develop pathologic angiogenesis in the inflammatory milieu. Consequently, the endothelial cell repair appears as a potent strategy. 1, 2, 3 Our work brings the means to elevate the pO2 level in the pathologic tissues leading to normalization/stable functionalization of the tumor vessels3. It strongly modifies the tumor microenvironment in terms of humoral/ cellular immune and inflammatory response with deep consequences on tumor outcome. This was particularly efficient in the case of melanoma and glioma growth as highly angiogenic tumors.

References:

1. Klimkiewicz, K. *et al.*. *Cancer Lett.* **396**, 10-20 (2017).
2. Collet, G. *et al*. *Cancer Lett.* **370**, 345-357 (2016).
3. Kieda, C. *et al. J. Mol.Med.* **91**, 883-899 (2013).

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THE POTENTIAL DIAGNOSTIC AND PROGNOSTIC ROLE OF PLASMA-DERIVED EXOSOMES IN CANCER

Theresa L. Whiteside[1]

[1]*University of Pittsburgh and UPMC Hillman Cancer Center, Pittsburgh, USA*

Plasma-derived exosomes are emerging as promising non-invasive correlates (liquid biopsies) of cancer progression. In patients with solid tumors, such as HNSCC, or with hematological malignancies, plasma exosomes contain subsets of tumor-derived exosomes (TEX) and normal cell-derived exosomes (non-TEX). TEX carry a cargo enriched in immuno-suppressive proteins and miRNAs that re-program functions of recipient cells, including immune cells. As immune suppression is one of the hallmarks of cancer progression, circulating exosomes rich in inhibitory molecules are implicated in mediating systemic immune suppression. We present evidence that the molecular cargo of plasma-derived exosomes and their effects on immune cell subsets correlate with disease activity, tumor stage and lymph node involvement in patients with HNSCC. Also, serially- monitored exosome levels and cargos of TEX in plasma of HNSCC patients treated with immunotherapies provide insights into resistance/response of patients to oncological treatments. Plasma exosomes are also non-invasive biomarkers of epithelial mesenchymal transition (EMT) in HNSCC.

SNAIL1 AS A KEY PLAYER IN A BIOLOGY OF RHABDOMYOSARCOMA

Marcin Majka[1], Klaudia Skrzypek[1], Anna Kusienicka[1], Aleksandra Ulman[1], Bogna Badyra[1]

[1]*Department of Transplantation, Institute of Pediatrics, Jagiellonian University, Medical College, Kraków, Poland*

The SNAIL family consists of 3 members: SNAIL (SNAI1), SLUG (SNAI2) and SMUG (SNAI3). SNAIL plays an eminent role in the epithelial to mesenchymal transition (EMT), the main mechanism responsible for both embryogenesis and the invasiveness and metastasis of neoplasms. Rhabdomyosarcoma (RMS) is a mesenchymal tumor of soft tissue in children that originates from a myogenic differentiation defect. Expression of SNAIL transcription factor is elevated in the alveolar subtype of RMS (ARMS), characterized by a low myogenic differentiation status and high aggressiveness. In RMS patients SNAIL level increases with stage. SNAIL silencing inhibits proliferation and induces differentiation of RMS cells *in vitro*, and completely abolishes the growth of human RMS xenotransplants *in vivo*. SNAIL silencing induces myogenic differentiation by upregulation of myogenic factors and muscle-specific microRNAs. SNAIL binds to the MYF5 promoter suppressing its expression and displaces MYOD from E-box sequences. SNAIL silencing allows the re-expression of MYF5. and canonical MYOD binding, promoting ARMS cell myogenic differentiation. SNAIL forms repressive complex with histone deacetylates 1 and 2 (HDAC1/2) and regulates their expression. SNAIL affects RMS metastasis by reorganization of actin cytoskeleton, regulation of ezrin expression and chemotaxis to HGF and SDF-1. Interestingly, in human myoblasts SNAIL silencing induces differentiation by upregulation of myogenic factors. These data clearly point to SNAIL as a key regulator of myogenic differentiation and a new promising target for future ARMS therapies.

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MECHANISMS OF ENVIRONMENTAL SIGNALING PROMOTING EPITHELIAL-MESENCHYMAL TRANSITION IN CANCER

Piotr Laidler[1]

[1]*Chair of Medical Biochemistry, Medical College, Jagiellonian University, Kraków, Poland*

Multiple studies have demonstrated the role of the acquisition of a mesenchymal-like phenotype by epithelial tumor cells in the metastasis of carcinomas, via a process designated as epithelial-mesenchymal transition (EMT). As cell undergo EMT, cell-cell and cell-extracellular matrix (ECM) interaction are altered. Therefore, both cadherins and integrins must function in a coordinated manner to effectively mediate phenotypic changes essential for triggering migration and tumor metastasis. EMT and malignant transformation among others are characterized by phenomenon known as cadherin switch, the decreasing expression of E-cadherin that is replaced by N-cadherin. Suppression of E-cadherin expression in various malignancies is caused by transcriptional factors including Snail, Twist, Zeb. The increased expression of N-cadherin is commonly followed by growing capacity for migration as well as resistance to apoptosis. Our own and literature data indicate the oncogenic role of N-cadherin in the activation of AKT and/or ERK pathway. Inhibition of N-cadherin expression by siRNA reduced proliferation through the decrease in the activity of certain protein kinases which was accompanied by reduced activity of some cyclins and the increased activity of the cell cycle inhibitors. Treatment of melanoma cells with siRNA against N-cadherin, also led to significantly decreased Matrix Metalloproteinases (MMPs) expression and activity, as well as diminished invasion. The molecular mechanism that promote the cadherin switch and all the above-mentioned consequences remain still unclear and elusive. We postulate that Integrin Linked Kinase (ILK) is a major signaling mediator involved this phenomenon. Upon its overexpression or/and activation by different stimuli, among which integrin mediated Extracellular Matrix Proteins (ECMP) signaling plays crucial role, ILK phosphorylates Akt and GSK-3beta and participates in the related signaling cascades. We showed that silencing of ILK expression by siRNA significantly abolished the presence of markers associated with EMT like Snail, vimentin or nuclear translocation of beta-catenin. ILK knockdown by siRNA suppressed N-cadherin expression in melanoma and bladder cancer and increased membrane re-expression of E-cadherin in the latter one. The presented results show that ILK pathway may regulate the cadherin switch through multiple mechanisms, including transcriptional and posttranslational regulation.

Session III: Metabolomics

chairperson: Piotr Widłak

MASS SPECTROMETRY IMAGING OF DRUGS, METABOLITES AND RESPONSE IN 3D CELL MODELS

Malcolm R Clench[1]

[1]*Centre for Mass Spectrometry Imaging, Biomolecular Research Centre, Sheffield Hallam University*

Typical two-dimensional cell culture systems are not considered good representations of the human response and environment due to characteristic differences in parameters such as gene expression, cell-cell/cell-matrix interactions. A new generation of three-dimensional (3D) cell culture systems is now emerging in response to these limitations with the potential to increase the translation of in vitro findings. The multiplexed nature of the data obtained from mass spectrometric imaging (MSI) experiments offers a powerful combination with 3D cell culture systems. Here data from experiments carried out using a commercial 3D living skin equivalent model and modifications of it to study drug absorption, drug metabolism, disease mechanisms and wound healing will be presented (1,2). In addition a new method for the absolute quantification drugs absorbed into a 3D osteosarcoma tumour spheroid and a living skin equivalent model using MSI will be described (3).

References:

1. Harvey, A., Cole, L.M., Day, R., Bartlett, M., Warwick, J., Bojar, R., Smith, D., Cross, N. and Clench, M.R. (2016) Proteomics 16(11-12), pp. 1718-1725
2. Lewis E.E.L., Barrett M.R.T., Freeman-Parry L., Bojar .RA. and Clench M.R. (2018) Int J Cosmet Sci. 40 (2) pp 148-156
3. Russo C., Brickelbank N., Duckett C., Mellor S., Rumbelow S. and Clench M.R. (2018) Analytical Chemistry 90(16), pp. 10031-10038

IN VIVO MOLECULAR IMAGING OF PROSTATE CANCER BY MRSI AND PET/MRI

Tone Bathen[1]

[1] *The Norwegian University of Science and Technology (NTNU), Trondheim, Norway*

The global burden of cancer continues to increase, largely because of the aging and growth of the world population. An estimated 14.1 million new cases of cancer occurred worldwide in 2012 (1). Prostate cancer is the 2nd most common cancer among men and constitutes a substantial health care problem. Given the huge number of affected individuals, current challenges in prostate cancer include imaging methods for improved diagnosis and risk stratification, assessment of response to therapy, and identification of early recurrence.

Magnetic resonance spectroscopic imaging (MRSI) enables the non-invasive assessment of specific metabolites. Several studies have shown the benefit of adding this metabolic information to MRI in the evaluation of prostate cancer (2-4). Further, recent technological advances allows for simultaneous acquisition of MR and positron emission tomography (PET) data, where a benefit arises from combining the excellent soft tissue contrast of MRI with the high molecular specificity of the PET imaging radiotracer. PET has a rapidly evolving role in the assessment of prostate cancer (5). The current talk will summarize results from our ongoing research using MRSI and PET/MRI in diagnosis and characterization of prostate cancer.

References:

1. Cancer Research UK. Worldwide Cancer Statistics. 2012.
2. Leapman MS, Wang ZJ, Behr SC, Kurhanewicz J, Zagoria RJ, Carroll PR, Westphalen AC: Impact of the integration of proton magnetic resonance imaging spectroscopy to PI-RADS 2 for prediction of high grade and high stage prostate cancer. Radiologia brasileira 2017, 50(5):299-307.
3. Selnaes KM, Heerschap A, Jensen LR, Tessem MB, Schweder GJ, Goa PE, Viset T, Angelsen A, Gribbestad IS: Peripheral zone prostate cancer localization by multiparametric magnetic resonance at 3 T: unbiased cancer identification by matching to histopathology. Investigative radiology 2012, 47(11):624-633.
4. Kobus T, Wright AJ, Weiland E, Heerschap A, Scheenen TW: Metabolite ratios in 1H MR spectroscopic imaging of the prostate. Magn Reson Med 2015, 73(1):1-12.
5. Wallitt KL, Khan SR, Dubash S, Tam HH, Khan S, Barwick TD: Clinical PET Imaging in Prostate Cancer. *Radiographics : a review publication of the Radiological Society of North America, Inc* 2017, 37(5):1512-1536.

METABOLOMICS OF BREAST CANCER

Guro Giskeødegård[1]

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Metabolomics is the branch of omics technologies that involves high-throughput identification and quantification of small molecular metabolites in tissues or biofluids. Reprogrammed metabolism is an emerging hallmark of cancer, typically characterized by deregulated uptake of glucose and amino acids and increased flux through anabolic metabolic pathways. Nuclear magnetic resonance (NMR) spectroscopy can be used to investigate such metabolic changes, and clear metabolic differences between breast cancer tissue and adjacent non-involved breast tissue have been described [1].

Liquid biopsies, such as serum and urine samples, are easily acquired and minimally invasive, and therefore excellent for patient screening and monitoring. Metabolic profiles of biofluids will reflect metabolic processes in the entire organism being studied - and not only those occurring in tumor [2]. Biofluid metabolomics can therefore provide valuable insight into how patients are affected by systemic cancer treatment. This is important as breast cancer survivors are shown to express treatment related disabilities that continue for years, limiting their functional capacity and reducing work activity. The current talk will provide an update on our ongoing research activities in the field of biofluid metabolomics of breast cancer.

References:

1. Bathen TF, Geurts B, Sitter B, Fjosne HE, Lundgren S, Buydens LM, Gribbestad IS, Postma G, Giskeodegard GF: Feasibility of MR metabolomics for immediate analysis of resection margins during breast cancer surgery. *PLoS One* 2013, 8(4):e61578.
2. Giskedegrd GF, Madssen TS, Euceda LR, Tessem MB, Moestue SA, Bathen TF: NMR-based metabolomics of biofluids in cancer. *NMR Biomed* 2018, In press:e3927.

MASS SPECTROMETRIC TECHNIQUES FOR STRUCTURAL CHARACTERIZATION OF NATURAL PRODUCTS FROM BIOLOGICAL MATERIAL, APPLICATIONS IN METABOLOMICS

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Structural characterization of natural products present in complex samples from biological material with mass spectrometric method is a very important task. Selectivity and sensitivity of analytical method applied for identification and structural characterization are very important parameters. These two parameters locate mass spectrometry among methods of choice applied in metabolomics during research conducted in the field of biological sciences: biomedicine, plant biology, agriculture, environmental research and broadly understand biotechnology. An additional advantage is easiness of mass spectrometer hyphenation with separation method (gas and liquid chromatography or electrophoresis). High sensitivity and selectivity of mass spectrometers results from the fact, that in the instruments are applied different physicochemical phenomenon for separation, excitation of the analyte and isolation of obtained ions in a mass analyzer. An important advantage is also connected with possible ionization of molecules under atmospheric pressure or without additional heating under decreased pressure in ionization chamber. It is important to underline that utilization of mass spectrometric databases for identification of the mixture components, statistical data processing and various bioinformatics tools for data interpretation are available.

Some examples of different methodical approaches for structural characterization of compounds present in the complicated mixtures with different kind of mass spectrometers hyphenated with chromatographic instruments will be demonstrated, especially from plant sciences and biomedicine metabolomics.

USE OF STABLE ISOTOPOMERS AND LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY (LC/MS) METHOD FOR CARDIAC SUBSTRATE PREFERENCE ANALYSIS IN MICE

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Heart is an organ characterized by prominent metabolic flexibility for utilizing all carbon substrates, including carbohydrates, fatty acids, ketones and amino acids. Cardiac substrate preference could be affected by factors such as the availability and regulation at different levels. It also might be deregulated in pathological conditions and determine condition of cardiac cells. However, current *in vivo* methods are complex and not suitable for high throughput analysis. Thus, we aim to develop method for the analysis of cardiac substrate preference using stable 13C glucose, 13C valine , 13C leucine isotopomers and mass spectrometry. Our assay allowed for investigation of contribution of specific substrates for glycolysis and Krebs cycle by analysis of 13C alanine and 13C glutamate enrichment. We optimized route of administration, concentrations and time of organ collection. Optimal procedure involved subcutaneous injection of glucose or amino acid isotopomers and collection of hearts after 60 min of injection. Blood samples were collected from a tail vein before and after 60 min from injection. Hearts and blood were extracted and analysed with LC/MS optimized to detect carbohydrates and amino acids with its isotopomers. Metabolic shifts were calculated from changes in 13C enrichment ratios. The method enabled estimation of glucose and amino acid use in cardiac metabolism in mice in several experimental studies relating to dyslipidemia, Huntington disease and heart failure.

SERUM LIPID PROFILE CAN DISCRIMINATE BETWEEN EARLY LUNG CANCER PATIENTS AND HEALTHY PARTICIPANTS OF A LUNG CANCER SCREENING PROGRAM

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Objectives: The role of a low-dose computed tomography (LD-CT) lung cancer screening remains a matter of controversy due to its low specificity and high cost. Screening complementation with blood-based biomarkers may allow a more efficient pre-selection of candidates for imaging tests or discrimination between benign and malignant chest abnormalities detected by LD-CT. We searched for a molecular signature based on a serum lipidome profile distinguishing individuals with early lung cancer from healthy participants of the lung cancer screening program.

Materials and methods: Blood samples were collected during a LD-CT screening program performed in the Gdansk district (Northern Poland). The analysis involved 100 patients with early stage lung cancer (including 31 screen-detected cases) and the pair-matched group of 300 healthy participants of the screening program. MALDI-ToF mass spectrometry was used to analyze the molecular profile of lipid-containing fraction of serum samples in the 320-1000 Da range. The LC-MS approach was used to identify and quantify selected lipids.

Results: Several components of the serum lipidome were detected, with abundances discriminating patients with early lung cancer from high-risk smokers. An effective cancer classifier was built with an area under the curve of 0.79 and 0.72 in the training and test groups, respectively. Corresponding negative predictive values were 100% and 92%, and a positive predictive value was 28% each. The downregulation of a few lysophosphatidylcholines (LPC18:2 and LPC18:1) in samples from cancer patients was confirmed using a complementary LC-MS approach.

Conclusions: Lipidome-based serum signatures showed potential usefulness in discriminating early lung cancer patients from healthy individuals. These signatures, though not validated in an independent dataset, deserve further investigation in a larger cohort study.

Session IV:
Radiation Oncology

chairperson: Krzysztof Składowski and Tomasz Rutkowski

DEVELOPMENTS IN RADIOTHERAPY: FROM HISTORY TO FUTURE

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Radiotherapy is in use as a cancer treatment for over 100 years. In the last 2 decades, developments have caused large changes in radiotherapy delivery, the role of the medical physicist and research. It is one of the most technological medical treatments. Important developments were computerized treatment planning, MV imaging, SRS, IMRT, inverse optimization, IGRT: planar kV imaging and CBCT, correlations between dose and toxicity, lung SBRT, IMPT, VMAT, other SBRT, multi-criteria optimization, knowledge based planning, completely automated planning, automated contouring, deep learning for contouring and dose prediction, kV tumor tracking, MRI guided RT and online adaptive RT. Although many techniques seem very new, some of the ideas date back 50-70 years.

With the automation, the field is going to shift and the role of physicists, planners and radiation oncologists will change. The technological development may help the introduction of high quality RT in currently underserved countries, where they have lack of skilled personnel.

MY VISION FOR CANCER TREATMENT

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Oncology is one of the most rapidly developing medical disciplines. Surgical oncology, in the past very aggressive and mutilating, has now changed its paradigm from maximum tolerated to minimal effective. Endoscopic surgery, including surgery through natural body orifices, has largely replaced open surgery. Robotic surgery has found its place in various indications, with further potential towards telesurgery and robots performing surgery. Recent developments in radiotherapy include improvements in biological, molecular and functional imaging, improvements in treatment delivery by real-time tracking of moving tumors or adaptive radiotherapy, tailoring treatment and doses according to specific tumor features, and the use of combined modality strategies. The discovery of DNA structure and function, identification and mapping all human genome, and the development of cancer genome atlas, have opened the door for unprecedented development of cancer systemic therapies. A spectacular example of this progress is a large array of effective molecularly targeting agents, with a huge potential for novel targets. Immunotherapy using both monoclonal antibodies or CAR T-cells has been found to enormously augment the ability to destroy cancer cells, with stunning effects of curing some patients with metastatic disease. In the future, treatment efficacy may be further dramatically increased by virtue of artificial intelligence algorithms, expediting in minutes all available evidence into real benefits and allowing the most personalized treatment. Discovery of new drugs may be highly accelerated by virtue of *in silico* trials using advanced biological networks or organs-on-a-chip technique.

ONCOLOGY WITHOUT IMAGING – MISSION IMPOSSIBLE ?

Barbara Bobek-Billewicz[1]

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Oncology without diagnostic imaging is a mission impossible without a question mark.

Why ?

In shortest without knowing the goal, it is difficult to achieve it and most of the goals of oncological treatment are defined by diagnostic imaging.

Medical imaging

- plays a crucial role in cancer detection and diagnosis
- allows to define exact anatomical location and extent of disease
- provides information on tumor response to therapies

Radiotherapy more than any other treatment modality relies heavily and often exclusively on diagnostic imaging .

All image modalities are used in the process of diagnosis, treatment planning, evaluation of treatment response and follow up of cancer patients

Session V:
Chromatin and Gene Regulation

chairperson Ronald Hancock and Joanna Rzeszowska

EFFECTS OF SELECTIVE DEGRADATION OF THE COHESIN COMPLEX ON HIGHER ORDER CHROMATIN STRUCTURES STUDIED WITH LIVE CELL AND SUPER-RESOLVED FLUORESCENCE MICROSCOPY

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Background: Current models postulate that cohesin, a multi-protein complex with multiple functions, is essential for the organization and shaping of the genome into chromatin loops or domains. Loss of cohesin was found to result in an immediate growth arrest of cells. Recent Hi-C data based on large cell populations showed that cohesin loss eliminates all loop domains *(Rao et al., Cell 171; 2017).* In our ongoing study we aim to investigate the effect of cohesin loss on the preservation/alteration of higher order chromatin structures on the single cell level with super-resolution microscopy and live cell studies. As reference structures we chose replication domains (RDs) with an average DNA content of ~1 Mb and a size of ~150 nm. RDs were previously shown to represent ~1 Mb chromatin domains (CDs), which become associated with the replication machinery during S-phase, but persist throughout interphase and during subsequent cell cycles. Hi-C defined topologically associating domains (TADs) likely correspond with ~1 Mb chromatin domains suggesting that TADs/CDs are the major organizational subchromosomal elements of chromosome territories.

Methods, Results: All experiments were performed with HCT-116-RAD21-mAID-mClover cells. In these cells RAD21 (a subunit of cohesin) fused to a conditional auxin inducible degron (AID + mClover) is rapidly depleted upon addition of auxin *(Natsume et al., Cell Reports 15; 2016).* Efficiency of RAD21 depletion was verified by loss of mClover fluorescence and lack of immunostaining with a RAD21 specific antibody. Live cell observations demonstrated the appearance of mitotic cells up to ~16h of auxin treatment with a strongly prolongated mitotic phase resulting in a high fraction of mitotic cells, which were unable to divide into two daughter cells and often formed a multilobulated or blebbed nucleus. Microscopic visibility of RDs was achieved with scratch-replication-labeling yielding the incorporation of fluorophore-labeled nucleotides into replicating DNA of S-phase cells. Typical RD patterns persisted after auxin treatment during a period of up to 46 h (longer times were not tested) and were transmitted over mitosis irrespective of highly abnormal nuclear morphologies. No significant difference of RD patterns and size of individual RDs was noted at the resolution level of 3D-SIM (xy ~120nm; xz ~300 nm) between control cells and cells which were first scratch-replication-labeled and then incubated with auxin. By contrast, when Rad21 proteolysis was first achieved by incubation with auxin and scratch-replication-labeling was performed thereafter in the continued prescence of auxin, we noted the formation of distinctly coarsened RD structures.

Conclusions, open questions and further work: Our microscopic observations provided information on the effects of a RAD21 proteolysis in individual cells, which were not recognized in Hi-C studies of entire cultures. These findings exemplify the importance to combine Hi-C with advanced 3D and 4D microscopy. Single cell Hi-C studies of individual interphase and mitotic cells identified by microscopy could help to further study connections between the level of chromatin loops and higher order chromatin arrangements in interphase and mitosis following cohesin depletion.

NUCLEAR ORGANIZATION AND FUNCTION STUDIED IN SPACE AND TIME

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A new international research field, called the 4D nucleome, is making a major effort to understand the implications of nuclear organization and function in space and time. The ANC-INC model predicts that the cell nucleus is partitioned into an active nuclear compartment (the ANC) and a co-aligned, mostly inactive nuclear compartment (the INC). The ANC is formed from two parts, a network of variably sized, irregularly shaped channels, called the interchromatin compartment (IC), and its lining, readily accessible and transcriptionally competent chromatin, termed the perichromatin region (PR). The IC starts/terminates at nuclear pores and permeates between chromatin domains (CDs). Its finest branches apparently extend between nucleosome clusters, which form building blocks of higher order chromatin organization. The INC harbors predominanantly transcriptionally silent chromatin. It is located more remotely from the IC and comprises chromatin with a higher compaction than the PR. We present and discuss current evidence for this model with special emphasis on a hypothesis predicting major functional roles of the IC including: (a) rapid intranuclear distribution of transcription factors and regulatory, non-coding RNAs to their target sites, and (b) channeled movements of RNPs towards nuclear pores.

References:

Cremer, T., Cremer, M., Hbner, B., Strickfaden, H., Smeets, D., Popken, J., Sterr, M., Markaki, Y., Rippe, K., and Cremer, C. (2015). The 4D nucleome: Evidence for a dynamic nuclear landscape based on co-aligned active and inactive nuclear compartments. FEBS Lett. *589*, 2931-43.

Kirmes, I., Szczurek, A., Prakash, K., Charapitsa, I., Heiser, C., Musheev, M., Schock, F., Fornalczyk, K., Ma, D., Birk, U., *et al.* (2015). A transient ischemic environment induces reversible compaction of chromatin. Genome Biol *16*, 246.

Cremer, M., Schmid, V.J., Kraus, F., Markaki, Y., Hellmann, I., Maiser, A., Leonhardt, H., John, S., Stamatoyannopoulos, J., and Cremer, T. (2017). Initial high-resolution microscopic mapping of active and inactive regulatory sequences proves non-random 3D arrangements in chromatin domain clusters. Epigenetics Chromatin *10*, 39.

Schmid, V.J., Cremer, M., and Cremer, T. (2017). Quantitative analyses of the 3D nuclear landscape recorded with super-resolved fluorescence microscopy. Methods *123*, 33-46.

Szczurek, A., Klewes, L., Xing, J., Gourram, A., Birk, U., Knecht, H., Dobrucki, J.W., Mai, S., and Cremer, C. (2017). Imaging chromatin nanostructure with binding-activated localization microscopy based on DNA structure fluctuations. Nucleic Acids Res *45*, e56.

Cremer, T., Cremer, M., and Cremer, C. (2018). The 4D Nucleome: Genome Compartmentalization in an Evolutionary Context. Biochemistry (Mosc) *83*, 313-325.

MAMMALIAN INO80-CLASS CHROMATIN REMODELERS IN THE MAINTENANCE OF GENOME INTEGRITY DURING S-PHASE

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We studied the involvement of the human INO80 chromatin remodeling complex during unchallenged replication and under replication stress. Fiber labelling analysis indicated that INO80 was specifically needed for efficient replication elongation, but not for initiation of replication. We found that cells deficient for Ino80 or Arp8 subunits had impaired replication restart after treatment with replication inhibitors and accumulated DNA damage as evidenced by the formation of phosphorylated H2AX and Rad51 foci. These data indicate that mammalian INO80 protects stalled forks from collapsing and allows their subsequent restart.

Further studies found that the knock-down of subunits specific to any of the mammalian INO80-class remodelers (SRCAP and Trrap-Tip60 in addition to INO80) resulted in impaired replication. A common feature of these remodelers is the split ATPase domain to which RUVBL-proteins bind, which led us to examine the role of RUVBL proteins in replication. We found that deregulated expression of these chromatin regulators resulted in transcription-dependent replication stress. We have examined the mechanisms underlying transcription-dependent replication stress in cells with deregulated expression of RUVBL proteins and the ways to exploit it.

TARGETING OF CHROMATIN REMODELING COMPLEXES AS A BASIS FOR NEW ANTICANCER THERAPIES

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The SWI/SNF- type ATP-dependent chromatin remodeling complexes (CRCs) are conserved from fungi to mammals and plants. SWI/SNF CRCs regulate the DNA accessibility by control of chromatin structure, activity and organization. The SWI/SNF impairment causes embryo lethality or severe defects in development, including carcinogenesis in animals. Recent study indicated that SWI/SNF complexes play important role in control of various regulatory processes like i.e. hormone signaling pathways and their crosstalk in both human and Arabidopsis. Furthermore, it has been reported that different classes of SWI/SNF complexes are involved in regulation of specific processes.

In yeast, the core of the SWI/SNF CRCs consists of SWI2/SNF2 subunit with ATPase activity, two SWI3-type proteins and one SNF5 protein. In humans, mutations in *SMARCB1*, the gene coding for SNF5/INI1/BAF47 homologue lead to the formation of extremely aggressive malignant rhabdoid tumors. As both human and Arabidopsis SNF5 type proteins are encoded by a single gene this subunit is likely the ideal marker of any SWI/SNF complex, however there are reports suggesting the existence of SWI/SNF complexes without INI1 in some types of cancer.

Here we show, using combined multidirectional approach, including molecular biology and cytology methods, classical genetics approaches and two models-Arabidopsis and human that the role of SNF5-type subunit is far more complicated as it was believed. We found that the loss of INI1 protein may be one of primary reasons for clear cell renal cell carcinoma (ccRCC) development but not for ccRCC with rhabdoid component. Furthermore, our genetic approaches demonstrated that indeed the Arabidopsis SNF5-type is not an essential subunit of SWI/SNF complex. Furthermore, our study demonstrated that the combined inactivation of various SWI/SNF subunits may cause synthetic lethality. Summarizing, our results show not only an important role of SWI/SNF CRCs in carcinogenesis but also indicate its subunits as perfect potential therapeutic targets for approaches based on i.e. synthetic lethality.

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REMODELING OF CHROMATIN DURING STRESS - A LESSON FROM NUCLEAR LAMINA PROTEINS

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Lamins and interacting karyoskeletal proteins are thought to play important role in chromatin organization, remodeling and regulation of gene expression. Lamins and lamin associated proteins have been implied in interaction with chromatin *in vivo* and their binding to DNA and/or location is regulated by different signaling pathways. Recent studies demonstrated that lamins A/C-type and lamin B1 type participate in translocation of genes within nucleus and are involved in epigenetic regulation of gene expression of many genes. Therefore we analyzed the role of lamins and interacting proteins in epigenetic regulation of transcription in fly model system under normal conditions, stress and recovery. We have chosen a well known and widely used heat shock method as an inducer of stress and fly model since in fly heat shock shuts down transcription of all loci except so called heat shock loci and the molecular background is sufficiently understood.

We found that stress changes the composition of protein complexes associated with lamins as well as properties of lamin Dm itself. Similarly, topoisomerase II proteome changes upon heat shock induction. Using immunoprecipitation, mass spectrometry, PLA and other *in vitro* methods we demonstrated that lamin Dm and topo II interact with each other. We found that lamin Dm - topo II interactions also take place directly at the chromatin/DNA. Changes in protein composition of lamin and topo II complexes upon stress induction correlated with increased solubility of lamin Dm. Similarly increased solubility was demonstrated for lamin Dm interacting proteins including such chromatin proteins as HDAC1, histone H3 and topoisomerase II. Changes in lamin Dm solubility and composition of lamin complexes upon stress induction correlate with general increase of association between lamin Dm and topo II with DNA. Based on our preliminary data we may assume a working hypothesis that stress inducs remodeling of post-translational modification (phosphorylation?) of lamins, which in turn affects lamin interactions (polymerization?) thus increasing the mobility of lamins and interacting proteins which, in turn, associate with chromatin.

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Session VI:
Evolution of Cancer

chairperson: Marek Kimmel

METABOLIC AND DEVELOPMENTAL REPROGRAMMING IN HEPATOCELLULAR CARCINOMA

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The hepatocyte, comprising up to 85% of the liver mass, plays an essential role in homeostasis performing a myriad of metabolic functions, synthesizing large quantities of protein which are excreted into the blood and bile acids for digestion, to name a few. As a consequence little energy is expended on cell division in the hepatocyte, unlike epithelia that lines other organs. Therefore conversion of the hepatocyte from its normal function into a rapidly dividing cancer cell requires extensive adaptation. Through large-scale multi-platform genomic analysis of hepatocellular carcinoma we identify some of the alterations that eliminate functions, which from the perspective of the cancer cell, are non-essential. The hepatocyte is effectively reprogrammed through genetic and epigenetic mechanisms to alter key metabolic and signaling pathways such as albumin production, the urea cycle and pyrimidine nucleotide synthesis, while up regulating the hedgehog pathway critical to stem cell renewal. This reprogramming exposes new vulnerabilities that might be exploited clinically through novel targeted therapies.

STUDYING TUMOURS IN 3.5D

Simon Tavare[1]

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Several groups have developed inferential methods for deducing features of tumour evolution, and for understanding tumour heterogeneity. Much of this information comes from DNA and RNA sequencing of bulk tumour samples, and from a theoretical viewpoint we know what is easy to estimate and what is not. More recently, the focus has moved to samples of single cells, producing data more reminiscent of those obtained in population genetics studies. In contrast to the latter setting, it has not until recently been possible to obtain molecularly annotated data from spatially resolved samples of cells. In this talk I will describe our Cancer Research UK Grand Challenge award that develops one approach to studying tumours in (a bit more than) 3D, producing novel in-situ molecular data on a vast scale. This resolution will allow us to identify cell types in a biopsy, and how those cells are interacting. I will show how we are using virtual reality platforms to display and interact with the data.

CANCER STEMNESS AS HALLMARK OF ONCOGENIC PROGRESSION

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Cancer progression involves the gradual loss of a differentiated phenotype and acquisition of progenitor and stem cell-like features. Here, we provide new stemness indices for assessing the degree of oncogenic dedifferentiation. We took advantage of an innovative one-class logistic regression machine learning algorithm (OCLR) to extract transcriptomic and epigenetic feature sets derived from non-transformed pluripotent stem cells and their differentiated progenies. Using OCLR, we were able to sort TCGA tumor samples by stemness phenotype and identify previously undiscovered biological mechanisms associated with the dedifferentiated oncogenic state. Analyses of tumor microenvironment revealed the correlation of cancer stemness with immune checkpoint expression and infiltrating immune system cells not previously anticipated. We have shown the de-differentiated oncogenic phenotype increased in the metastatic tumor that further justify their more aggressive phenotype. Application of our stemness indices reveals features of intra-tumor heterogeneity in molecular profiles obtained from the single-cell analyses. Finally, the machine learning-based indices allowed for the identification of chemical compounds and novel targets for the cancer therapies aiming at tumor differentiation. Our findings provide new prognostic signatures that enable cancer biologists and oncologists to quantify the impact of tumor stemness on outcome across cancer types and may help to pave the way for progress in treatment strategies for cancer patients.

EXTRACTING TUMOR GROWTH AND MUTATION HISTORIES FROM SEQUENCING DATA

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Recent abundance of bulk sequencing data from human tumors has prompted questions concerning their interpretation. It is a complex problem, not only because of biological complexity of tumors themselves. Sampling inherent in sequencing introduces biases and uncertainties. A recent Nature Genetics paper by Williams et al. provides a comprehensive introduction to simulation and estimation techniques that might be used to analyze the site frequency spectra (SFS) of mutations present in tumor genomes. The present talk analyses in what way existing mathematical models might help the effort. We focus on two of these, based on the Tajima-Kingman coalescent and birth-death process, developed correspondingly by Griffiths and Tavare and Lambert and his co-workers. They lead to similar SFS forms, both in simulations and analytically, although they differ in assumptions. We also consider different read (i.e. DNA fragments used in sequencing) sampling schemes which involve rejecting different portions of data deemed unreliable, and show their effect on SFS of genomes available via The Cancer Genome Atlas (TCGA). We also attempt to correlate parameters extracted from the SFS with presence or absence of major driver mutations in a given tumor. Aside from importance for cancer research, the topic reveals interesting connections with unsolved issues in coalescent theory and branching processes. Collaboration with Simon Tavare, Amaury Lambert, Khanh Dinh and Roman Jaksik is acknowledged.

Poster sessions

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

Session I:
Regulation of cellular processes

[I-1] NEUTRALIZATION OF REACTIVE OXYGEN AND NITROGEN SPECIES MAY DEPEND ON THE CELL LINE

Sylwia Ciesielska[1], Patryk Bil[1], Joanna Rzeszowska[1]

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Redox reactions in cells are reduction and oxidation reactions in which electron transport occurs and are connected with the appearance and neutralization of Reactive Oxygen Species (ROS). Regulation of cellular redox state seems to be a key element which decides about responses to different environmental factors or to therapeutic measures. Changes of redox state in both normal and cancer cells may occur during their normal life cycle and under the influence of chemical or physical factors such as UV radiation.

We assayed ROS and Reactive Nitrogen Species (RNS) in two cancer cell lines, HCT116 (Human Colorectal Carcinoma) and Me45 (Human Melanoma), after UV irradiation. We compared the influence of different UVA doses on cell proliferation (no effect, inhibition of proliferation, or stimulation of proliferation). Different doses of UVA caused different effects as observed for the entire cell population.

The highest doses of UVA caused a decrease of clonogenicity in both types of cells, but exposure to some lower doses, specific to each cell line, caused stimulation of proliferation. Analysis of ROS showed that for each cell line the level of ROS fluctuated during growth. Exposure to high doses caused inhibition of proliferation and changes in free radical dynamics, but lower doses which stimulated proliferation did not cause such changes.

The two types of cells also showed differences in the expression of genes the products of which participate in antioxidant systems, suggesting that the mechanisms of neutralization of ROS and NOS may differ among cell lines.

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[I-2] MODULATION OF THE DNA DAMAGE RESPONSE PATHWAY BY MDM2 IN BREAST CANCER CELLS

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MDM2 protein is an E3 ubiquitin ligase, a chief negative regulator of p53 tumor suppressor protein. It has been shown that MDM2 protein is involved in proper folding of wild-type p53 demonstrating a chaperone-like activity. This activity requires the binding of ATP by MDM2, however does not depend on its E3 ubiquitin ligase function. Apart from p53, it interacts with many proteins involved in all kinds of cellular pathways. Many studies implied that both p53-dependend and independent activities of MDM2 are involved in cancer development and progression. Also, clinical data suggest that MDM2 overexpression could be a negative prognostic marker for cancer patients. Our bioinformatics analysis performed on The Cancer Genome Atlas data set confirms that elevated level of MDM2 transcript correlates with worse prognosis for survival of breast cancer patients.

Our research is focused on p53-independent role of MDM2 protein in DNA damage response and its implications in breast cancer cells drug sensitivity. Utilizing a panel of breast cancer cell lines with different p53 status we demonstrated by co-immunoprecipitation the interaction between MDM2 and MRN (Mre11-Rad50-NBN) complex. To determine the impact of MDM2 on activation of DNA damage response pathway we performed a screening experiment with clinically used DNA damaging agents such as Camptothecin, Etoposide, Doxorubicin and analyzed the phosphorylation level of histone H2AX. We have shown that, upon MDM2 silencing with specific siRNA, phosphorylation of histone H2AX and other proteins of homologous recombination pathway was impaired. Moreover, cells with low MDM2 level were more sensitive to tested drugs in cell viability assay. Reduction of MDM2 expression results in features similar to homologous recombination deficiency. Thus, our findings suggest that MDM2 could be an important modulator in homologous recombination pathway and potentially prognostic marker for selection of cancer chemotherapy.

[I-3] PRO-DEATH SIGNALING DURING STRESS – MECHANISMS OF PMAIP1/NOXA ACTIVATION BY HEAT SHOCK TRANSCRIPTION FACTOR 1

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PMAIP1/NOXA is a proapoptotic protein, a member of the Bcl2 family. We found that Pmaip1 was the most induced gene in testes of transgenic mice expressing constitutively active HSF1 (Heat Shock Factor 1). HSF1 is the main mediator of the heat shock typically inducing cytoprotective Heat Shock Proteins (HSPs). However, cell death is induced by active HSF1 in mouse spermatocytes, possibly via PMAIP1.

Based on genomic studies we stated heat induced HSF1 binding to its consensus binding sites (HSEs), interestingly located in the *Pmaip1* introns, and upregulation of the *Pmaip1* transcription in spermatocytes and in certain somatic mouse and human cell lines and tissues subjected to heat shock. We found the PMAIP1 activation in so called heat-sensitive organs. TUNEL test demonstrated that apoptosis was rapidly induced by heat shock in tissues exhibiting the highest *Pmaip1* activation i.e. testes, thymus, and spleen.

Among cell lines, the highest *Pmaip1* induction was observed in HECa10 murine endothelial cells. Using HECa10 cells with decreased HSF1 expression (by shRNA) we confirmed that HSF1 is important for *Pmaip1* activation. Hemideletion of the HSE from the second *Pmaip1* intron (CRISPR/Cas9) also led to diminished activation of *Pmaip1* by heat shock, however we cannot exclude a clonal selection effect. Using live imaging microscopy and Western blot analyses we proved that *Pmaip1* overexpression led to caspase-3-dependent apoptosis. Furthermore, we deleted the *Pmaip1* gene in HEca10 cell to validate its participation in the regulation of apoptosis after heat shock.

Our findings support the idea that HSF1 may play a dual role in response to heat shock: cytoprotective, mediated by HSPs or proapoptotic mediated by PMAIP1. The final response to stress could be determined by the balance between by the antiapoptotic and proapoptotic factors differentially regulated by HSF1.

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[I-4] miR-20b-5p and miR-363-3p as candidate oncomiRs in pediatric T-cell acute lymphoblastic leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) is a rare and aggressive cancer, originating from T-cell precursors. T-ALL is characterized by marked heterogeneity on genetic and immunophenotypic level. MicroRNAs (miRNAs) belong to the class of small noncoding RNAs. They act as posttranscriptional regulators of gene expression by blocking translation of messenger RNAs (mRNAs) or promoting their degradation. Some miRNAs are encoded within polycistronic clusters and are transcribed as one pri-miRNA, giving rise to several mature miRNAs. Such miRNAs may co-regulate the expression of genes involved in certain biological processes. miRNAs are important regulators in a multitude of biological processes, including hematopoiesis, while aberrations of miRNA expression or function contribute to diseases, including leukemia.

In our recent study we performed miRNA profiling in pediatric T-ALL with the use of Next-Generation Sequencing. We identified miRNAs differentially expressed in T-ALL. The set of overexpressed miRNAs included, among others, miR-20b-5p, miR-363-3p and miR-18b-5p, belonging to a cluster of six miRNAs: miR-106a-363. This cluster is a paralog of miR-17-92 cluster, a prototypic oncogenic cluster of eminent importance in human hematopoietic cancers. Despite the similarity of seed sequences between miRNAs from both clusters, the significance of miR-106a-363 cluster in T-ALL remains to be elucidated.

In this study we investigated the expression of the miR-20b-5p and miR-363-3p and miR-18b-5p in children with T-ALL, healthy donor thymocytes, normal bone marrow samples and T-ALL cell lines. RT-qPCR confirmed overexpression of 2 miRNAs from cluster miR-106a-363 (miR-20b-5p and miR-363-3p) in children with T-ALL and in T-ALL cell lines. To predict potential target genes of overexpressed miRNAs belonging to miR106a-363 cluster, we applied 8 target prediction algorithms and pathway enrichment analysis. This revealed the enrichment target genes of miR-20b-5p and miR-363-3p in positive regulation of apoptosis Gene Ontology term. We further validated predicted miRNA-mRNA interactions by Dual Luciferase Assay, confirming the majority of them (e.g. *PTEN*, *FBXW7*). Finally, we assessed the effect of mimicry/inhibition of overexpressed miR-20b-5p and miR-363-3p on proliferation, cell cycle distribution and apoptosis in two T-ALL cell lines. Overexpression of miR-20b-5p and miR-363-3p resulted in increased proliferation and inhibited apoptosis.

Here we showed that miRNAs belonging to miR-106a-363 cluster directly interact with mRNAs implicated in the regulation of apoptosis. We demonstrated *in vitro* that miR-20b-5p and miR-363-3p act as pro-proliferative and anti-apoptotic regulators, thus are candidate oncomiRs and may have an oncogenic role in the biology of T-ALL.

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[I-5] THE PROTEIN MCPIP1 REGULATES THE MIGRATION ACTIVITY AND THE MALIGNANT PHENOTYPE OF CCRCC CELLS

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Introduction/Rationale: Tumorigenesis is a complex, multi-step process which depends on the acquisition of certain characteristics: the ability of uncontrolled growth, the production of enzymes and the ability to migrate. One of the factors that stimulate the development of cancer is inflammation. Inflammation is one 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 migration and invasion. The negative regulator of inflammation is the MCPIP1 protein (Monocyte Chemoattractant Protein-1 Induced Protein), induced by many pro-inflammatory factors. MCPIP1 degrading, among others IL-1, IL-6 as well as regulating the NFB pathway plays a role during the development and course of inflammation.

Methods: Normal and tumor epithelial cell lines were transduced with lentiviral vectors, the doxycycline-dependent TetON system. The levels of genes and proteins were studied by real-time PCR and Western blot. Microarray analysis was conducted on samples from tumors of patients suffering from ccRCC. Motility assays were performed to check migration and invasion. The location and distribution of EMT markers in cells was checked by immunofluorescence staining.

Results: This study investigated the correlation of the level of MCPIP1 protein and the process of EMT in normal kidney and tumor cell lines. Low expression of MCPIP1 protein increases the character of mesenchymal cells in tumor and normal cells. Downregulation of MCPIP1 suppressed E-cadherin and upregulated -catenin and vimentin. In contrast, overexpression of MCPIP1 causes a decrease of transcription factor Snail, vimentin and -catenin and an increase of E-cadherin. Microarray analysis of ccRCC tumor tissue revealed high transcripts level of mesenchymal phenotype and also correlates with the degree of tumor progression, metastasis and changes in cellular connections

Conclusions/Novel aspect: MCPIP1 overexpression significantly decreases the motile activity in ccRCC. The results indicate that the level of MCPIP1 is critical in the regulation of EMT and carcinogenesis. The results obtained may contribute to a better understanding of clear cell renal cell carcinoma progression, which may help in the development of more effective therapy in the future.

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[I-6] THE SURVIVAL KINASE PIM2 IS EFFICIENTLY STIMULATED BY STRONGLY ACTIVATED P53 PROTEIN

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The p53 protein is an inducer of apoptosis, acting as transcriptional regulator of apoptotic genes, which are induced by p53 when it is hyperactivated. This enables p53 monomers to form a tetramer and work in cooperative fashion on p53 response elements, which do not perfectly fit to the consensus sequence. Strong activation of p53 is marked by Ser46 and Ser392 phosphorylation. We observed that in various cell lines co-treatment with actinomycin D and nutlin-3a (A+N) synergistically hyperactivated p53. Actinomycin D by some unknown mechanism activates kinases, which phosphorylate p53, while nutlin-3a inhibits a negative regulator of p53 MDM2 protein and exposes p53 to the catalytic activity of kinases.

We found that agents which strongly activated p53 also induced inactivating phosphorylation of proapoptotic BAD protein on Ser136. We started our study with investigation of the source of this phosphorylation of BAD. This protein is phosphorylated by so-called survival kinases AKT and PIM2.

Cells in culture were treated with actinomycin D and nutlin-3a or with camptothecin - an anticancer drug and strong inducer of apoptosis. To obtain insight into the protein expression, the arrays of antibodies against apoptosis-related proteins were used. The protein expression was also examined by Western blotting and changes in the levels of mRNA were measured by semi-quantitative RT-PCR. Knockdown of p53 or PIM2 was performed using transduction-ready lentiviral particles containing constructs encoding shRNA targeting *TP53* mRNA or *PIM2* mRNA. The influence of p53 on the gene regulatory regions of *PIM2* was measured using dual-luciferase reporter assay system. For this purpose, the regulatory regions of *PIM2* with the potential p53 binding sites were cloned into pGL3-Basic reporter vector.

We found that strong activation of p53 was associated with upregulation of the *PIM2* gene. The level of PIM2 accumulation was associated with the degree of p53 activation in three cell lines and knockdown of p53 prevented strong accumulation of PIM2. Moreover, we demonstrated that exogenous p53 activated regulatory element from *PIM2* and PIM2 protein did not accumulate after stress in a p53-null cell line. In addition, we confirmed and extended the observations of other researchers, who found a p53 binding site in *PIM2* gene. This demonstrates that *PIM2* is another p53-regulated gene.

Based on our results, we conclude that *PIM2* gene is efficiently stimulated only by strongly activated p53 molecules. The biological role of activation of prosurvival protein (PIM2) by proapoptotic p53 is unknown. Because PIM2 expression is stimulated early after p53 activation (6 hours), we speculate that it promotes the survival of cells, which may be repaired if the stress conditions disappear. Otherwise the proapoptotic program of p53 overpowers the prosurvival activity of PIM2.

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[I-7] LACK OF P53 ALTERS THE BASAL REDOX ENVIRONMENT IN HUMAN COLON CANCER CELLS

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The role of p53 protein in the repair of DNA damage, cell cycle inhibition, apoptosis, and carcinogenesis has been intensively studied since its discovery in the 1970s. The concept of p53 as a regulator of the redox environment appeared much later and at first assumed a prooxidative function only. A relatively new idea is the ability of p53 to function both as pro- and anti-oxidant.

Here we present results showing that lack of p53 in human colon cancer cells causes alterations in the redox environment. In p53lacking RKO and HCT116 cells we observed a decrease in the total level of reactive oxygen species despite a significant increase in superoxide anion production. Overrepresentation analysis of microarray gene lists revealed that p53-deficient cells are characterized by altered gene ontologies related to superoxide anion production, lipid oxidation, and cellular response to hydrogen peroxide as well as ATP biosynthesis. Several aspects of mitochondria functioning seem to be affected in p53-/- cells judging by the changes in the mitochondrial mass and membrane potential as well as altered expression of several genes involved in the regulation of mitochondrial organization, membrane potential and permeability. In p53-/- cells, we also identified several differentially (compared to cells with functional p53) expressed antioxidants including overexpressed glutathione-disulfide reductase (GSR) and underexpressed glutathione peroxidase 4 (GPX4).

Our results implicate that under normal (non-stressful) conditions p53 is a factor involved in regulation of redox homeostasis and that its status is important for reactive oxygen species production and antioxidative activity in human colon cancer cells.

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[I-8] SCAFFOLDS FOR CONTROLLED OLIGOMERIZATION OF FGFR1 LIGANDS

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Fibroblast growth factor receptors (FGFRs) together with extracellular fibroblast growth factors (FGFs) transmit signals through the plasma membrane. FGFR signaling regulates pivotal cellular processes like proliferation, migration and apoptosis. The ultimate effect of FGFRs signaling depends on the spatial distribution of the receptor in the plasma membrane.

Here we utilize various protein-based scaffolds as a tool for controlled oligomerization of FGFR1 ligands. Our strategy is based on four different oligomerization scaffolds: streptavidin (SA), coiled-coil motifs (cc), GFP polygons (GFPp) and antibodies (Ab). SA scaffolds allow for controlled oligomerization of biotinylated ligands up to tetramers. SA tetramers with varying number of biotin-binding sites are obtained by simultaneous refolding of wild-type SA and mutant defective in biotin binding. CC motifs are genetically fused to ligands and allow for formation of oligomers up to hexamers. GFPp is specific variant of GFP where one -strand was moved into position that doesnt allow for folding into fluorescent -barrel, but facilitates intramolecular folding, leading to assembly of GFPp into various oligomeric structures. Ligands can be fused with GFPp either genetically or by interaction between GFPp-ProtG and ligands containing Fc fragment of IgG. Finally, FGFR1 ligands are successfully oligomerized by sequential fusion with Fc fragment of IgG.

Constructed oligomeric ligands by binding to FGFR1 will adjust spatial distribution of the receptor, regulating receptor lifetime, trafficking and function and thus outcome of FGFR-dependent signaling.

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[I-9] SPATIAL REGULATION OF FIBROBLAST GROWTH FACTOR RECEPTOR 1 BY EXTRACELLULAR GALECTINS

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Fibroblast growth factor receptors (FGFRs) are integral membrane proteins that transmit signals through the plasma membrane. FGFR signaling needs to be precisely controlled as aberrant FGFR signaling is observed in numerous human cancer types. The function and trafficking of FGFRs relies on the formation of multiprotein complexes. Here we used mass spectrometry-based identification of FGFR1 interaction partners.. We identified galectin-1 and galectin-3, lectins implicated in cancer development and progression as novel FGFR1 interactors. We demonstrated that galectin-1 and galectin-3 directly bind to sugar chains of the glycosylated extracellular region of FGFR1. Studied galectins regulate FGFR1 activity and distribution, but to different extent. Extracellular galectin-1 efficiently activates FGFR1 and receptor-downstream signaling pathways in the absence of the growth factors and as a result induces cell proliferation and protects cells from apoptosis. In contrast, galectin-3 causes extensive receptor clustering that results in only very weak activation of signaling cascades. Galectin-3 induced FGFR1 clusters are highly dynamic as that are efficiently disassembled by FGF2. Summarizing, our data demonstrate that galectin-1 and galectin-3 are novel FGFR1-binding proteins that differentially regulate receptor distribution on the plasma membrane and receptor function. Galectin-FGFR1 interplay may contribute to uncontrolled FGFR signaling in various cancers.

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[I-10] INFLUENCE OF THE MICROENVIRONMENT OF SKOV-3 OVARIAN CANCER CELLS ON NORMAL FIBROBLASTS

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The microenvironment of cells is a complex structure that may directly or indirectly influence the development and changes occurring in individual cells. Exosomes are vesicular particles 30-100 nm in diameter which may, among other things, mediate the transfer of information between cells thus being one of the microenvironmental elements. Ovarian cancer is currently the fifth leading cause of morbidity among women in Poland. Late diagnosis and associated high mortality rate make understanding of the mechanisms of this cancer very important. Due to many side effects of chemotherapy researchers are looking for novel natural substances suitable for cancer treatment that can be found in herbs. Plants of the family *Clusiaceae* Lindl. are a rich source of compounds termed xanthones. One of the most active compounds is -mangostin with high anticancer potential.

The aim of the study was to evaluate the effect of exosomes from treated SKOV-3 ovarian cancer cells on normal fibroblast cells.

The SKOV-3 ovarian carcinoma cell line and normal NHDF fibroblasts were used in the study. XTT test was used to determine IC50 values. SKOV-3 cells were treated with cisplatin (IC25) and -mangostin (IC25). Exosomes were isolated from supernatants of ovarian cancer cells and fibroblasts (Total Exosome Isolation Reagent, Invitrogen). The NTA (Nanoparticle Tracking Analysis) technique was used to assess and characterize isolated exosomes. The uptake of exosomes derived from ovarian cancer cells by fibroblasts was evaluated. Measurements were made after 1, 2, 3 and 4 hours. Exosomes were stained with Dil dye (Invitrogen) and cells with SYTO RNASelect dye Green Fluorescent cell Stain. A wound healing test was also performed. Measurements were performed at the following time points: 0h, 2h, 6h, 12h, 24h, 36h, 48h and 72h.

In ovarian cancer, the IC 50 value for -mangostin (7.3 M) was significantly lower compared to cisplatin (50 M). NTA analysis of exosomes revealed that untreated ovarian cancer cells secrete 49.9 times more exosomes than normal fibroblasts (average molecular concentration in the 35 nm - 95 nm range for SKOV-3 47 519 milion molecules/ml exosomes, for fibroblast exosomes 952 milion molecules/ml).There was a significant decrease in ovarian cancer cell exosomes secretion due to treatment of cells with cisplatin (by 87.1%) and -mangostin (by 85.3%). Analysis of the uptake of exosomes of tumor origin by normal fibroblasts showed that exosomes from non-treated ovarian cancer cells were found within fibroblasts after an hour. In turn, exosomes from cells treated with -mangostin and cisplatin appeared inside fibroblasts in the fourth hour of incubation. The wound healing test showed differences in overgrowth of the scratch depending on the origin of exosomes (from treated or untreated cells).

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[I-11] THE INFLUENCE OF SPENT HOPS OF HUMULUS LUPULUS L. EXTRACT ON THE VIABILITY OF CANCER AND NORMAL HUMAN COLON CELLS

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Introduction: The hop (*Humulus lupulus* L.) is used in the production of beer and is responsible for its taste and specific aroma.The female cones of this plant as well as the spent hops after the hops extraction by supercritical CO2 are the source of the substances with high biological activity. These include phenolic compounds among others: catechin, epicatechin, quercetin, kaempferol having a lot of properties which could be used in the medicine. They also demonstrate anti-proliferative activity which is responsible for the inhibition of the cancer cells growth.

Aim: The aim of the present study was to evaluate the effect of spent hops extract on the viability of cancer and normal colon epithelial cells.

Materials and methods: Three cell lines were tested: two colon cancer lines (SW-480 and HT-29) and normal epithelial colon (CCD841CoN) cell line. The activity of the spent hops extract was tested on the basis of cell growth by means of the MTT test. The cells were incubated with the tested extract at 37°C with the constant of CO2 content in atmosphere for 24, 48, 72 hours.

Results: The results showed that tested extract inhibited the growth of two colon cancer cell lines (SW-480 and HT-29) more than the growth of normal cell line (CCD841CoN).The IC50 value for SW-480 cell line was obtained at the concentration 400 g/mL after 48-hours incubation, for HT-29 cell line at the concentration 200 g/mL after 72-hours incubation while for normal epithelial CCD841CoN cell line the IC50 value was not received.

Conclusions: The spent hops extract has anti-proliferative activity. The most susceptible to extract was SW-480 cell line. The normal CCD841CoN epithelial cells were the least sensitive to the extract activity.

[I-12] THE ELEVATED LEVEL OF HSF1 DIRECTS HUMAN MAMMARY EPITHELIAL CELLS TOWARDS MESENCHYMAL PHENOTYPE

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Heat Shock transcription Factor 1 (HSF1) is ubiquitously expressed protein. It is well-known regulator of Heat Shock Protein (HSPs) expression in response to elevated temperature. Beyond, several studies on hsf1-null organisms pointed an HSF1 role in development and growth. High level of HSF1 expression has also been found in a broad range of tumors and tumor cell lines. Its activity was found to be implicated in signaling pathways associated with growth and proliferation, apoptosis, glucose metabolism, angiogenesis and cell motility.

We observed that the level of HSF1 varies in different cell lines established either from non-cancerogenous (MCF10a and MCF12a) or cancerogenous (MCF7, SKBR3, MDA-MB-468 and BT-549) tissues of mammary gland. We aimed to study how the HSF1 can affect two non-tumorigenic human mammary epithelial cell lines (MCF10a and MCF12a). We noticed that these two lines differ by HSF1 level, morphology, expression of E-cadherin (epithelial marker) and vimentin (mesenchymal marker), and growth in matrigel. MCF10a cells has more epithelial properties while MCF12a more mesenchymal. We down-regulated HSF1 expression in these two lines using specific shRNAs. This led to inhibition of the proliferation and spheroid formation in matrigel by MCF10A cells. However, down-regulation of HSF1 did not affect the proliferation of MCF12A cells but led to formation round spheroids in matrigel in contrast to non-modified cells, which form mass-shaped acini. Overexpression of HSF1 led to change in cell morphology towards more elongated fibroblast-like cells in MCF10a, but no effect on morphology and proliferation was observed in MCF12a. We determined expression of several markers associated with epithelial-to-mesenchymal transition and we noticed that HSF1 overexpression led to E-cadherin reduction and vimentin increase in MCF10a cells, while HSF1 silencing resulted in E-cadherin up-regulation and vimentin down-regulation in MCF12a. Above results suggest that elevated level of HSF1 may direct human mammary epithelial cells towards the mesenchymal phenotype while HSF1 lowering could reverse mesenchymal phenotype to epithelial one.

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[I-13] ERALPHA-SIGNALING IS DIRECTLY REGULATED BY TRANSCRIPTION FACTOR HSF1

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HSF1 is a transcription factor, which is activated by environmental stress. In turn, it activates expression of HSPs (Heat Shock Proteins), which participate in the folding of other proteins and protect cells from stressinduced apoptosis. Moreover, HSF1 and HSPs are overexpressed in many types of tumors. Among others, a supportive role of HSF1 was established for breast cancerogenesis. High level of HSF1 was found in estrogen receptor positive (ER+) breast cancer patients, which was correlated with worse prognosis. ER+ breast cancers represent up to 80% of all cases and rely on supplies of the estrogen to grow.

We noticed, that 17b-estradiol (E2) treatment led to rapid phosphorylation of HSF1 on S326 (activation site) in ERs-positive mammary breast adenocarcinoma MCF7 cells, but not in ER-negative normal breast MCF10A and MCF12A cells nor in ovarian ER-negative cancer cell lines, such as ES2 or OVCAR3. HSF1 phosphorylation also occurred in MCF7 treated with other ERa agonists, such as propylpyrazoletriol (PPT) or bisphenol A (BPA). Furthermore, silencing of ER expression by specific siRNA resulted in inhibition of HSF1 activation after E2 treatment. Using specific inhibitors for ERK1/2, PI3K and mTOR we showed, that ERK1/2 and mTORdependent signaling pathways are involved in the phosphorylation of HSF1 by E2. Chip-Seq analyses revealed that E2activated HSF1 is transcriptionally potent and binds to the regulatory regions of some genes. Furthermore, RNA-seq analyses performed on cells with silenced HSF1 confirmed that HSF1 could directly affect the expression of E2-regulated genes, among others *GREB1* (growth regulating estrogen receptor binding 1).

To summarize, we found that E2 leads to HSF1 phosphorylation in ERa-dependent manner. In turn, HSF1 can affect some genes involved in E2-dependent signaling.

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[I-14] IONIZING RADIATION PROPAGATES CELLULAR FERROPTOSIS UNDER OXIDATIVE STRESS IN NORMAL HACAT AND CANCER ME45 CELLS

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Ferroptosis is a non-apoptotic form of regulated cell death dependent on iron and characterized by the accumulation of lipid peroxides. Ferroptosis can be triggered by small molecules or conditions that inhibit glutathione biosynthesis or the glutathione-dependent antioxidant enzyme glutathione peroxidase 4 (GPX4) [1, 2]. Ferroptosis is driven by lipid oxidative degradation resulting from GPX4 activity depletion which is responsible for enzymatic lipid repair [3].

Aim of the studies: the ferroptosis activator 1S,3R-RSL 3 (RSL3) binds and inactivates GPX4 and thus mediates GPX4-regulated ferroptosis. RSL3 exhibits selectivity for tumor cells bearing oncogenic RAS. Ferrostatin (Fer-1) is a potent and selective inhibitor of ferroptosis. We determined the effects of ionizing radiation (IR) and different doses of RSL3 and Fer-1 on reactive oxygen species (ROS) and apoptosis.

Materials and methods: melanoma (Me45) cells and normal keratinocytes (HaCaT) were exposed to 4 Gy of IR. Levels of reactive oxygen species in cells were estimated at different time points following IR exposure (1, 6, 12 and 24 h). Apoptosis and ROS were tested by flow cytometry using Annexin-V for apoptosis and CellROX Green for ROS assays, respectively. Additionally, specific ferroptosis inducer RSL3 and inhibitor Ferrostatin-1 were examined under the oxidative conditions, followed by 24h MTT viability assay.

Results and conclusions: after 24h of RSL3 treatment surviving fractions decreased by about 20% in both cell lines. Finally, IR exposition resulted in the occurrence of ferroptosis pathway markers and GPX4 expression. ROS production in 12h in Me45 cells was lower than in HaCaT cells, as expected from their radioresistance profile.

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References:
[1] Stockwell, B. R. et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell. 171(2), 273285 (2017).
[2] Cao J. Dixon S. Mechanisms of ferroptosis, Cell. Mol. Life Sci. 73, 2195-2209 (2016).
[3] Yang, W.; Stockwell, B. Ferroptosis: Death by Lipid Peroxidation . PubMed Central, (2015).

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[I-15] IONIZING RADIATION INDUCES RELEASE OF EXOSOMES ENRICHED IN PROTEINS INVOLVED IN CELLULAR RESPONSE TO GENOTOXIC STRESS

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Ionizing radiation (IR) as the basis of radiotherapy is one of the most commonly used tools in anti-cancer therapy. It consists of photons or charged particles which interact with molecules in irradiated cells causing damage to DNA and cell death. IR can act on cellular molecules directly, or indirectly by producing free radicals. Although radiation therapy has been improved by many technological advances over the years, poor treatment outcome in many patients remains unexplained. Deep characteristics of defense mechanisms in cancer cells induced during radiotherapy treatment may contribute to improvement of cancer curability. Exosomes reported as important players in intercellular communication seem to be a promising subject of a study from the point of view of their role in cellular response to genotoxic agents.

In our study we characterized the proteome profile of exosomes released in vitro by irradiated oral cancer cells and we identified proteins potentially associated with exosome-driven cellular response to genotoxic agents. Exosomes and other small extracellular vesicles were purified by size-exclusion chromatography from cell culture media collected 24 hours after irradiation of UM-SCC6 cells with a single 2, 4, and 8 Gy dose, and then proteins were identified using a shotgun LC-MS/MS approach. In general, exosome-specific proteins encoded by 1217 unique genes were identified. There were 472 proteins the abundance of which in exosomes was significantly affected by radiation, including 425 upregulated and 47 downregulated species. Several functional GO terms were associated with radiation-affected exosome proteins. Among over-represented processes were those involved in response to radiation, metabolism of reactive oxygen species, DNA repair, chromatin packaging and protein folding.

Qualitative and quantitative analysis of proteins identified in exosomes released from irradiated UM-SCC6 cells showed significant influence of ionizing radiation on the proteome composition of exosomes which supports the assumption about the possible influence of these proteins on the cellular response to the examined factor commonly used in anti-cancer therapy.

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[I-16] INDUCED NEURAL STEM CELLS WITH DOXYCYCLINE-INDUCIBLE EXPRESSION OF IDH1R132H AS A NEW TOOL FOR THE STUDY OF MUTANT PROTEIN FUNCTION

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The physiological function of isocitrate dehydrogenase 1 (IDH1) is to catalyze the conversion of isocitrate to -ketoglutarate with formation of NADPH. Mutations in *IDH1* gene are often detected in gliomas. 90% of all *IDH1* mutations are heterozygous point mutations that result in the substitution of arginine to histidine at codon 132 (R132H). As a result of the mutation -KG level decreases and D2HG oncometabolite is formed. At the same time, there is also a reduction in the level of NADPH, which participates in the creation of D2HG. Accumulation of D2HG and inhibition of -KG-dependent dioxygenases is associated with the risk of neoplastic transformation, although the exact mechanism has not yet been discovered.

The analyses were performed on a model of induced neural stem cells (iNSc) with introduced Tet-On 3G regulatory elements. Expression of IDH1R132H was induced with doxycycline (1 g/ml, added with medium change every 48 hours) and its impact was assessed with Sanger sequencing, Real-time Quantitative Reverse Transcription PCR and immunocytochemistry at different incubation time points (24h, 48h, 72h and 120h). In the case of 72-hour incubation with doxycycline, we have observed two similar peaks of A/G at codon 132. Real-Time qRT-PCR analyses showed the highest expression of IDH1R132H mRNA following 72-hour incubation with doxycycline and decreased significantly after 120 hours, which may suggest the extinction of induced expression, mRNA degradation or occurrence of cellular apoptosis caused by the presence of this mutated protein. A similar result was observed on the protein level in the ICC analysis.

The cell model based on iNSc with introduced Tet-On system provides opportunity to control the level of the mutant IDH1R132H protein and to further analyze the activity of -KG-dependent dioxygenases. Obtaining a cellular model similar in terms of IDH1R132H and IDH1wt expression levels to the *in vivo* environment will contribute to broadened knowledge of biological function of this particular mutation in gliomas.

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[I-17] INTERPLAY BETWEEN MCPIP1 AND C-MET RECEPTOR IN TUMOR RESISTANCE TO RTKS INHIBITORS

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Clear cell renal cell carcinoma (ccRCC) treatment with small molecules that inhibit multiple receptor tyrosine kinases (RTKs), showed in approximately 38% of patients significant tumor control. However, despite the efficacy of treatment, ccRCC often develops resistance to targeted drugs and the majority of patients who receive such treatment exhibit progressive disease after 1 year. Several hypotheses have been proposed regarding the mechanisms underlying resistance to RTKs inhibitors, but the precise pathways have not yet been fully elucidated.

The main objective of our study was to determine the role of c-Met and anti-inflammatory protein MCPIP1 in the acquisition of resistance to RTKs inhibitors in ccRCC.

Microarray and Western blot analysis show that in ccRCC patient samples, together with MCPIP1 downregulation, level of phosphorylated and total c-Met receptor are increased. Our *in vitro* study shows that short term (24h) and long term (3 weeks) stimulation with sunitinib or sorafenib, induced phosphorylation of c-Met receptor, STAT3 and Src kinase. Furthermore, sunitinib treatment resulted in the acquisition of cancer stem cells features and the formation of clones in normal and tumor cell lines. In addition, after sunitinib and sorafenib treatment we observed a decrease in the protein level of MCPIP1, a postulated tumor suppressor. Interestingly, after MCPIP1 overexpression, we observed phosphorylated c-Met downregulation, however, after c-Met inhibition with specific siRNA we did not observe any changes in MCPIP1 level, which indicates that only phosphorylation of c-Met receptor may regulate MCPIP1.

We showed that acquisition of therapy resistance in ccRCC may be affected by MCPIP1 decrease, together with phopsphorylation of c-Met receptor and it can be partially reversed by overexpressing the MCPIP1 protein, which may act as a potent tumor suppressor. The proposed research may help in understanding the mechanisms responsible for tumor resistance to targeted therapy. The obtained results may contribute to increased understanding of the biology of clear cell renal cell carcinoma, which in the future may help in identifying new, more effective therapeutic goals or improving existing ones.

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[I-18] THE P53 TUMOR SUPPRESSOR PROTEIN STIMULATES THE EXPRESSION OF BLNK – GENE IMPORTANT IN HUMORAL IMMUNE SYSTEM

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Introduction: Humoral immunity is a type of immunity response, which is mediated by antibodies binding to the antigens recognized as potentially dangerous. The most important elements of this system are B cells. The *BLNK* (B cell linker) gene encodes cytoplasmic adaptor protein, which is primarily known by its critical role in B cell development and receptor signaling. The main function of BLNK is a regulation of biological outcomes of B-cell function. BLNK participates in orchestrating the pro-B cell to pre-B cell transition. The protein also plays a role in basic cell functions, for example activation of: ERK/EPHB2, NF-kappa-B kinase, JNK or intracellular calcium mobilization response. The highest expression of *BLNK* was observed in the spleen, tonsils and lymph nodes. Therefore, BLNK may be important in the pathogenesis of many diseases. Its mutations cause hypoglobulinemia, whereas its deficiency has been found in lymphoblastic leukemia. Moreover, studies show that the down-regulation of *BLNK* may be related to lymphocyte transformation mediated by Rel proteins. On the other hand, it acts as protein adapter in TREM2 pathway, which is associated with higher risk of Alzheimers disease.

We developed a hypothesis according to which *BLNK* is regulated by tumor suppressor protein p53. We observed that two substances: actinomycin D and nutlin-3a synergistically stimulate activation p53 in A549 cells. The analysis of transcriptome sequencing (RNA-Seq) of A549 cell line (lung cancer) exposed to actinomycin D and nutlin-3a (A+N) revealed a significant increase in the expression of over 2000 genes, including expression of 500 genes upregulated at least 10-fold. Surprisingly, the gene for BLNK was among the most strongly activated genes.

Methods: The cells in culture: A549 (lung cancer), A375 (melanoma) and U-2 OS (osteosarcoma) were treated with: actinomycin D and nutlin-3a. The protein expression was examined by Western blotting. In order to confirm the hypothesis, the gene regulatory region of BLNK with a potential p53 binding site was cloned into pGL3-Basic reporter vector. Additionally, we mutated the putative p53 binding site using site-directed *in vitro* mutagenesis system.

Results: We confirmed our hypothesis, that p53 affected the induction of BLNK following co-treatment with actinomycin D and nutlin-3a. Consistent with our hypothesis, A+N treatment resulted in strong up-regulation of BLNK in A549. In addition, the effect increases with the duration of cell exposure to A+N. Moreover, we observed the influence of p53 knock-down on expression of BLNK protein. The cloned promoter of BLNK contains bona fide p53 response element.

Conclusions: This recently identified new biological link between p53 and humoral immunity deserves more detailed exploration in further studies using models of B-cells.

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[I-19] THE APOPTOTIC PROPERTIES OF MOUSE LOC66598 PROTEIN AND ITS DELETION VARIANTS

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The mouse *3110001I22Rik* gene encoding the LOC66598 protein is located in the first intron of the *Bfar* (bifunctional apoptosis regulator) gene. Thus recently it was renamed to BFAR isoform 3 although the encoded protein is completely distinct from other BFAR isoforms and is rather similar to Periphilin-1 (PPHLN1). Mice with the *3110001I22Rik* gene knockout did not show any significant phenotypic changes, and the function of this gene remains unknown.

We found that the *3110001I22Rik* gene transcription can be induced by heat shock in some mouse organs, however, at the protein level, we observed an opposite effect. This could result from the existence of the transcribed pseudogene which is reported in the Ensembl database. Our preliminary experiments with the use of a live imaging microscopy suggested that the LOC66598 protein may have pro-apoptotic properties. *In silico* search indicated the presence of two potentially functional domains: LGE and Serine-rich. Thus we cloned the coding sequence of the gene and two clones with deletion of the above domains to establish their significance in the activity of the LOC66598 protein. We used a lentiviral expression system induced by doxycycline or transient transfections (to NIH-3T3 mouse embryonic fibroblast) of vectors encoding the full LOC66598/EGFP protein or its deletion clones and EGFP as a control. After transient transfections, all constructs with LOC66598/EGFP (but not EGFP alone) induced caspase-3-dependent apoptosis. However, apoptosis was not observed after induction with doxycycline. We postulate that the level of protein in the cell may be crucial for such an effect. Only at the high protein concentration that is achieved after transient transfection, apoptosis is induced.

Looking for proteins interacting with LOC66598, we performed co-immunoprecipitation (after induction of LOC66598/EGFP or EGFP alone with doxycycline, thus in conditions in which apoptosis is not induced) using GFP-trap (Chromotek) and subsequent protein analysis on the Orbitrap spectrometer. Initial analysis revealed interactions with 40S ribosomal proteins (S25, S27, S14, S19, S3), histones (H1.2, H1.4, H3.3C), proteins involved in splicing (SFPQ, SNRPA1, HNRNPA3, NONO, KHDRBS3) and proteins involved in calcium signaling (HPCAL1, PTK2B). Interestingly SFPQ (Splicing factor, proline- and glutamine-rich) and NONO (Non-POU domain-containing octamer-binding protein) proteins belong to the *Drosophila* behavior human splicing (DBHS) family of RNA-binding proteins and it was already reported that SFPQ-NONO heteromer may be involved in DNA unwinding and in DNA non-homologous end joining (NHEJ) as well may play a role in nuclear retention of defective RNAs. Our results suggest that LOC66598 could be an additional component of this complex. Observed LOC66598 interactions with histones could be mediated by LGE domain since it is known that this domain could be involved in the ubiquitination of histones. On the other hand, interactions with proteins involved in calcium signaling are consistent with the expression pattern of 3110001I22Rik which is the highest in cells with high levels of calcium ions.

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[I-20] CELLULAR LEVELS AND LOCALIZATION OF SUPEROXIDE AND NITRIC OXIDE RADICALS

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Introduction: Reactive oxygen and nitrogen species (ROS) play important roles in regulation of cell signaling and survival but can also exert opposing effects, inducing cell damage and death. Superoxide and nitric oxide (NO) are the main cellular radicals; the main source of superoxide is leakage from the mitochondrial respiratory chain, but it also is purposely produced by enzymes from the NOX family. NO, a highly reactive gas acting as a messenger for paracrine and autocrine communication, is synthesized from L-arginine by members of the nitric oxide synthase family (NOS). In some conditions, NOS may produce superoxide instead of NO. The aim of this work was to check how the levels and distribution of superoxide and NO differ between different cell types.

Materials and methods: Two human cultured cell lines, Me45 and HCT116, were compared. Superoxide and NO were detected by the fluorescent dyes MitoSOX Red and DAF-FM diacetate. Observations were performed on living cells in time lapse fluorescence microscopy experiments or on cells fixed with ethanol. MATLAB software was used to calculate Pearsons correlation coefficient between superoxide radical and NO localisations on the basis of signal intensity in all pixels in images.

Results and conclusions: In both cell types, most NO and superoxide were co-localized as shown by a high positive correlation of their signals in single pixels. However, Pearsons correlation coefficients for the intensity of both dyes was higher in Me45 cells, indicating more frequent co-localization of superoxide and NO radical sources in these cells. Analysis of the results of microarray experiments in which the transcriptomes of both cell lines were studied showed that Me45 cells express significantly less than HCT116 cells transcripts coding for the first and rate-limiting enzyme in biosynthesis of tetrahydrobiopterin (BH4), a cofactor required for activity of nitric oxide synthases. We conclude that in Me45 cells NOS, the NO producing enzymes, switch to production of superoxide. more frequently than in HCT116 cells.

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[I-21] THE ROLE OF MCPIP1 IN THE PROCESS OF EPITHELIAL TO MESENCHYMAL TRANSITION

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Introduction: MCPIP1 protein (Monocyte chemotactic protein-induced protein 1) functions as tumor suppressor and negative regulator, involved in the control of inflammation and the maintenance of homeostasis. In addition MCPIP1 inhibits the NF-B transcriptional nuclear signaling cascade, while activation of the NF-B / CSN2 / Snail signal pathway induces an epithelial-mesenchymal transition (EMT). It can therefore be stated that the level of MCPIP1 is important in regulating the EMT process. The epithelial-mesenchymal transition is characterized by the disappearance of intercellular interactions, resulting in the disturbance of the structure characteristic for epithelial tissue. The level of epithelial cell markers such as E-cadherin decreases, whereas the level of mesenchymal markers increases.

Methods: The aim of this study was to check if TGF affects the level of MCPIP1 protein and if overexpression of MCPIP1 influences epithelial to mesenchymal transition in RPTEC/TERT1 cell line. The TGF also may be responsible for the induction of EMT, so we checked whether the level of MCPIP1 changes after stimulation TGF. Models overexpressing MCPIP1 and D141 (carrying a point mutation that completely abolishes MCPIP1 RNase activity) were also created and we checked the effect of protein overexpression on cell morphology. The level of EMT markers was checked by qRT-PCR and Western Blot analysis.

Results: In wild type cells we observed an increase in the level of mesenchymal markers after stimulation of TGF, while the level of MCPIP1 decreased. We repeated that stimulation using cells with overexpression of MCPIP1. In this case the level of mesenchymal markers was significantly lower than in control cells. In addition, we obserwed significant differences in the morphology between MCPIP1 and D141N cells.

Conclusion: The obtained results confirm that MCPIP1 is crucial for maintaining the epithelial phenotype.

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[I-22] PHLDA1 OVEREXPRESSION CAN INDUCE DETACHMENT OF CELLS BUT NOT CASPASE-3 DEPENDENT APOPTOSIS

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PHLDA1 (pleckstrin-homology-like domain family A, member1, also called TDAG51, T-cell death-associated gene 51 protein) is an evolutionarily conserved proline-histidine and proline-glutamine rich protein. PHLDA1 may have both pro- and antiapoptotic functions and the exact role of its expression in apoptotic cell death remains controversial.

It was proposed that PHLDA1, which was identified as a direct target of Heat Shock transcription Factor 1 (HSF1), could be responsible for heat shock-induced cell death in heat-sensitive cells. Thus, we checked the activation of the *Phlda1* gene by heat shock and its correlation with heat-sensitivity (assessed by Tunel-test) in mouse organs. The level of *Phlda1* expression is very low and it is hardly induced by heat shock in heat-sensitive testes while it is strongly activated in the heat-resistant liver. The *Phlda1* expression was also up-regulated by heat shock in some other organs (epididymis, lung, kidney, colon, small intestine, spleen, heart, brain). Then, we aimed to compare the ability of PHLDA1 and proapoptotic PMAIP1/NOXA (both induced by heat shock in HSF1-dependent manner) to induce apoptosis, thus we checked the level of active caspase-3 after transient transfection of vectors coding for EGFP/PHLDA1, PHLDA1/EGFP, or PMAIP1/EGFP. Only PMAIP1 overexpression led to activation of caspase 3. We additionally observed NIH3T3 cells after transient transfections of the above vectors using life imaging microscopy with Caspase-3/7 Red apoptosis assay reagent. PMAIP1/EGFP overexpression induced apoptosis and green cells disappeared from a field of view soon after typical membrane blebbing lasting for 40-60 minutes. EGFP/PHLDA1 fusion protein (very effectively produced already after several hours of transfection) led to detachment of cells while caspases-3 and -7 were not activated. Furthermore, almost all detached green cells still existed until the end of the observation for 48 hours in contrast to cells undergoing the typical apoptosis which disappeared. The PHLDA1/EGFP fusion protein was not so effectively produced and the first few green cells appeared after 24 hours from the start of transfection. In this case, most green cells had a normal morphology with nuclear/cytoplasmic localization of the fusion protein indicating that PHLDA1/EGFP does not cause cell detachment. Thus, we conclude that the PHLDA1 protein is not responsible for the initiation of the HSF1-dependent heat shock-induced apoptosis.

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[I-23] HSPA2 CHAPERONE PROTEIN IS HIGHLZ EXPRESSED IN MALIGNANT ASTROCYTOMAS BUT NOT INVOLVED IN REGULATION OF GLIOBLASTOMA MULTIFORME CELL PROLIFERATION AND MOTILITY.

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Glioblastoma multiforme (GBM) is the most common subtype of the primary malignant astrocytic brain tumors. Despite the advances made in multimodal treatment consisting of surgical techniques, radiotherapy and chemotherapy, the median survival for GBM patients is 16 months. GBM, as the most aggressive brain tumor with the highest number of tumor-related deaths in rankings over the last 10 years, represents a major socioeconomic burden and unmet medical need. Uncontrolled cell proliferation and widespread single-cell invasion into normal brain parenchyma are regarded as primary cause of tumor recurrence and the patients poor prognosis. Therefore, there is an urgent need to define and understand the underlying mechanisms of GBM malignancy.

HSPA2 chaperone protein, the least characterized member of the HSPA (HSP70) multigene family, was originally described as a testis-specific and crucial for male fertility. We previously reported that HSPA2 is expressed in selected somatic tissues, including epidermis, and is involved in the control of differentiation process in keratinocytes. A high level of HSPA2 was also detected in oligodendroglial cells in human brain. Furthermore, HSPA2 has attracted increased interest due to its alleged supportive role in the maintenance of invasive phenotype of epithelial cancer cells derived from various non-testicular tissues. At present, the knowledge of expression and involvement of HSPA2 in the CNS biology is unknown.

Our immunohistochemical analysis of HSPA2 expression in gliomas revealed changes in the localization pattern of immunolabeling for HSPA2, compared to normal brain tissue. We found that elevated level of the HSPA2 protein correlated with the increased histologic grade in primary astrocytomas. In GBM tissue specimens we also detected a high level of HSPA2 in non-endothelial, perivascular cells (CD31-/SMA+) of neoplastic blood vessels. These observations suggested a possible role for HSPA2 in GBM biology. Dual immunofluorescent staining showed that HSPA2 is highly expressed in malignant astrocytes (GFAP+) in the astrocytomas. Interestingly, HSPA2 was not detected in proliferating cells positive for Ki67 marker. In order to reveal a potential contribution of HSPA2 to glioblastoma multiforme cell phenotype we used RNAi technology to stably down-reglulate HSPA2 expression in tumor cells. We found that HSPA2 depletion did not affect proliferation of cells grown either in monolayer or in 3D culture *in vitro*. Moreover, we observed that deficiency in HSPA2 expression had no impact on glioblastoma multiforme cell migration and invasion.

Taking this into account the results of immunohistohemical studies suggest a potential contribution of HSPA2 to GBM biology. The ongoing study shows that further investigation is needed to clarify the functional significance of HSPA2 in malignant gliomas development, angiogenesis and/or progression.

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[I-24] DISTINCT AND REDUNDANT FUNCTIONS OF THE HSPA2 PROTEIN IN NORMAL AND CANCER CELLS

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Heat shock proteins (HSPs) are a large group of chaperones considered critical for maintaining cellular proteostasis. It is commonly accepted that aberrant expression of HSPs in cancer cells can modulate the course of all processes defined as hallmarks of cancer. Previously, we showed that HSPA2, one of the least characterized member of the multigene HSPA (HSP70) family, is expressed in selected human organs in a cell type-specific manner. Beside testis and brain, we detected a high level of the HSPA2 protein in the cells located in the basal layer of stratified epithelium of the skin and pseudostratified epithelium of the bronchus. Also, it has been reported that HSPA2 is frequently overexpressed in various types of tumors, including non-small cell lung cancer (NSCLC). We found that a high expression of HSPA2 in NSCLC tumors correlated negatively with patients prognosis. Bearing in mind that knowledge of HSPA2 functions in extra-testicular cells is limited, we aimed at investigating a potential impact of HSPA2 on the phenotype of cells originated from normal multilayered epithelia and corresponding malignant tissues.

We found that paralog-selective RNAi-mediated depletion of the HSPA2 protein from immortal cells of human epidermal keratinocytes line (HaCaT) and bronchial epithelial line (Beas-2B) had negligible effect on cell proliferation and migration. Simultaneously, epithelial cells deficient in HSPA2 showed reduction in their ability to form colonies and adhere to extracellular matrix components. However, when HSPA2 was depleted from a number of NSCLC cell lines we did not detect any changes in proliferative, clonogenic, migratory and adhesive potential of the cancer cells. These results suggest that epithelial cell-specific activities of HSPA2 can be lost or compensated by other chaperones in NSCLC cells. Bearing in mind that HSPA family in humans consists of twelve similar members, several of which are highly overexpressed in NSCLC cells, we assumed that HSPA paralogs might compensate for the HSPA2 protein deficiency. To confirm our assumption we used VER-155008, a specific pan-inhibitor of the HSPA family proteins. We found that VER-155008 exerted potent anti-proliferative activity selectively on NSCLC cells, but not on normal bronchial epithelial cells. Thus, our results indicate that HSPA chaperones form a highly redundant network of growth-promoting factors in NSCLC cells. In turn, HSPA2 activity is crucial for supporting clonogenic potential and adhesive ability of normal epithelial cells. Given that characteristic feature of undifferentiated cells in multilayered epithelia is high clonogenicity and adhesiveness, our results suggest that HSPA2 may participate in regulating epithelial cells differentiation.

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[I-25] INTERACTION OF LET-7 WITH REPORTER GENES IN TWO CELL LINES - DIFFERENCES IN TRANSCRIPTION AND TRANSLATION PROCESSES.

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The let-7 family was the first known human miRNA. This microRNA family contains 12 members and is highly conserved both in sequences and functions across species. In mammals, let-7 expression is high during embryogenesis and brain development, remains high in adult tissues, and regulates hematopoietic stem cell self-renewal, proliferation, quiescence, and differentiation. The aim of the present study was to compare the effects and mechanisms of let-7 action in two human cell lines, Me45 and HCT116. Cells were transfected with the plasmid psiCHECK2 (Promega) containing Renilla luciferase genes, with or without Let-7- targeted sites in its 3UTR. Levels of mRNA and reporter proteins were measured 24 hours after transfection by RT-qPCR and luminescence of a luciferase substrate, respectively. Transcripts containing Let-7-targeted sequences showed higher levels in HCT116 cells and lower in Me45 cells than transcripts without such sequences. In both cell lines the presence of Let-7 targeted sequences in Renilla transcripts caused change in protein level in comparison to unregulated transcripts, but HCT116 and Me45 cells differed in reporter gene translation efficiency. Searching for the mechanisms underlying these differences at the translation level, we assessed the distribution of Renilla luciferase mRNA in polysome fractions from both cell types transfected with plasmids regulated or not regulated by Let-7. Polysome fractions were obtained by ultracentrifugation of cytoplasm extracts in sucrose gradients for 4 hours at 4C and 40 000 rpm in an SW41Ti rotor. In HCT116 cells the presence of Let-7-targeted sequences inhibited the creation of ribosomes containing Renilla mRNA, which was found mainly in fractions containing small ribosomal subunits. In contrast, in Me45 cells inhibition of translation was detected after the formation of entire ribosomes, and Let-7-regulated reporter gene mRNA was found in the monosome fraction. Analysis of the transcriptomes of both cell lines from previous microarray experiments showed differences in expression of some factors engaged in regulation of translation. Taken together, our results suggest that the mechanisms of Let-7 action are different in each cell line.

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[I-26] P2X7 RECEPTOR AS TUMOR MICROENVIRONMENT REGULATOR

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P2X7 is a ionotropic, nucleotide receptor located on the cell surface and activated by extracellular ATP. Many studies reported that P2X7R stimulation is crucial for development of pathological states like chronic inflammation and cancer. Gliomas are the most common and most aggressive tumors of the central nervous system with high metastatic potential and resistance to chemotherapy and radiotherapy. Interestingly, many malignant glioma cell lines are characterized by P2X7 expression. The aim of presented work was to investigate the activity of P2X7 receptor in human and rat glioma cells. In vitro studies have shown increased proliferation and massive calcium influx after P2X7 stimulation in rat glioma C6 cells. In vivo studies were performed on C57BL/6J mice with subcutaneously injected glioma C6 cells. We observed that administration of Brilliant Blue G (BBG), a selective inhibitor of P2X7, effectively reduced tumor growth and distant infiltration from the primary tumors. Further experiments revealed that these P2X7-dependent effects were accompanied by decreased p38 MAPK phosphorylation as well as lowered expression of chaperone proteins. Inhibition of P2X7 also reduced the amount of active matrix metalloproteinase-2 in tumors. What is more, in tumors treated with BBG we observed a decreased level of macrophage marker CD68 and T-regulatory cells marker FoxP3. At the same time, a higher level of hematopoietic growth factors (IL-2, IL-7) and TIMP metallopeptidase inhibitor 1 was found in the serum of BBG-treated animals.

The obtained results show that P2X7R is involved in modulation of phenotype and tumor microenvironment of rat glioma C6. Pharmacologically modified receptor may be a potential therapeutic target in glioblastoma multiforme treatment.

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[I-27] ADAPTATION OF GLIOBLASTOMA CELLS WITH AMPLICONS TO IN VITRO CONDITIONS – LOSS OF AMPLICONS IN A FORM OF EXTRACHROMOSOMAL VESICLES AND EGF-MEDIATED SENESCENCE

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Introduction: *In vitro* cell cultures constitute an important model for cancer cells analyses as well as for preliminary screening of molecules with possible antineoplastic potential. Stable cancer cell lines are most commonly applied for such analyses, however, the rationale for their usage is often undermined. Primary cancer cell cultures tend to be considered better *in vitro* model, as due to their heterogeneity these cultures resemble *in vivo* tumor state more adequately. Nevertheless, their stabilization and culturing is troublesome. This is especially important in case of glioblastoma (GB) cells with amplification of oncogenes in various analyzed culture conditions. We previously suggested that this may be associated with loss of amplicons encoding oncogenes in a form of extrachromosomal vesicles (EVs) and therefore, intended to test such hypothesis. Additionally, we analysed the impact of specific growth factors that are commonly used in GB cell culturing on an example of epidermal growth factor (EGF).

Methods: For this purpose EVs were firstly isolated from DK-MGhigh cell line. This is one of the very few stable cancer cell lines characterized with amplicons encoding oncogenic variant of epidermal growth factor receptor EGFRvIII, an alteration commonly detected in this tumor type. Two methods were applied for isolation of EVs from culture medium one employing commercially available kit (exoEasy Maxi Kit, Qiagen) and the second approach using aqueous two phase system. Following EVs selection, DNA and RNA were isolated and *EGFRvIII* expression was analyzed using RT-Real-time-PCR. When the most optimal protocol was established, analyses were also conducted using culture medium obtained from primary glioblastoma cultures adherent culture and fresh neurospheres. The impact of EGF was evaluated *via* real-time *in vitro* observations and senescence analysis (SA--Gal detection).

Results: Analyses indicated that *EGFRvIII* mRNA can be detected in EVs in case of both DK-MG cell line as well as adherent glioblastoma cultures, while expression of this oncogene is absent in case of EVs from fresh neurospheres. Lack of expression of housekeeping genes excluded possibility of genomic DNA/RNA contamination. These results strongly indicate that amplicons encoding oncogenes may be extruded and hinder establishment of reliable cancer *in vitro* models, with neurosphere culture conditions being an exception, somehow inhibiting amplicon loss. EGF does not influence amplicon deprivation in a statistically significant manner, however, tends to increase the overall percentage of senescent GB cells in culture.

Conclusions: The obtained data suggest that GB cells adaptation to *in vitro* conditions is associated with oncogene amplicon loss in the form of EVs. Undoubtedly, this phenomenon may be overcome by the optimization of culture conditions.

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[I-28] EXTREMELY LOW CONCENTRATION OF EGFRvIII mRNA IS SUFFICIENT TO KEEP THIS ONCOGENE PROTEIN AT DETECTABLE LEVEL

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Introduction: EGFRvIII is known as extra or intrachromosomally amplified oncogene. There exist many premises that amplicons fulfill important role in oncogenesis. For example, they are considered as the centers of transcription. However, the detailed mechanism of their action remains unclear. We made an attempt to precise the role of EGFRvIII and amplicons in glioblastoma cells.

Methods: Experiments were done on DK-MG cell sublines with high and low concentration of EGFRvIII protein. Moreover, primary cancer cells were analyzed. DNA amplicons of EGFRvIII/EGFR were detected by Real-time PCR and FISH. mRNA of EGFRvIII was detected by qRT-PCR. Quantitative RT-RT-PCR was performed to determine the numbers of EGFRvIII mRNA molecules per single cell. EGFRvIII protein was detected by Western blot and immunocytochemistry.

Results: EGFRvIII amplicons were detected in DK-MG sublines with high and low average EGFRvIII protein expression. Subline with high average protein expression showed only a two-times higher number of amplicons than subline with low protein expression. In contrary to subline with high protein concentration, average of mRNA EGFRvIII expression in subline with low protein expression was almost undetectable. Level of EGFRvIII mRNA was more than thousand times higher in subline with high concentration of EGFRvIII protein than in subline with low concentration, respectively. Real-time qRT-PCR analysis suggests that even single molecules of EGFRvIII mRNA were sufficient to protect detectable EGFRvIII expression level. For cell cultures with low protein expression immunocytochemistry allowed to discriminate between cultures with small number of such cells that are highly EGFRvIII- positive from cultures with majority of cells showing minimal concentration of this protein.

Conclusion/Novel aspect: Minor differences between number of amplicons in DK-MG sublines with high and low mRNA concentration implies that amplicons can be epigenetically silenced in neoplastic cells. Apparently, glioblastoma cells sustain high number of inactive amplicons which seems to be unreasonable. The obtained results indicate that amplicons may play a different role, other than providing high EGFRvIII mRNA and protein expression. Extremely low concentration of mRNA is sufficient to keep EGFRvIII protein expression at detectable level in Western blot analysis. This phenomenon can be explained as a consequence of EGFRvIII resistance to EGFR degradation.

This work is continuation of analyses published in J of Cancer
Peciak J, Stec WJ, Treda C, Ksiazkiewicz M, Janik K, Popeda M, Smolarz M, Rosiak K, Hulas-Bigoszewska K, Och W, Rieske P, Stoczynska-Fidelus E.

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[I-29] THE EGFRvIII GENE EXPRESSION ITSELF IS INSUFFICIENT TO ASSURANCE THE INCREASED SURVIVAL AND PROLIFERATION OF GLIOBLASTOMA CELL LINES.

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Introduction/Rationale: Increase cell survival and proliferation rate are one of the most common carcinogenesis hallmarks. It is believed that expression of EGFRvIII in glioblastoma cells contributes to occurrence of that processes. The mentioned above phenomenon can be observed *in vitro* on stable cancer cell lines. One of the most prominent example is DK-MG cell line. This line could be characterized by elevated survival and proliferation ratio. Mentioned premises prompted us to verify if expression of EGFRvIII can involve the increase of survival and proliferation ratio in cells with low expression level of examined oncogene.

Methods: To obtained DK-MG subline carries low EGFRvIII expression the genetic subcloning were performed. After that the EGFRvIII gene was introduced into this subline via Gateway Cloning System and lentiviral vector. Next to antibiotical selection cells were analysed by Western Blotting and Real Time PCR. Moreover the proliferation ratio and cell survival was assessed via microscopic observation. Analyses were performed at various time intervals for half a year after the transduction process.

Results: Obtained DK-MG sub-line could be featured by decrease the cell survival and proliferation ratio according to parental line. Furthermore this cells showed susceptibility to undergoing an apoptosis process. After introduction of EGFRvIII into DK -MG sub-line the restoration of parental cell line phenotype was not observed. Apoptosis was still occurred despite the relatively high expression level of examined oncogene.

Conclusion/Novel aspects: Though the extensive knowledge in oncology field some phenomenon are still unclear and mysterious. Expression of EGFRvIII gene in glioblastoma is often associated with increased of proliferation, survival and invasiveness. However, this correlation seemed to be more complex than we expected. It turns out that expression of EGFRvIII itself is insufficient to involve changes characteristic for cancer cells. Recently, there have been some reports suggested that the co-expression of EGFRvIII with other genes, like SEC61G-EGFR or EGFR-AS1, is required to fulfill his role in carcinogenesis process. In the future, we will attempt to verify this hypothesis. We believe that the knowledge gained in this way will help in broadening science about the cancer process and contribute to the development of effective and modern anti-cancer therapy.

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[I-30] MICRORNAS AS A POTENTIAL REGULATORS OF EPITHELIAL TO MESENCHYMAL TRANSITION IN COLORECTAL CANCER

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Introduction: Colorectal cancer (CRC) is the third common type of cancer and the fourth cause of death worldwide. Tumor growth and metastasis is a multi-step process. There are numerous factors responsible for this process, including small RNA. As the most studied type of non-coding RNA, microRNAs (miRNAs) are thought to regulate the post-transcriptional expression of tumor suppressor genes and oncogenes involved in CRC development. Considering the above, miRNAs may play a role as promising therapeutic target and diagnostic factor. The aim of our studies was to discover novel miRNAs, which are associated with clinical parameters and may play roles as promising therapeutic target or diagnostic factor in CRC as regulators of epithelial to mesenchymal transition (EMT).

Methods: MiRNA expression profile was analyzed in paraffin-embedded CRC samples from 50 patients by the next generation sequencing. These results generated potential microRNA candidates for further investigation *in vitro*. CRC cell lines, authenticated by STR profiling, were cultured in vitro for isolation of RNA and protein. After reverse transcription, expression levels of interesting miRNAs and genes of interest were measured by qPCR. Expression of EMT-associated proteins was analyzed by Western blot and by immunocytochemistry (ICC) staining.

Results: Sequencing data enabled us to select microRNA candidates, which expression profiles correlated with different clinical parameters in CRC. Subsequently, their expression was validated in CRC cell lines *in vitro* that displayed different expression of EMT associated factors. Epithelial phenotype of the cells was characterized by validation of expression of E-cadherin and vimentin. One of the interesting miRNA candidates was miR-193a-3p that displayed lower expression in cell lines expressing both E-cadherin and vimentin. Therefore, it was hypothesized that it may be a regulator of EMT in CRC. Moreover, expression of miR-193a-3p was positively associated with higher expression of SLUG, an important regulator of EMT.

Conclusions/Novel aspect: Our studies discovered microRNAs that are involved in CRC development and can be used as therapeutic target or diagnostic factor. One of the interesting candidates is miR-193a-3p that may be a regulator of EMT in CRC. Its role will be validated in further research to evaluate mechanism of its action.

[I-31] MONOMERS AND HOMODIMERS OF EGFRVIII

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Introduction: Epidermal Growth Factor Receptor (EGFR) amplifications and rearrangements are common and lead to generation of constitutively active oncogenic protein, e.g. EGFRvIII. This variant is characterized by deletion of exons 2-7 of wild-type EGFR which results in the presence of unpaired cysteine 16 (C16) in its monomer form. Previous works suggested that C16 remains unpaired, whereas other cysteines form disulfide bonds within monomers. Our team tested those revelations and our recently published results show the opposite, i.e. C16 can directly participate in covalent homodimerization of EGFRvIII. Dimerization is likely to impact EGFRvIII activity and stability, creating a promising target for a novel therapy, we hypothetized that C16 is not the only cysteine important for this process.

Methods: Based on the EGFRvIII structural model developed by our team, we selected other cysteines to be modified into serine, what might resemble removing C16. Therefore, we created various mutants of EGFRvIII as well as wild-type EGFR, and each was overexpressed in AD293 cell line by lentiviral vector. AD293 cell line is characterized by a negligible amount of endogenous EGFR. Further, we used various tyrosine kinase inhibitors and chemical compounds to analyze undiscovered nature of EGFRvIII dimerization, applying e.g. standard and semi-native western blot techniques.

Results: Point mutations of selected cysteines in extracellular part of EGFRvIII showed similar effect to the previously shown influence on stability and amount of homodimer formed by EGFRvIII by mutated C16. We noticed impairment of homodimer formation and its stability, i.e. there is a significant decrease of EGFRvIII homodimers and increase of monomers in mutated samples. On the contrary, we show that wild-type EGFR extracellular part hinders stable dimer formation due to possibly much more rigid structure. EGFRvIII not only covalently homodimerizes cysteines on the extramembrane, but also receptor plasticity is much more complex.

Conclusions/Novel aspect: To conclude, EGFRvIII dimerizes covalently several core amino acids, which is not the case in wild-type EGFR. Cysteine to serine mutation of any of several amino acids impair EGFRvIII homodimer stability and this, according to our preliminary data, suggests impact on cell growth rate and EGFRvIII phosphorylation level. It is obvious that this critical finding not only will let us to understand deeper the nature of EGFRvIII, but also brings hope to the creation of successful targeted therapy.

[I-32] ACCELERATED DEVELOPMENT OF DMBA/TPA INDUCED TUMORIGENESIS UPON LOSS OF EPIDERMAL MCPIP1 FUNCTION

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Monocyte chemoattractant protein-induced protein 1 (MCPIP1) is encoded by the Zc3h12a gene, and as an RNase regulates the levels of transcripts coding for inflammation-related factors. The key role of MCPIP1 is controlling physiological and pathological processes of inflammation, but recent studies also proved its role in regulating many other biological processes, such as differentiation, lipid metabolism, angiogenesis and tumor growth. *In vitro* and *in vivo* studies with the use of carcinoma cell lines (neuroblastoma, ccRCC and breast cancer) indicated that MCPIP1 displays antitumor potential via inhibiting proliferation of cancer cells, selectively enhancing mRNA decay of antiapoptotic gene transcripts and regulating the rate of metabolism and angiogenesis.

To determine the role of MCPIP1 in skin tumor development we generated conditional knockout mice lacking Zc3h12a in the epidermal basal keratinocytes (Mcpip1EKO). We observed that loss of epidermal MCPIP1 does not lead to any obvious phenotypic changes in newborn and young mice. In contrast, adult Mcpip1EKO mice displayed reduced body weight and developed chronic skin lesions around their cheeks, ears and neck from around 3-4 months of age.

We next utilized a well characterised 7,12-dimethylbenzanthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA) approach to induce skin carcinogenesis in mice. Our results demonstrated that control and knockout mice treated only with TPA develop epidermal hyperplasia, which is more prominent in the Mcpip1EKO mice. The epidermis of the Mcpip1EKO mice upon TPA treatment was 1,5-fold thicker than that of the control mice. Subsequently, after 9 weeks of DMBA/TPA stimulation 33% of the Mcpip1EKO mice developed papillomas, while no phenotypic changes were observed in the control mice. Furthermore, Mcpip1EKO mice developed chronic wounds on entire back skin and partially lost their hair. At the histological level, skin lesions were characterized by hyperkeratosis, appearance of keratin pearls that are characteristic for squamous cell carcinoma and aberrant clusters of pigment-filled melanocytes. Immunohistochemical staining of Keratin 14 and Keratin 10 showed disturbed processes of keratinocyte proliferation and differentiation in the *papillomas* of the DMBA/TPA treated Mcpip1EKO mice.

In conclusion, our studies indicate that MCPIP1 is an important factor in maintaining proper skin homeostasis. Furthermore, our pre-eliminary data show that Mcpip1EKO mice are more susceptible to the chemically induced skin tumorigenesis.

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Session II:
New molecules
and experimental therapies

[II-1] METABOLISM OF UNSYMMETRICAL BIS-ACRIDINE ANTITUMOR AGENTS WITH HUMAN RECOMBINANT P450 AND UGT ISOFORMS AND THEIR IMPACT ON UGT ACTIVITY

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Introduction: Unsymmetrical bis-acridines (UAs) are a novel class of antitumor agents with marked antitumor efficacy *in vitro* and *in vivo*. During the drug development particular attention is paid to drug metabolic pathways. Thus, two human recombinant P450 isoforms and eight UGTs were screened for their ability to transformation of the selected UAs. The influence of the tested bis-acridines on the activity of UGT isoenzymes was also investigated.

Methods: The studies were performed with the selected UAs (C-2028, C-2045, C-2053). All derivatives were incubated for specified time with human recombinant P4503A4, P4502C19, UGT1A1, UGT1A4, UGT1A6, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, UGT2B17 and cofactors (NADPH/UDPGA) at 37C. Isoenzyme activities were studied by the comparison of metabolic rates of specific substrates, namely, imipramine (P4502C19), testosterone (P4503A4), SN-38 (UGT1A1), trifluoperazine (UGT1A4), epirubicin (UGT2B7) and standard substrate for UGT - 7-hydroxy-4-(trifluoromethyl)coumarin (UGT1A6-10 and 2B15, 2B17). The chemical structures of the observed metabolites were identified using mass spectrometry.

Results: Multiple metabolite peaks of various intensities were observed after 30 min of incubation of C-2028, C-2045 or C-2053 with P4502C19 or P4503A4. The compound C-2045 demonstrated significantly lower susceptibility to metabolism compared with C-2053 (analogue without hydroxyl group) and its demethyl analogue, C-2028. Screening research also indicated that only compound C-2045 (analogue with hydroxyl group) was glucuronidated by UGT1A1, UGT1A9 and UGT1A10. The glucuronidation rate with UGT1A10 was the highest for this compound. Furthermore tested bis-acridines inhibited activity of UGT1A1 and UGT2B7.

Conclusions: The results showed that the metabolic pathways of the tested UAs are extremely complicated and its recognition will require extensive research, particularly in tumor cells. Also it seems that the studied compounds modulates activity of UGT1A1 and UGT2B7 which may effect on the treatment with multi-drug therapies.

[II-2] PRELIMINARY ASSESSMENT OF THE IN VITRO ACTIVITY OF NEW QUINOLINE GLYCOCONJUGATES WITH BUILT-IN 1,2,3-TRIAZOLE RING

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Cancer is one of the main causes of mortality in the world. Therefore, the development of new pharmaceuticals characterized by increased efficacy and reduced toxicity has become the aim of research conducted in many scientific centers. Glycoconjugate chemistry has been gaining a lot of interest in recent years with respect to the treatment of cancer. Prodrugs conjugated to sugar units are very interesting, because they can increase the uptake of a drug by cancer cells which display an increased need for glucose and over-expression of their transporters [1,2]. Our research group has been involved in the synthesis of derivatives of 8-hydroxyquinoline (8HQ). The broad spectrum of activity of quinoline derivatives, especially glycoconjugates, is often associated with their ability to chelate metal ions or with the ability to intercalate into DNA. [3,4] Recently, we have demonstrated that the presence in the structure of the 1,2,3-triazole linker between sugar and quinolone aglycone is essential for their antiproliferative activity.

The aim of this work was to extend the compound library based on the glycoconjugates derivatives of 8HQ containing a triazole ring in the linker structure. As many biologically active compounds contain amide moiety, it was decided to check whether the introduction of this structural element to the quinoline glycoconjugates would improve their activity as compared to derivatives obtained previously. On the other hand, the introduction of an ethoxy linker between the triazole unit and sugar or chain extension between triazole and quinoline, could increase the flexibility of the obtained compound.

The new compounds were tested *in vitro* for their inhibitory potency against commercially available -1,4-galactosyltransferase as well as antiproliferative activity against a wide array of cancer cell lines (in which overexpression of this enzyme was observed). We also checked the cytotoxicity of the tested compounds against healthy human cells. Some new derivatives appeared to be active on the tested cell lines, being at the same time less toxic for normal cells. The results of evaluating cytotoxic activity of these compounds will be presented.

References:

1. E.C. Calvaresi, P.J. Hergenrother, *Chem. Sci.*, **2013**, *4*, 23192333.
2. C.C. Barron, P.J. Bilan, T. Tsakiridis, E. Tsiani, *Metabolism*, **2016**, *65*, 124139.
3. V. Milacic, P. Jiao, B. Zhang, B. Yan, Q.P. Dou, *Int. J. Oncol.*, **2009**, *35*, 1481-1491.
4. S. Zhai, L. Yang, Q.C. Cui, Y. Sun, Q.P. Dou, B. Yan, *J. Biol. Inorg. Chem.*, **2010**, *15*, 259-269.

[II-3] IL-12 SECRETED BY MODIFIED MSC SIGNIFICANTLY REDUCES THE VOLUME OF PRIMARY TUMORS AND THE NUMBER OF LUNG METASTASES OF MURINE MELANOMA

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Due to immunosuppressive properties and confirmed tropism towards cancer cells mesenchymal stromal cells have been used in many therapeutic trials, also as carriers of therapeutic compounds. In our study we attempted to use these cells as carriers of IL-12 in the treatment of mice with metastatic B16-F10 melanoma. IL-12 has confirmed anti-cancer activity. As a mediator of inflammation it interacts with many immune cells. The cytokine induces a strong immune response against cancer cells and acts as an anti-angiogenic agent. The cytokine acts locally, in the vicinity of cancer cells and a major limitation of the use of IL-12 therapy is its systemic toxicity.

The aim of the study was to develop a system of mesenchymal stromal cells secreting IL-12 that administered systemically exert therapeutic properties without toxicity.

The cells were isolated from murine bone marrow. Their phenotype was determined, the potential for differentiation and the migration capacity were tested. The IL-12 cDNA was introduced into MSC with adenoviral vectors. We confirmed the effectiveness of our system in inhibiting the growth of primary mouse melanoma tumors. Tumors in mice treated with modified MSC were 8-fold smaller than in mice from control groups. We also observed 2-fold decreased vascular density and 4-fold increased number of anticancer M1 macrophages in tumors after administration of modified MSC.

MSCs infused into the bloodstream of an animal localize in lung capillaries. MSC secreting IL-12 were administered to the tail vein of mice bearing melanoma metastases. We observed a significant 4-fold reduction in the number of metastases in mice treated with modified cells compared to control mice.

Therapeutic effect (reduction in the number of metastases) is the result of pleiotropic (proinflammatory and anti-angiogenic) properties of IL-12 administered locally released by modified mesenchymal stromal cells.

The work is a result of the research project No. UMO-2015/17/N/NZ4/02738 financed by National Science Center, Poland.

[II-4] THE EFFECTIVENESS OF ANTI-VASCULAR DMXAA COMBINED WITH BRACHYTHERAPY IN ANTICANCER THERAPY

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A therapeutic strategy with the use of anti-vascular drugs seems to be a promising anticancer approach. The therapy destroys tumor blood vessels and leads to the necrotic areas formation in the central part of the tumor and, as a consequence, tumor volume reduction. However, on the periphery of the tumor remains a layer of living cells - tumor rim cells, which are responsible for tumor re-growth. To prevent this, combinations of anti-vascular drugs with anti-angiogenic drugs, chemotherapy or ionizing radiation are made.

The aim of the work was to explain the mechanism of action of anti-vascular drug DMXAA combined with brachytherapy.

The experiments were conducted on B16-F10 murine melanoma model. In the studies, DMXAA (an agent that specifically destroys tumor blood vessels) was combined with brachytherapy (8Gy dose in 4 fractions).

Two therapeutic regimens were scheduled. In the first scheme (S1), the tumors were exposed to radiation and then DMXAA was administered. In the second (S2) scheme the order of proceedings was reversed.

It was shown that only the regimen with brachytherapy followed by anti-vascular agent (S1), effectively reduced the number of blood vessels in tumors. Brachytherapy itself also reduced the number of blood vessels in tumors but not as effectively as the combination of both.

In tumors treated with DMXAA alone and in combination with brachytherapy, an influx of M1 cytotoxic macrophages was observed. Brachytherapy alone slightly increased the percentage of M1 macrophages.

The highest percentages of macrophages were reported in the groups where DMXAA was administered alone and where mice were first irradiated and then received DMXAA. The reverse order of the application of therapeutic factors resulted in significantly lower percentages of M1 macrophages.

The proposed therapeutic strategy based on the combination of anti-vascular factor and brachytherapy is an effective solution. As a result, the immune system is activated to destroy surviving cancer cells.

The work is a result of the research project no. UMO-2015/17/N/NZ4/02738 financed by National Science Center.

[II-5] EFFECT OF STRUCTURAL MODIFICATION AND TYPE OF PROTECTING GROUPS IN URIDINE GLYCOCONJUGATES WITH 1,2,3-TRIAZOLE IN THE LINKER ON THEIR ANTIPROLIFERATIVE ACTIVITY

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Disorders in the glycosylation process, listed among the most important post-translational modifications, initiate the development of numerous disease states, bacterial and viral infections, as well as intensify the phenomena of malignant transformation and proliferation of tumor cells [1]. For the formation of a glycosidic bond in the living system, a large group of enzymes classified in glycosyltransferases group (GTs) are responsible [2]. For this reason, GTs are targets for the development of a method allowing to control their activity. Selective GTs inhibitors can provide the control of glycosylation associated with cancerogenesis and may lead to the development of new therapeutic agents.

The designing of GTs inhibitors is generally based on similarity to their natural substrates: NDP-sugars and acceptor. Designing of NDP-sugar analogues is generally based on the modification of one of three structural parts: carbohydrate part, the diphosphate linkage or the nucleoside moiety. In natural GTs substrate, the pyrophosphate moiety interacts with a bivalent metal cation present in an enzyme active site. Analogues of such compounds that have an anionic character face problems with entering cells through the phospholipid bilayer. This is why analogues containing a neutral diphosphate surrogate which would interact with metal bivalent cation are being designed more and more often. Such a surrogate may be a heteroaromatic system, especially a pyridine and/or 1,2,3-triazole ring [3].

To synthesize this type of compounds click-chemistry reaction can be used. Owing to our previous experience with the application of the copper-catalyzed azide-alkyne cycloaddition for synthesis of potential GTs inhibitors [4], a series of uridine derivatives was prepared in which diphosphate bridge was replaced with a linker containing 1,2,3-triazole unit and amide bonds or ether moiety which connects the sugar and uridine moieties. In order to check the compounds lipophilicity effect on their biological activity, both types of derivatives i.e. glycoconjugates with different types of protecting groups as well as completely unprotected glycoconjugates were obtained.

The new uridine glycoconjugates were tested as -1,4-galactosyltransferase inhibitors as well as their cytotoxicity was determined toward selected cancer cell lines (HCT-116, MCF-7) and normal cell line (NHDF). The results of assessing the biological activity of these compounds will be presented.

References:

1. M. Ko, C.-H., *Beilstein J. Org. Chem*., **2015**, *11*, 1547-1552.
2. K.H. Jung, R.R. Schmidt, Carbohydrate-based drug discovery, in: C.-H. Wong (Ed.), vol. 2, Wiley-VCH Verlag, Weinheim, **2003**, 609659.
3. S. Wang, J.A. Cuesta-Seijo, D. Lafont, M.M. Palcic, S. Vidal, *Chemistry* **2013**, *19*, 15346-15357.
4. G. Pastuch-Gawołek, M. Pleśniak, R. Komor, A. Byczek-Wyrostek, K. Erfurt, W. Szeja, *Bioorg Chem*. **2017**, *30*, 80-88.

[II-6] TETRAVALENT BISPECIFIC ENGINEERED ANTIBODY AS A TARGETING MOLECULE FOR SELECTIVE DELIVERY OF CYTOTOXIC DRUGS INTO CANCER CELLS OVERPRODUCING FGFR1

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Fibroblast growth factor receptor 1 (FGFR1) together with extracellular fibroblast growth factors (FGFs) form a complex signaling system responsible for regulation of a variety of biological processes. Importantly, aberration in FGFRs-FGFs signaling leads to the severe developmental disorders and cancer. Recent studies have shown that FGFR1 can be overexpressed in certain types of cancer, including lung cancer or breast cancer, therefore it constitutes a good molecular target for selective anti-cancer therapy. The purpose of this study was construction and characterization of the bispecific scFvD2C1Fc engineered antibody fragment directed against FGFR1 and its conjugation with a cytotoxic drug. Our results confirmed bispecific character of scFvD2C1Fc and its very high affinity towards FGFR1. In the next stage we demonstrated that scFvD2C1Fc does not cause receptor activation. Interestingly, at the same time scFvD2C1Fc is rapidly and efficiently internalize into the cells via FGFR1-dependent endocytosis. We studied intracellular trafficking of scFvD2C1Fc and revealed that engineered antibody is efficiently delivered to the lysosomes. Next, we conjugated antibody with a strong cytotoxic drug, monomethyl auristatin E (MMAE), yielding scFvD2C1Fc-based ADC (Antibody Drug Conjugate) - scFvD2C1Fc-vcMMAE. Cytotoxic assays demonstrated high cytotoxic potential of scFvD2C1Fc-vcMMAE against the cells which overproduce FGFR1. Cytotoxic effect of scFvD2C1Fc-vcMMAE is highly selective as control cells devoid of FGFR1 are resistant to applied ADC. Summarizing, our data suggest that scFvD2C1Fc has a great therapeutic potential. Due to its high specificity towards the FGFR1, it can be used in targeted drug delivery system in selective anticancer therapies.

[II-7] PROTEOMIC PLATFORM TOGETHER WITH OPTICAL TWEEZERS TECHNOLOGY AS A NEW APPROACH TO STUDY ADHESION ABNORMALITIES IN SINGLE CANCER CELL

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Introduction: The measurement of cell-to-cell adhesion forces is essential to understand the physical characteristics of living cells. During the last decade, several methods for measuring and studying single cell adhesion properties were developed, including optical tweezers technology. The wide range of applications of optical tweezers includes delivering cells to specific locations, sorting cells and transporting foreign materials into single cells. Here, we developed the cell model mimicking in-vivo like interactions between cancerous cells and the surrounding microenvironment.

Methods: In our study double wavelength multifunctional optical tweezers were used to optically trap and assemble individual lymphoma cell (DLBCL) on the stromal cell and to enable their interactions by applying trapping force to maintain contact until adhesive interactions between lymphoma and stromal cells were formed. After we characterized the adhesive properties of the panel of diffuse large B cell lymphoma cell lines in time-scale, we performed the proteomics investigations on cellular adhesion proteins using liquid chromatography- tandem mass spectrometry (LC-MS/MS).

Results: We established that the average time of nascent adhesion formation of lymphoma cell to stromal cell was from 15.5 8.4 s to 132.9 48.8 s for Ri-1 and Toledo cells, respectively. Based on precisely determined contact time values, we were able to distinguish cell lines with high and medium adhesive properties (Ri-1, SUDHL-10, U2932, and Pfeiffer), and the cell lines where evident lower adhesion properties were observed (U2904 and Toledo). Next, we identified several significantly deregulated proteins among cell lines, and by correlating data obtained from large-scale proteomics and optical trapping study, we were able to indicate which proteins are critical for maintaining cellular adhesion.

Novel aspect: The reported approach provides a new opportunity to investigate adhesion abnormalities in lymphoma at a single cell level which may indicate more invasive phenotype of cancer.

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[II-8] EFFECT OF A RADIATION DOSE ON THE TYPE OF INDUCED CELL DEATH AND RATE OF CANCER CELLS REPOPULATION

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An important task that any cancer therapy aims at is the elimination or significant limitation of cancer cells repopulation after treatment. The effectiveness of radiotherapy is affected by many physical factors, including: the total dose, dose per fraction, dose rate, beam energy and geometry, irradiation techniques and others. These parameters affect the type of biological response of irradiated cancer cells. Ionizing radiation used in radiotherapy induces cytogenetic damage and cell death. Tumor cell killing is mostly represented by a direct cytotoxic effect, including the induction of irreparable DNA damages, through double-strand break, leading to cell-cycle arrest and cell death. Ionizing radiation leads to cell death by many mechanisms, including mitotic catastrophe, apoptosis, necrosis, autophagy and senescence.

The aim of the study was to investigate the effect of the radiation dose on the type of induced cell death and the rate of repopulation of cancer cells.

Irradiation of human A549 lung carcinoma cells in T25 culture flasks was performed with single doses of 2.5Gy, 5Gy, 10Gy and 15Gy. The flasks with cells were placed in a water phantom, at the depth of 10cm. 6 MV photon radiation (X-rays) with a beam power of 400 MU/min, was used.

It was shown that irradiation of the cells induces a dose-dependent cell cycle arrest in G2/M phase.

With the increasing dose, raised percentage of apoptotic cells is observed. However, the number of cells killed by apoptosis is not high. As a result of irradiation, cells change their morphology. Cell sizes are increasing, numerous granules appear in the cytoplasm. Studies using acridine orange indicate that after irradiation in most cells the number of lysosomes responsible for autophagy process increases. On the other hand, cells stained with x-gal solution indicate aging process. Increase in the number of cells in the autophagy/aging state was dependent on the radiation dose: the higher dose, the more altered cells.

Further observation showed that after irradiation, the number of cells in the autophagy/aging state decreased as the time of cell culture was prolonged. After a few days of culture, cells proliferated and recovered normal morphology prior to irradiation. With increased radiation doses received by cells, the time of cell recovery also increased.

Therefore, on the basis of these preliminary studies, the suggestion appears that the dose of radiation received by tumor cells seems to be directly proportional to the time elapsed from the end of radiotherapy to the moment of repopulation initiation. In an effort to reflect conditions of processes occurring in actual patients we will take into account in our *in vitro* experiments further extension of cells incubation period after irradiation.

[II-9] ANTIPROLIFERATIVE MECHANISM OF NOVEL STYRYLQUINOLINE DERIVATIVES

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In relation to societal problems associated with constantly increasing level of cancer incidence, searching for new pharmaceuticals with antiproliferative properties is extremely important. One of the strategies of designing potential drugs in medical chemistry and pharmacology is the concept of privileged structure, which refers to molecular fragment that occurs in compounds with biological activity [1]. Among heterocyclic organic compounds one of the well-known privileged structure is quinoline. Diverse biological features of quinoline compounds are determined by the localization of substituents. Recently, 8-hydroxyquinoline (8-HQ) and styrylquinoline derivatives have gained attention due to their broad range of pharmacological activity such as antibacterial, antifungal, antiprotozoal and neuroprotective agents [2] as well as HIV-1 inhibitors [3]. In recent years our research was focused on antitumor activity of some novel styrylquinoline derivatives[4].

In this study, a series of novel synthesized styrylquinoline derivatives based on 8-hydroxyquinoline and 8-acetoxyquinoline scaffold were tested for their antiproliferative activity against a panel of human cancer cell lines including colon carcinoma (HCT116), glioblastoma (U-251) and two pancreatic cancer (PANC-1 and AsPc-1). Furthermore, the most active compounds were applied to normal human dermal fibroblast (NHDF). Cell lines selection was determined based on p53 status and related TP53 gene expression which was confirmed quantitatively. Some of the tested compounds were more active against cell lines with mutation of p53 than against wild type cells. Next, using flow cytometry, we studied the influence of the selected derivatives on the cell cycle and type of the induced cell death. Moreover, Western blot analysis was carried out, which allowed to determine the possible mechanism of action of the tested compounds and to assess the effect of p53 mutations on this mechanism. Additionally, preliminary results of time-dependent measurement of ROS level are presented.

References:

1. L. Costantino, D. Barlocco, *Curr. Med. Chem.*, **2006**, 13, 6585
2. Song Y., et.al., *Medicinal Chemistry Communications*, **2015**, 6, 61-74
3. Zouhiri F., et al., *Tetrahedron Letters*, **2005**, 46, 2201-2205
4. Mrozek-Wilczkiewicz A., et al., *PLOS ONE*, **2015**, 10(11), 1-14

[II-10] NOVEL THIOSEMICARBAZONE DERIVATIVES IN PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is characterised by low invasiveness and high selectivity towards normal cells, making this modality an attractive form of treatment compared to chemotherapy and radiotherapy. The essence of this therapy is a singlet oxygen and other free radicals generation by absorption of appropriate wavelength of light by photosensitizer.

A variation of PDT therapy is ALA-PDT in which, instead of exogenous photosensitizer a prodrug - 5-aminolevulinic acid (5-ALA) is administered. This compound is a precursor of protoporphyrin IX (PpIX), a natural endogenous photosensitizer [1,2]. Although cancer cells have elevated levels of PpIX, its biosynthesis is often insufficient to reach therapeutic concentrations due to its conversion to heme. For this reason, iron chelating compounds are attempted to be incorporated into PDT, with the aim of inhibiting PpIX conversion [3].

The research conducted by our team has so far concentrated on biologically active thiosemicarbazone (TSC) derivatives in combination with exogenous and endogenous photosensitizers. Currently, research is focused on biologically inactive TSC derivatives (IC5025 M) in ALA-PDT therapy, which are characterized by good complexing properties of iron ions. The effect of new TSC derivatives on the accumulation of protoporphyrin IX on selected cell lines (breast, lung, colorectal and brain tumors) was investigated. Due to the fact that the accumulation of PpIX is probably dependent on the enzymes involved in heme biosynthesis, the gene expression pattern of heme biosynthesis pathway was examined before and after the ALA and TSC application. The obtained results show a significant increase in PpIX accumulation after treatment with 5-aminolevulinic acid as well as in combination with some TSC derivatives. Expression of heme biosynthesis pathway genes correlates with the results of PpIX accumulation and the results show the particular importance of ferrochelatase and heme oxygenase in this process.

References:
1. Mrozek-Wilczkiewicz A., ACS Med. Chem. Lett., 5, **2014**,336
2. Millon SR, et al., J. Biomed. Opt., 15, **2010**, 18002
3. Mrozek-Wilczkiewicz A, et al., J. Cancer, 8, **2017**, 1979

[II-11] ONCOLYTIC MYXOMA VIRUS SHIELDED BY MESENCHYMAL STEM CELLS DESTROYS MELANOMA TUMORS

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Introduction: oncolytic viruses are a great promise of anti-cancer therapy as they are able to target hard-to-reach aggressive or disseminated tumor beds, trigger oncolysis and elicit antitumor immune response. One of challenges still facing oncolytic therapy is the success of systemic delivery of the benevolent virus. Bearing in mind that human bone marrow-derived mesenchymal stem cells (MSCs) are capable of homing *in vivo* to various tumors we previously characterized MSC-mediated transfer of oncolytic myxoma virus (MYXV) to cultured melanoma cells. Next, we investigated kinetics of *in vivo* distribution of MYXV in murine tissues and treated mice bearing experimental lung melanoma lesions. **Methods:** MSCs isolated from healthy human bone marrow donors were infected with vMyx-Fluc/tdTr (MYXV encoding firefly luciferase protein and tomato red fluorescent protein under poxvirus synthetic early/late promoter) or vMyxIL15R-tdTr (MYXV encoding IL-15 complex with subunit of its receptor and tdTr). *In vivo* studies using competent melanoma-challenged (+MET) or unchallenged (-MET) C57BL6 mice were performed with *in vitro* MYXV-preinfected MSCs. **Results:** animal studies demonstrated that B16-F10 cells preloaded *in vitro* with MYXV-infected MSC can effectively inhibit melanoma foci formation in lungs. The MSC-shielded MYXV constructs upon iv. infusion quickly accumulated in murine lungs (as opposed to naked virus) and their level there remained high enough to warrant a successful therapeutic attempt to destroy experimental melanoma lesions in murine lungs. **Conclusions:** we show that human bone marrow mesenchymal stem cells are suitable cell carrier (i.e. Trojan horse) to ferry advanced therapeutic MYXV constructs to sites of disseminated cancer such as metastatic lung melanoma.

This study was supported by grant No. UMO-2016/22/M/NZ6/00418 from National Science Centre, Poland, to JJ-R.

[II-12] MINOCYCLINE PROTECTS NEUROVASCULAR UNIT FROM SUBSTANTIAL DAMAGES PRODUCED BY SUBARACHNOID HAEMORRHAGE IN RATS

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Minocycline belongs to tetracycline antibiotics and has been widely studied as a neuroprotective agent, but we have limited knlowedge about its action in subarachnoid hemorrhage (SAH). In most cases SAH arises from aneurysm rupture, when arterial blood fills subarachnoid space surrounding brain. This fact makes SAH an untreatable and highly morbid disease of very complicated pathology. Neurovascular unit (NVU) consists of endothelial cells sealed by tight junctions (TJs) forming the blood-brain barrier (BBB). Abluminal face of NVU consist of pericyte and astrocyte feet aligning basal lamina of capillary and closest neuron. Components of NVU are connected together by extracellular matrix proteins that makes them critical substrates for proteases like MMP-2, MMP-9 and EMMPRIN. They play significant role in rebuilding the neurovascular unit under physiological and pathological conditions.

The aim of the present study was to determine whether minocycline could prevent neurovascular unit from ultrastructural remodeling and biochemical changes induced subarachnoid hemorrhage in rat brain.

Pre-chiasmatic SAH was produced by injection of 200uL of fresh autologous arterial blood into pre-chiasmatic cistern in Wistar rat brain. Minocycline was injected 10 min after ictus (1mg/kg). 24 hrs following the surgery animals were: (i) transcardialy perfused with fixative and whole brains were collected for transmission electron microscopy and confocal microscopy observation; (ii) basal brain cortex was cut on dry ice for Western Blot, gelatin zymography and qPCR.

Minocycline administration protected NVU from ultrastructural abnormalites resulting from SAH such as: (i) capillaries collapse, (ii) basal lamina deformation and delamination (iii) abnormal vacuoles and vesicle production in endothelium, (iv) pericytes of abnormal morphology, (v) far-reaching neuropil damage in form of different size electron lucent areas, (vi) degenerative changes among neurons and astrocytes. We did not observe differences in TJ morphology or length and tortuosity between the groups. However, SAH lead to reduction in TJ proteins, Occludin and Claudin-5 protein level but upregulation of *Ocln* and *Cldn5* expression with respect to control values. Minocycline administration had no effect on Occludin and Claudin-5 protein level, yet it limited only *Ocln* expression. Minocycline neuroprotective effect did not rely on MMP-2 and MMP-9 proteolytic action, for which level of protein active form and gene expression did not differ between groups. Similarly, EMMPRIN protein level was unchanged but we observed *bsg2* downregulation, both after SAH or SAH and minocycline, as compared to control. Minocycline decreased previously elevated EMMPRIN expression in capillaries after SAH *in situ,* at the same time protecting Collagen IV and Laminin from degradation.

Results indicate definite neuroprotective effect of minocycline which makes it a promising candidate for SAH treatment.

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[II-13] ON THE WAY TO DISCOVER MOLECULES WITH THERAPEUTIC POTENTIAL FOR TUMORS CARRYING KRAS G12V MUTATION

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Introduction: *KRAS* oncogene mutations are the most prevalent genetic alterations in multiple malignancies, including colorectal, pancreatic and lung cancer. The vast majority of mutations in *KRA*S occurs at positions 12, 13 and 61, that are crucial for proper protein activation. One of the most frequent is the G12V mutation and results in a substitution of glycine (G) to a valine (V) and makes the growth pathway resistant to be turned off. It has been demonstrated that tumors harbouring mutations in *KRAS* are intrinsically insensitive to anti-EGFR antibodies-based therapies, such as cetuximab or panitumumab. A lot of effort is put into understanding the biological function of *KRAS* mutants in determining the therapeutic response of these cancers. Among approaches that are used in searching for an effective KRAS blockers are inhibition of mechanisms responsible for anchoring protein to the cell membrane or blocking its effectors. On the basis of *in silico* studies, molecules that seem to be of great importance for drug development are selected for further *in vitro* testing. One of the most important steps during the process of drug discovery is to determine the cytotoxic effect of tested compounds. One of the tools, the half maximal inhibitory concentration (IC50) is a quantitative measure that indicates the concentration in which molecule of interest inhibits biochemical or biological function by half. The aim of the study was to analyze *in silic*o selected small-molecule compounds in order to assess their therapeutic potential as anti-cancer agents.

Methods: For this purpose two cancer cell lines carrying *KRAS* G12V mutation (SW620 and SW480 colorectal cancer cell lines) and one normal cell line (NIH/3T3) were used in the study. Prior to seeding the cells in 96-well plates, density tests were performed. Simultaneously, percentage content of serum in culture medium was determined to avoid cell number decrease due to the lack of this component. After seeding and serum starvation, cells were exposed to molecules in a wide range of concentrations. Cytotoxicity assessment of tested compounds on both normal and cancer cells was performed using crystal violet viability assay standard protocol.

Results: Determination of IC50 values indicates that some of them are toxic for cancer cells expressing specific KRAS mutants and at least 5 times less toxic for normal cells.

Conclusions: The outcome suggests that some of the selected molecules exhibit therapeutic potential.

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[II-14] THE HSPA PROTEIN FAMILY ARE INVOLVED IN PROTECTION AGAINST MANUMYCIN A TREATMENT IN NON SMALL CELL LUNG CARCINOMA (NSCLC)

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Heat Shock Proteins are highly conserved molecular chaperones considered crucial for protection against proteotoxic stress. The proteins belonging to the HSPA (HSP70) family are well-known cancer-related proteins and pro-survival factors frequently overexpressed in various types of cancer cells. HSPAs contribute to cytoprotection, have anti-apoptotic activity, and their increased levels have been frequently associated with poor patient prognosis and resistance to chemotherapy. Since lung cancer is the leading cause of cancer-related mortality among men and women, there is an urgent need to invent an effective, novel therapeutic strategy for lung cancer. Natural products are well-recognized sources of anticancer drugs. Manumycin A, an antibiotic synthesized by *Streptomyces parvulus*, is one of such natural compounds with anti-cancer activity. It acts as a selective and competitive inhibitor of Ras farnesyltransferase, and can effectively induce apoptosis in some types of cancer cells such as triple negative breast cancer or malignant pleural mesothelioma. This antibiotic is also known as an inhibitor of the Sp1 (specificity protein 1) transcription factor.

The aim of this work was to characterize the response of HSPA cytoprotective system in NSCLC cells exposed to manumycin A treatment, and to explore potential role of HSPAs in intrinsic resistance of NSCLC cells to this antibiotic. We found that manumycin A caused massive reduction in the viability of NSCLC cells. Exposure of NSCLC cells to manumycin A led to induction of classical stress response, as concluded from high induction of the *HSPA1 (Hsp70)* and *HSPA6 (Hsp70B)* gene expression. Interestingly, we also observed a significant decrease in the *HSPA2* gene expression. Assuming that the massive induction of *HSPA1* and *HSPA6* gene expression is regulated by Heat Shock Factor 1 (HSF1) transcription factor, and that the activity of Sp1 transcription factor plays an important role in the constitutive expression of *HSPA2* gene, our results suggest that manumycin A may inhibit the activity of Sp1 while increasing the activity of HSF1 in NSCLC cells.

Considering that upregulation of HSPA1 and HSPA6 proteins can exert protecting effect against manumycin A toxicity, we decided to inhibit HSPAs activity using the small molecule pan-HSPA inhibitor VER 155008. We found that combination of manumycin A with a low dose of VER 155008 had additive toxic effect on NSCLC cells. This observation confirms that HSPA chaperones protect NSCLC cells against manumycin A toxicity. Therefore, it seems that combination of manumycin A with HSPAs-targeting compound may provide an interesting novel therapeutic strategy in NSCLC.

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Session III:
Bioinformatics
and mathematical modeling

[III-1] HEAT SHOCK PROTEINS – POSITIVE OR NEGATIVE REGULATORS OF CANCER DEVELOPMENT?

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Heat shock proteins (HSPs) belong to a highly conserved family of proteins that act as molecular chaperones in conditions of stress, including carcinogenesis. Most of previous studies reported correlation of high expression of HSPs with cancer aggressiveness and poor prognosis. In contrast, some research has shown that reduced expression of HSPs is associated with poor prognosis in cancer patients. Therefore, the biological mechanisms of HSPs and their role in cancer development still remain to be investigated**.**

Utilizing the Cancer Genome Atlas (TCGA) and KM plotter databases we analyzed 96 eukaryotic heat shock genes and selected six of them the expression of which was the most significantly correlated with survival of breast cancer patients in both datasets. Interestingly, patients overexpressing two identified HSPs exhibited longer survival, whereas overexpression of other four HSPs resulted in unfavorable prognosis for breast cancer patients. Furthermore, we revealed that positive and negative role of survival-associated HSPs was reflected in clinicopathological traits. High expression of tumor suppressive HSPs was correlated with smaller tumors and luminal phenotype of breast cancer. In contrast, high expression of oncogenic HSPs was correlated with advanced tumor stage, lymph node involvement and aggressive subtypes of breast cancer.

We also developed a novel signature that can be used to calculate risk score for breast cancer patients basing on the expression of identified HSPs and cancer stage. The effectiveness of our predictive model was confirmed in an independent validation data set in the TCGA.

Finally, we analyzed the association of six prognostic HSPs expression with survival of patients suffering from other types of cancer. We revealed the ambiguous role of HSPs in carcinogenesis. Depending on the specific cancer type, each identified HSP can work either as a tumor suppressor or an oncogene.

[III-2] ANALYSIS OF EGFRvIII ECTODOMAIN CONFORMATIONAL TRANSITION WITH TARGETED MOLECULAR DYNAMICS

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Introduction: EGFR extracellular domain spanning over 600 amino acids has two functionally important conformations (or states) inactive, autoinhibited or tethered (T) monomer and ligand bound, active or untethered (UT) form which promotes EGFR dimerization. Deletion of exons 2-7 of wild type EGFR causes presence of unpaired cysteine in EGFRvIII monomer. Previous works suggested that Cysteine 16 is unpaired, whereas other cysteines form disulfide bonds within monomers. This model suggests that only C16 can directly participate in covalent homodimerization of EGFRvIII [1]. Despite mutation, hinge region responsible for conformational transition between T and UT states remains intact in EGFRvIII mutant. Crystallographic model of EGFRvIII covalent homodimer is not available. Homology model based on EGFR homodimer (PDB ID: 3NJP) does not show conformation suitable for covalent homodimerization trough C16. The main goal of this work was to simulate conformational transition between UT and T states to capture conformation in which EGFRvIII most likely homodimerize covalently.

Methods: Timescales of large conformational transitions are usually unavailable to classical Molecular Dynamics (MD) therefore Targeted Molecular Dynamics (TMD) has been used. In TMD additional force proportional to the RMSD from desired, final conformation is applied to the molecular system. This additional force allows the system to cross energy barriers and the conformational transition can be observed in tens of nanoseconds of simulation.

Results: Using TMD simulation (30 ns) trajectory of conformational transition between UT and T states was registered. It can be observed that C16 in monomers are getting closer to each other during transition than in any of the final states (UT or T).

Conclusions: EGFRvIII covalent homodimerization is most likely a dynamic process and requires some conformational adaptation of the ectodomain in the hinge region.

References:
1. Stec W. et al. Cyclic trans-phosphorylation in a homodimer as the predominant mechanism of EGFRvIII action and regulation. Oncotarget. 2018; 9:8560-8572.

[III-3] THYROID CANCER CLONAL STRUCTURE ANALYSIS BASED ON THE NEXT GENERATION SEQUENCING DATA WITH CORRECTION FOR TUMOR PURITY

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We present an approach for clonal structure analysis of neoplastic cells population based on the next generation sequencing (NGS) data. Clonality analysis provides plenty of information supporting studies of , e.g., drug resistance or cancer evolution. However, this type of analysis is quite sensitive to tumor purity. By collecting samples from tumor tissue we very often include microenvironment of cancer cells (eg.: immune cells, blood vessels cells, fibroblasts, stromal cells), so sometimes non-cancer cells are also gathered. Underestimation of this problem could lead to improper and distorted outcomes of time-consuming and complex analyses.

In order to make calculations related to estimation of existing subclones more reliable, we decided to emphasize detection of copy number alterations (CNA). Accuracy in detecting such variants is one of the most important points to clonality analysis and also one of the most critical disturbing features. Due to this, our analysis pays a lot of attention to estimation of cancer samples contamination and uses it to correct calling of CNA. The whole study was based on using free and publicly available tools and also our own algorithms to improve tumor purity estimation and searching for existing subclones. Our approach was examining the structure of the papillary thyroid carcinoma (PTC) among 10 patients. Data come from The Cancer Genomic Atlas (TCGA).

Results show that analysis with corrections for tumor purity can lead to very stringent results. Outcomes, from the developed algorithm for clonal structure estimation, complies with literature reports. The primary conclusion from the whole analysis is that while analyzing sequencing data, especially in such complex task as clonal structure, there is a need to take into account the tumor purity.

This work has been supported by the Silesian University of Technology Grant:BK-200/RAU1/2018/8 to JP and by NCN, OPUS grant (2016/21/B/ST6/02153) to AP.

[III-4] APPLICATION OF SENSITIVITY ANALYSIS FOR POTENTIAL MOLECULAR DRUG TARGETS SEARCHING

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Sensitivity analysis (SA) methods have been in use for over half a century. In recent years these methods have been applied also to perform analysis of biological systems models including signaling pathways. SA methods can serve a number of useful purposes, e.g. uncover technical errors in the model, identify critical regions in the parameter space or establish priorities for research [1]. One of such purposes may be searching for the crucial processes in a given signaling pathway. Altering these processes, e.g. by using pharmacological agents, may result in drastic changes in cell responses and therefore indicate a potential target for new drugs.

In this report we present a novel method of sensitivity analysis based on the frequency distribution of a model transient response. The method can be used to examine the sensitivity of the model to changes in parameters in the range that may represent the effect of drug administration (such as blocking/suppression of selected chemical processes). The range of parameter changes can be adjusted to what is observed clinically after drug administration, including differences between cell responses in a heterogeneous cell population.

The applicability of the proposed method is illustrated with an example of Interferon-beta induced JAK-STAT signaling pathway [2], which contributes to mucosal immune recognition and an anti-viral state. The innate immune response is the first line of defense to protect against an infection that results in the expression of cytokines that serve to limit viral replication until the adaptive immune response develops [3]. Considering the clinical significance of this signaling pathway, analysis of its model may provide valuable information.

Analysis of the Interferon-beta induced JAK-STAT model allowed to select two parameters corresponding to the processes in the signaling pathway that have the greatest impact on the cellular response. Reducing the values of these kinetic rates results in a drastic change in the model response: either suppressing or enhancing it. Therefore, the method indicates molecules involved in these processes as potential drug targets.

References:

1. Saltelli, A., M. Ratto, T. Andres, F. Campolongo, J. Cariboni, D. Gatelli, M. Saisana and S. Tarantola (2008). Global Sensitivity Analysis: The Primer. Wiley.
2. Smieja, J., M. Jamaluddin, A.R. Brasier and M. Kimmel (2008). Model-based analysis of interferon-beta induced signaling pathway. Bioinformatics 24(20): 23632369.
3. Akira, S., Takeda, K. (2004). Toll-like receptor signaling. Nat Rev Immunol. 4(7): 499-511.

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[III-5] NORMALIZATION BY COMBINATION OF MICRORNA EXPRESSION FOR HUMAN SERUM AND PLASMA RT-QPCR QUANTIFICATION DATA

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Introduction: Quantification of microRNAs using real-time PCR shows huge potential for diagnostic test development. However, a major obstacle in translating this class of potential biomarkers into clinical practice persists as there is no consensus on choosing the reference for data normalization. In contrast to mRNA quantification in specific types of tissues where housekeeping genes are fairly well-validated, the search for a stable, endogenous reference microRNA or a set of microRNAs, particularly in biofluids was mostly unsuccessful as numerous candidates did not pass validation or were shown to be disease-specific. In this work, we address this issue by a combination-based approach to normalization.

Methods: We conducted a literature search to gather available datasets of microRNA qPCR profiling from serum or plasma. We applied three different normalization algorithms (GeNorm, BestKeeper, NormFinder) to each dataset to calculate the most stable single microRNA. Then we repeated the analysis for all possible 2- and 3-microRNA combinations to find the most stable one. We calculated a ranking showing the relative position of single microRNAs and pairs determined in a ranked list of references sorted by stability value. We verified our approach on three independent validation sets.

Results: In the Gene Expression Omnibus database, we identified 11 datasets, which followed our inclusion criteria: less than 20% of missing data, 5 or more samples, 170 or more microRNA present in the array. For these datasets, both 2- ($p=0.0021$) and 3-microRNA ($p=0.0003$) combinations significantly outperformed single miRNAs in terms of stability and did not differ significantly from mean of all microRNAs ($p=1.0000$ and $p=0.9899$). We chose a set of candidate microRNAs, which were components of the most stable combinations based on our algorithm. Most stable microRNAs were more often part of the most stable combinations of 2 and 3 microRNAs.

Conclusions: We outlined a new normalization scheme and performed the analysis on 11 available datasets, which showed that pairs of microRNAs are more suitable for as reference factors for qPCR than single microRNAs. We chose the most powerful components of stable combinations and positively evaluated their performance on independent validation datasets.

[III-6] THYROID CANCER CLONAL STRUCTURE ANALYSIS BASED ON THE NEXT GENERATION SEQUENCING DATA WITH CORRECTION FOR TUMOR PURITY

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We present an approach for neoplastic cells population clonal structure analysis based on the next generation sequencing (NGS) data. Clonality analysis provides plenty of information supporting studies of, e.g., drug resistance or cancer evolution. However, this type of analysis is quite sensitive for tumor purity. When collecting samples from tumor tissue very often tumor microenvironmental cells (eg.: immune cells, blood vessel cells, fibroblasts, stromal cells) are included, so that non-cancer cells are present. Underestimation of this problem could lead to improper and distorted outcomes, during a time-consuming and complex analysis.

In order to make calculations related to estimation of existing subclones more reliable, we decided to emphasize detection of copy number alterations (CNA). Accuracy in detecting such variants is one of the most important points in clonality analysis and also one of the most critical disturbing features. Due to this, our analysis pays a lot of attention to estimation of cancer samples contamination and uses it to correct calling of CNA. The whole study was based on using free and publicly available tools and also our own algorithms to improve tumor purity estimation and searching for existing subclones. Our approach was examining the structure of the papillary thyroid carcinoma (PTC) from 10 patients. Data come from The Cancer Genomic Atlas (TCGA).

Results show that analysis with corrections for tumor purity can lead to very strict results. Outcomes, from the developed algorithm for clonal structure estimation, comply with he literature reports. The primary conclusion from the whole analysis is that in analyzing sequencing data, especially in such a complex task as clonal structure, there is a need to take into account the tumor purity.

This work has been supported by the Silesian University of Technology Grant:BK-200/RAU1/2018/8 to JP and by NCN OPUS grant (2016/21/B/ST6/02153) to AP.

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[III-7] ADAPTIVE PREPROCESSING OF MICRORNA SEQUENCING DATA WITH AUTOMATED IDENTIFICATION OF ADAPTER SEQUENCE

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Introduction: Next generation sequencing is nowadays one of the most common ways of analyzing miRNAs, as it provides reliable and extensive information. Preprocessing of sequencing data is a crucial moment of an analysis, allowing for noise reduction that occurs due to the nature of the process. Filtration of the data using set cut-offs is currently the gold standard, which does not consider differences between distinct designs, and thus, is not always the best decision. Another common issue in NGS data analysis is a lack of knowledge about the adapter sequence used in the experiment. Here we propose an analysis pipeline that allows for automated identification of adapter sequence and adaptive preprocessing of the data.

Materials and methods: RNA material was isolated from 18 samples of head and neck cancer cells (FaDU cell line) with the use of miRCURYTM RNA Isolation Kit (Exiqon, 300110). Library preparation allowed for selection of miRNAs of size 15-30 nt. The sequencing was performed with Illumina NextSeq550, read length 1x75 bp resulting in 8,307,000 to 51,240,000 unique reads per sample.

The proposed analysis pipeline consisted of i) quality control, ii) automated identification and filtering of adapter sequence, iii) mapping to the newest reference genome (*GRCh38*), iv) reference to *miRBase release 22* in order to obtain miRNA counts and v) adaptive filtration of count data using GMM decomposition. Automated identification of adapter sequence was based on suitable alignment of the reads and k-mer identification. Adaptive cut-offs for count data filtration were obtained by decomposing read distribution with GMM method and choosing the resulting threshold for filtering redundant components.

Results: Thanks to the applied analysis pipeline we were able to identify and filter the used adapter sequence, which resulted in preprocessed reads of median length equal to 22. The use of GMM method allowed for adaptive and experiment-dedicated identification of thresholds for count data filtration and enabled the reduction of noise in the data and lowering its dimensionality. The proposed analysis pipeline allowed for performing reliable downstream analysis.

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[III-8] IN-VITRO BLOOD FLOW AND CELL-FREE LAYER FORMATION IN HYPERBOLIC MICROCHANNEL: CFD MODEL VALIDATION

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Over the years, progress in microfabrication technologies has attracted the attention of researchers in several areas. Microfluidic devices could be useful to provide powerful tools for better understanding of the biophysical behavior of the blood flow in microvessels. Such devices can be, as well, used to separate the suspending physiological fluid from the whole *in-vitro* blood. Therefore, it is essential to acquire a detailed description of the complex interaction between erythrocytes (RBCs) and plasma. Experimental data [1,2] show that RBCs tend to move to the center of the channel due to the parabolic velocity profile, which results in a high shear stress around the walls. This movement results in the formation of the cell-free layer (CFL) with an extremely low volume fraction of cells.

The scope of this work was to develop a CFD model capable of reproducing the multiphase flow behavior in a microchannel obtained under laboratory conditions [1], where the CFL formation is visible downstream of the hyperbolic contraction of the channel. The fluid used in the experiment was dextran, the properties of which are consistent with those of plasma, containing about 5% of hematocrit by volume of RBCs. Dextran is assumed to behave as a Newtonian fluid and the RBCs are modeled as a suspension of rigid spherical particles with a viscosity dependent on the shear-rate and the hematocrit. The multifluid Euler-Euler approach [3] was used to model both continuous phase (plasma) and suspended within particles (RBCs). The obtained results show CFL thickness of ~6m downstream of the hyperbolic contraction. The volume fraction of the RBCs in CFL is less than 1% compared to 5% in the core flow. This is in good agreement with the experimental data published in [1].

Refrences:

1. Rodrigues, R. O., Lopes, R., Pinho, D., Pereira, A. I., Garcia, V., Gassmann, S., Lima, R. In vitro blood flow and cell-free layer in hyperbolic microchannels: Visualizations and measurements. *Biochip Journal*, **10**(1), 915 (2016)
2. Yaginuma, T., Oliveira, M. S. N., Lima, R., Ishikawa, T., Yamaguchi, T. Human red blood cell behavior under homogeneous extensional flow in a hyperbolic-shaped microchannel. *Biomicrofluidics*, **7**(5), 054110 (2013)
3. B. Melka, M. Gracka, W. Adamczyk, M. Rojczyk, A. Golda, A.J. Nowak, R.A. Białecki, Z. Ostrowski, Multiphase simulation of blood flow within main thoracic arteries of 8-year-old child with coarctation of the aorta, *Heat and Mass Transfer* **54**(8):2405-2413 (2018)

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[III-9] STUDY OF DIFFUSIVE MOTION OF A TRACER PARTICLE IN HYBRID POLYMER MEMBRANE

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Nowadays research draw an attention to the role of the structure on which the transport of penetrant takes place since the structure is the element which can be influenced. Such approach can bring scientists closer to designing membranes (the thin films) with specific and desired transport properties.

Hybrid alginate membranes containing dispersed various amount of magnetite (Alg/Fe3O4) and crosslinked using four different agents, i.e. calcium chloride (AlgCa), phosphoric acid (AlgP), glutaraldehyde (AlgGA) and citric acid (AlgC) were prepared and described in [1].

In this work, the membranes are characterized by different parameters like: the amount of polymer matrix, the fractal dimension of polymer matrix, the average size of polymer matrix domains, the average number of obstacles in the proximity of each polymer matrix pixel. Determination of the above mentioned characteristics base on the image analysis of a sufficiently large cross-sections of the membranes.

Diffusive transport is investigated by simulation of a particle motion in the membrane environment. Diffusion driven by Gaussian random walk is shown in order to check if the effective diffusion exponent at long time limit is subdiffusive and if it depends on the details of the underlying random process causing diffusion.

Thanks to such investigations the relationship between chemical composition, structure and morphology, and separation properties of the thin films, can be determined.

Refrences:

1. G. Dudek, M. Krasowska, R. Turczyn, M. Gnus, A. Strzelewicz, Structure, morphology and separation efficiency of hybrid Alg/Fe3O4 membranes in pervaporative dehydration of ethanol, Separation and Purification Technology 182 (2017) 101109
2. M. Krasowska, A. Strzelewicz, G. Dudek, M. Cieśla, Structure-diffusion relationship of polymer membranes with different texture, Physical Review E 95, 012155 (2017)
3. A. Strzelewicz M. Krasowska, G. Dudek, A. Rybak, R. Turczyn, M. Cieśla, Anomalous diffusion on fractal structure of magnetic membranes, Acta Physica Polonica B, 44 (2013) 955-965
4. M. Krasowska, A. Strzelewicz , A. Rybak, G. Dudek, M. Cieśla, Structure and transport properties of ethylcellulose membranes with different types and granulation of magnetic powder, Physica A: Statistical Mechanics and its Applications, 452 (2016) 241250

[III-10] MATHEMATICAL MODEL OF THE INTRACELLULAR PROCESSES AS SYSTEM WITH SWITCHINGS – STOCHASTIC APPROACH

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Biological systems are stochastic by their nature. Every reaction, which occurs in cell, is a result of a random event. Consequently the high concentration of the transcription factor inside cell does not necessarily mean that given gene is active, but it only increases the probability of gene activation at the given time. Complexity of the intracellular signaling pathway usually leads to complex stochastic models which are hard to analyze. One can try to analyze the deterministic approximation of the stochastic model but it usually leads to the analysis of system with many highly nonlinear differential equations. In [1] we presented another approach using linear systems with switchings, where the whole state space is divided into the smaller regions in which system is described by simple linear equations. Between regions are switchings dependent on the given conditions. The disadvantage of that approach was the lack of stochasticity. In the current work we fill this gap.

We consider a simple model of p53 signaling pathway with 4 variables: p53, Mdm2 in cytoplasm, Mdm2 in nucleus and PTEN. Each can be at low or high level and the level determines its function e.g. high p53 produces Mdm2 and PTEN. The switching between the processes rate is stochastic and its probability depends on the switching function which follows the Hill dynamics. The various values of the Hill coefficient (n) reflect the various sensitivities of the switchings in the different cell lines. We examined how the cells response on various levels of input (increased Mdm2 degradation caused by external stress) depends on the value of the Hill coefficient.

Deterministic approximation shows that the limit value of external stress R = 6.34 a.u.; lower stress doses induce system oscillation which are related to cell cycle blockade and higher activate increase of p53 level and apoptosis. In the stochastic case, with R=5 a.u. all cells oscillate and with increase of the Hill coefficient we observed higher amplitude of these oscillations. When R is close to the limit and n is low all cells choose apoptotic solution. With the increase of n we can notice growing fraction of the cells which oscillate instead of dying. With subsequent growing of R all cells go to the apoptosis regardless of n value.

Our results may provide explanation why the p53 oscillation amplitude differs in different cell lines. In our opinion it may be caused by different cell sensitivity to the switching threshold reflected in our work by Hill coefficient value. It may also explain the different fraction of apoptotic cells in different cell lines in response to the same stimuli.

Refrences:

1. Ochab M et al. *Variety behavior in the piece-wise linear model of the p53-regulatory module*, BIOINFORMATICS AND BIOMEDICAL ENGINEERING, IWBBIO 2017, pp. 208-219

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[III-11] DESCRIPTION OF THE MORPHOLOGY OF HYBRID ALGINATE MEMBRANES BY MEANS OF STEREOLOGICAL-FRACTAL ANALYSIS

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Nowadays, there has been a noticeable increase in the application of biopolymers in the field of nanotechnology as well as environmental and medical sciences. The biomaterials are more and more often used in place of synthetic polymers due to their excellent biocompatibility, biodegradability, high hydrophilicity, relative non-toxicity and low costs [1]. An example of such biopolymer is a biodegradable alginate with antibacterial properties.

This study investigated hybrid alginate membranes filled with various amounts of magnetite Fe3O4 and crosslinked using four different agents, i.e. calcium chloride (AlgCa), phosphoric acid (AlgP), glutaraldehyde (AlgGA) and citric acid (AlgC). These alginate membranes can be used to dehydrate ethanol in the process of pervaporation [2-3]. One of the most significant problems in membrane technology is the preparation of a membrane with required properties. Such properties can control mass transport and decide about the time and number of particles released from the membrane. A precise description of the morphology of a material is necessary in order to establish structural and functional relationships in the membranes [1]. Methods of morphological analysis should involve quantitative techniques, which would yield objective and reproducible values for any morphological structure and enable statistically defined comparisons. Such a tool is provided by the combination of a stereological analysis and fractal analysis. The morphology of the studied membranes was characterized on the basis of the membranes cross-section image analysis performed using a scanning electron microscope (Phenom Pro-X). The structure and morphology of the above-named materials were examined by means of the stereological-fractal analysis. The stereological analysis was based on shape descriptors (elongation factor f1, surface factor f2, irregularity parameter f3 and bulkiness f [2, 4]. Generalized fractal dimension [5] and lacunarity [6] constitute the basis of fractal analysis. With respect to the tested membranes, it was possible to identify the correlation between transport properties (pervaporation separation index (PSI)) and morphological parameters. The morphology of the membrane with the best separation properties was determined as a result. The membranes crosslinked using orthophosphoric acid with 15% and 20% addition of magnetic powder are characterized by the highest separation properties. The morphology of these membranes showed the highest self-similarity.

Refrences:

1. R.D. Noble, Perspectives on mixed matrix membranes, J. Membr. Sci., 2011, vol. 378, no. 1-2, p. 393-397.
2. G. Dudek, M. Krasowska, R. Turczyn, M. Gnus, A. Strzelewicz, Structure, morphology and separation efficiency of hybrid Alg/Fe3O4 membranes in pervaporative dehydration of etanol, Sep. Purif. Technol., 2017, vol. 182, p. 101-109.
3. G. Dudek, M. Gnus, A. Strzelewicz, R. Turczyn, M. Krasowska, The influence of metal oxides on the separation properties of hybrid alginate membranes, Sep. Sci. Technol., 2018, vol. 53, no. 8, p. 1178-1190.
4. M.N. Pons, J. Dodds, Particle Shape Characterization by Image Analysis, In Progress in Filtration and Separation, edited by Steve Tarleton, Academic Press, Oxford, 2015, p. 609-636.
5. Z.J. Grzywna, M. Krasowska, Ł. Ostrowski, J. Stolarczyk, Can generalized dimension (Dq) and f(a) be used in structure-morphology analysis?, Acta Phys. Pol. B, 2001, vol. 32, no. 5, p. 1561-1577.
6. M. Krasowska, P. Borys, Z.J. Grzywna, Lacunarity as a measure of texture, Acta Phys. Pol. B, 2004, vol. 35 no. 4, p. 1519-1534.

[III-12] REMUS: A WEB-BASED TOOL FOR BUILDING A TISSUE-SPECIFIC MAP OF REGULATORY ELEMENTS FOR A SET OF GENES

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Frequent somatic mutations are the hallmark of cancer genomes. Thus far, the main focus has been on somatic mutations recurring in the coding sequence of genes. Recently however, several studies have also identified recurrent non-coding somatic mutations, with the most notable example of the TERT promoter. Changes of non-coding regions can be specific to certain types of cancers and tissues, may contribute to tumorgenesis by acting in concert with changes of the coding regions, and potentially provide better insight into prognosis when evaluated together with coding mutations. Finding non-coding mutations with a causative role in tumorgenesis is difficult though, mainly due to a large number of such events and limited understanding of their functional impact. One of the key challenges is linking mutations of the non-coding regions with their potential effector genes, thus predicting the outcome of such variants.

We present Remus, an on-line tool that may facilitate identification of regulatory regions potentially associated with the expression of input genes. Although the tool is primarily developed for searching pathogenic variants in rare-disease studies, it can be useful in cancer research, for instance to study tissue-specific gene regulatory interactions. Starting from a small set of input genes implicated in pathogenesis of a disease, Remus allows creating a list of regulatory features active in a set of tissues of interest. Coordinates of regulatory regions are extracted from large repositories of tissue-specific regulatory data generated by genome-wide assays, such as ChIP-seq, DNase-seq, and CAGE-seq. The regulatory elements are selected based on experimental evidence of interaction or spacial proximity to the input genes Customizable search and step-by-step process allows for iterative building of a list of coordinates representing genomic locations of elements that likely play a role in regulating expression of the input genes in the user-selected tissues. The coordinates can subsequently be used to filter variants in search for mutations with pathogenic potential.

Remus is still in active development. The growing inventory of available regulatory data includes coordinates of tissue-specific enhancers, transcription start sites, and regions of accessible chromatin from ENCODE and FANTOM5 repositories. Recently, microRNA mRNA interactions from miRTarBase and miRWalk have been added. Future releases of Remus will enable inclusion of relevant transcription factor binding sites, as well as locations of TAD boundaries adjacent to the input genes. The software is available at https://github.com/seru71/Remus

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[III-13] A MODELING APPROACH OF CYTOSINE METHYLATION AND DEMETHYLATION

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The goal of this study was, using experimental data, to propose a mathematical model of methylation and demethylation of cytosine forms, which would be able to predict levels of different cytosine forms based only on the knowledge of enzyme transcript levels and selection of the model structure, which would allow to find out which TET protein work on successive stages of 5 methylcytosine transformation.

Methylation process during replication is conducted mainly by DNA methyltransferase DNMT1. Much less is known about the demethylation and enzymes participating in this process. Active demethylation of 5-methylcytosine in DNA occurs by oxidation to 5-hydroxymethylcytosine and further oxidation to 5-formylcytosine and 5-carboxylcytosine, and is performed by enzymes of the Ten-Eleven Translocation family (TETs 1, 2 and 3), but the particular role of each enzyme from TET family in each step is not known. Deoxycyditines can also be deaminated and transformed to uracil by Activation-Induced Cytidine Deaminase (AID). All these modified cytidine forms except methylated one are recognized by DNA repair systems and converted back to cytidine in DNA

Here we propose the approach of modeling in which known cytidine modification pathways are presented by 343 possible model versions with assumed different combinations of TET1, 2 and 3 activities in different pathways. Model parameters were calculated on the basis of 5-methylcytosine, 5-hydroxymethylcytosine, 5-formylcytosine, 5-carboxylcytosine and 5-hydroxymethyluracil levels experimentally assessed in 5 human cultured cell lines. Selection of the model version giving in simulations the best fit to experimental data suggested that not all TETs participate in some modification reactions.

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[III-14] EVALUATION OF SYSTEM DESIGN METHOD PERFORMANCE IN ANALYSIS OF ODE BASED MODELS OF BIOCHEMICAL SYSTEMS.

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Qualitative methods of analysis of dynamic system properties possess a number of advantages over traditional methods based on quantitative models. Parameter estimation can be particularly problematic in cases of more complex or strongly non-linear models. System design space methodology proposed by Savageau and co-workers allows for efficient identification and examination of qualitatively different system behaviors observed for varying values of parameters. The basic concept behind the method relies on approximation of the dynamics of system in question with dynamics of simplified models called S-systems, in so-called dominance regions, e.g. regions of parameter space where certain assumptions regarding parameter and variable values are met.

The aim of the presented work is to evaluate limitations of the design space method by examination of properties of a well-known biochemical system and its respective S-systems.

Selkovs model of glycolytic oscillations is a classical example of a structurally simple biological system exhibiting rich qualitative behavior. Ordinary differential equations governing its dynamics can be derived from stoichiometric chemical equations in accordance with mass action law. In that form there is a constant chemical reaction rate assigned for every term of each equation, hence there are five parameters associated with transformation of the substrate into product and with influx and efflux of both substances. The system is known to undergo Hopf bifurcations in response to a change of parameter governing the rate at which substrate is being delivered to the system from the external environment. Detailed analysis of S-systems and the original system dynamics highlights the discrepancies in the dynamics of S-systems and full system both inside and outside regions of dominance.

[III-15] PRELIMINARY STUDY FOR AUTOMATED DETECTION OF CEREBRAL MICROBLEEDS ON MR IMAGES

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The objective of the work was to perform parameter sensitivity analysis of the implemented system for the automated detection of cerebral microbleeds (CMB) on MR images. The implemented tool could increase the sensitivity of CMB detection and improve the accuracy of diagnostics.

Cerebral Microbleeds are caused by structural abnormalities of the brains small vessels. CMB are often linked with many diseases but they can also be present in healthy brain and lead to cognitive impairment, disability or death. They are visible on specific MRI sequences as round or ovoid areas with lower signal intensity and diameter up to 10 mm.

In the study, MRI images from Fudan University of China are used. Susceptibility Weighted Imaging (SWI) sequences were collected for a group of 81 healthy donors and CMB patients. Each image has a resolution of 240x320x72 voxels. Data preparation includes brain extraction and standardization step.

The proposed method is divided into 3 steps: segmentation based on MiMSeg algorithm, filtration of segmented objects in 2D space and the number of False Positives (FP) reduction in 3D space. MiMSeg uses Gaussian Mixture Models with K-Means to finding an optimal global threshold for segmentation of potential CMB with SWI modalities. Selected objects include CMB and a lot of artefacts with similar signal intensities. Filtration step contains roundness, size and core intensity criteria which reduces the number of false discoveries. The last step allows decreasing the number of FP based on their location and structure in 3D space. Length and centroids criteria exclude too long objects and those with a centre point shift between slices more than 1 pixel. The brains symmetry line is calculated to reject mirrored objects.

Using three patients who have CMB marked by an expert the filtration parameters were fine-tuned in numerical simulation. The final detection results are very sensitive to these parameter values, making tuning an important step in the development of the automatic CMB detection tool. The best results obtained from the optimal parameter set for patients with marked CMB are presented.

This work was partially financed by SUT BK-200/RAU1/2018/8.

[III-16] THE IMPORTANCE OF LIGANDS PRESENCE IN ENZYME CRYSTAL STRUCTURES FOR MOLECULAR DYNAMICS STUDY

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Protein Data Bank (PDB) provides more than 145 thousand structures. Many of the deposited proteins are represented by multiple structures that differ by resolution, additional mutations, or presence of small molecules. Specific proteins can be crystalized only in the presence of additives which may significantly perturb the native structure. To evaluate such effect, we have compared two structures of the same protein deposited in PDB one crystalized without, and the other with additional ligand in the active site.

As a study case we selected two structures of *Pseudomonas aeruginosa* epoxide hydrolase; one with urea inhibitor bound into the active site (PDB ID: 5HK9), and one without any ligand (PDB ID: 3KD2). This is a small enzyme with active site buried inside the protein core, which makes it sensitive for changes caused by inhibitor presence. The active site is located between two domains, the /-hydrolase core domain and the cap (lid) domain, and is connected with the surroundings by tunnels.

To evaluate the differences between both structures we run five 50-ns repetitions of MD simulations of both structures. We compared the access to the enzyme active site and the usage of those pathways using CAVER [1] and AQUA- DUCT [2], respectively.

Our results indicate differences in the distribution and the usage of the pathways leading to the active site. We also noticed distinct conformational changes in both systems, related mainly to the cap domain movements and the increase of water molecules flow. Such observation suggests that the co-substrates presence during protein crystallization should be accounted during *in silico* experiments. We hypothesize that our observations can be also related to the limited sampling of short molecular dynamics simulations which cannot capture long timescale effects. Systems with bound molecules require longer simulations to equilibrate system to mimic the native protein structure behavior.

Refrences:
1. Chovancov et al. 2012. PLoS Computational Biology 8: e1002708, 8(10).
2. Magdziarz et al. 2017. Bioinformatics 33 (13): 2045-2046.

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[III-17] DEVELOPMENT AND VALIDATION OF THE NUMERICAL MODEL FOR THE ARTIFICIAL AORTIC VALVE

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The cardiovascular system diseases (CVDs) are the leading cause of death in developed countries, where 31% of deaths are caused by CVDs. One cause of CVD is arteriosclerosis, causing among others myocardial infarction, heart failure, heart valve stenosis and stroke, and some other frequent affections, as valvular diseases. Nowadays, various diagnostic techniques are used, but all suffer from some imperfections. Generally, diagnostic procedures involve high examination costs, required qualified medical personnel and expensive equipment. Therefore, there is a room for developing virtual diagnostic procedures, such as numerical modeling, which will provide a complex insight into a medical problem without any potential risk. This fact could reduce patient mortality and provide a tool improving the safety of the patients examination.
The aim of the presented work is to develop a 2D numerical model of the blood flow through the artificial aortic valve, where the cardiac cycle will be mimicked using measured profile representing pulsatile flow. Digital model is developed using two different approaches, while their accuracy will be validated against experimental data collected at in-house test-rig. It is equipped with peristaltic pump (Harvard Apparatus) used to mimic the heart pulsation, aortic valve (Medtronic, Inc.) built in a transparent pipe, pressure transducer and PIV system to observe the flow field through the valve.

The first numerical model was performed with 2-way iteratively implicit approach of Fluid-Structure Interaction. This approach is connected with two applications: ANSYS Mechanical (Finite Element Method) and ANSYS Fluent (Finite Volume Method). The Fluent solver calculates the forces, coming from pressures and wall shear stresses. The Mechanical solver will use these forces for determining the new position of the artificial valve, and send this information back to Fluent, which in the next step will create the new numerical mesh of the fluid. This sequence is repeated several times during every time step, until the results from the Fluent, Mechanical as well as transferred values will be converged. The second model will engage the sheer Fluent solver, using the six degrees of freedom (6DOF) solver. The trajectory of the moving (in this case: rotating) objects calculations are based on the forces and moments balances acting to the rigid body. The new positions are updated by the dynamic mesh (smoothing and remeshing).

The success at the mentioned stages will lead to development of fully 3D multiphase model for predicting blood flow through aortic or mitral valve.

[III-18] HAIR-REMOVING ALGORITHM IMPACT FOR THE QUALITY OF SEGMENTATION PROCESS IN DERMATOSCOPY IMAGES OF SKIN LESIONS.

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Introduction: Segmentation of images, which show different skin lesions, is an important process of classification in skin cancer diagnosis. It is helpful for a specialist in fast and correct diagnosis, which is very important for proper therapy.

The main goal of this project was to investigate hair-removing algorithm for improving segmentation and gaining as good values of Jaccard index as possible, by comparing two images: the reference mask, which was made by dermatology specialist and a mask obtained in the process of image segmentation by using a computerized algorithm on skin lesions.

Methods: For the experiment images from PH2 database were used, and the whole process was prepared in Python programming environment. For segmentation, three special algorithms were implemented and one simple one, based on thresholding. These algorithms include several operations, like for example Gaussian blurring, converting to greyscale, gamma correction edges detection by using Laplacian operator or Canny-edge detector, histogram equalisation for better image contrast, then simple binarization by Otsu method, and applicable morphological operations for making the edges of object smooth and homogeneous. Received mask was compared to the reference one by Jaccard index.

The whole process was repeated by using the hair-removing algorithm. This algorithm simultaneously performs two combinations of operations on the input image. The first combination consists of defining the boundaries of the objects present in the image and removing them to reduce the surface of the hair. The second combination focuses on the detection of hair in the image. Then a common part of both images obtained from the combinations is removed resulting in a picture that does not contain hair.

Results: The results of the experiment are very promising as the received masks, in comparison with the references, gave Jaccard index noticeably higher than the one obtained without using the hair-removing algorithm.

Conclusions: The Scores, resulted in the Jaccard index show that the hair-removing algorithm allows a better segmentation effect on dermatoscopy images.

[III-19] ASSESEMENT OF THE LOW-DOSE DEPENDENCE OF THE ACUTE RESPONSE TO IRRADIATION OF MURINE HIPPOCAMPAL TISSUE

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The complex response of the irradiated central nervous system tissues involves the neurochemical mechanisms responsible for neuroinflammation or neurodegenerative illnesses. Poorly understanding of effects caused by low dose ionizing irradiation, which could induce either the negative or positive response on the health, resulted in searching its genomic and proteomic signatures.

The study was aimed at identifying protein signatures and their functions responsible for the occurrence of significant differences at the hippocampal low-dose irradiated samples on the molecular level.

Hippocampal tissues were harvested from wildtype mice 24 hours after exposure to the ionizing irradiation. LC-MS/MS was performed to deliver the protein expression level for different doses of irradiation (0 Gy, 0.063 Gy, 0.125 and 0.5 Gy). The raw data was subjected to the conservative data filtration. 1184 proteins were taken to the statistical and functional analysis to detect the dose-dependent signatures. Non-parametric ANOVA with the stepwise comparisons was implemented, subsequently, the Benjamini-Hochberg procedure was applied to correct the p-values for multiple testing.

Since, according to the previous studies on the same dataset [1], the impact of sex on the response of irradiated murine hippocampal tissues was not significant, it was decided to analyze male and female samples together. This analysis reviewed that 154 proteins changed significantly in-between the doses. 44 of them showed the additive response with the increasing dose of irradiation. U-shape expression profile was indicated for 102 proteins, for only 8 the decreasing linear trend was observed.

We observed that dose-only dependence is mainly responsible for the axonal regulation, peptide chain elongation and cell migration. Increasing and decreasing linear trend is not predominant for the low-dose response.

Refrences:

1. R. Piecyk *et al.*, Impact of sex and recovert time on the molecular profile of murine hippocampal tissue after irradiation, AGH ISC 2018. Books of abstracts, p. 57, e-ISBN 978-83-944254-7-0
2. MM. Acharya *et al*., Consequences of Low Dose Ionizing Radiation Exposure on the Hippocampal Microenvironment, PLoS One 2015, 10(6), e0128316
3. SJ. Kempf *et al*., Chronic low-dose-rate ionising radiation affects the hippocampal phosphoproteome in the ApoE/ Alzheimers mouse model, Oncotarget 2016, 7(44), 71817-718232

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[III-20] PROTEIN HOT–SPOTS IDENTIFICATION – CAN WE REPLACE LONG SIMULATIONS BY SERIES OF SHORT RUNS?

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Molecular dynamics (MD) simulations are used to understand macromolecular structures and interactions between them. It is an easy and fast way to introduce theoretical models to experimental results. By analyzing MD simulations we can detect amino acids (i.e. hot-spots) that may significantly affect enzymes activity, selectivity or stability. Hot-spots identification helps planning and performing further experiments. Numerous tools are able to detect potential hot- spots on the basis of crystal structure. However, analysis of crystal structures does not allow to capture the flexibility of proteins which, for many systems, might be essential.

More precise hot-spots detection is possible by MD simulations analysis. Obvious question emerges, what is sufficient length of MD simulation to provide necessary information for hot-spots detection. Longer simulations are able to capture transformations occurring in longer timescale. On the other hand, shorter simulations require less CPU/GPU time and explore wider conformational space.

We compared results of five repetitions of short simulations (50ns) with a single long run (500ns). As a model system we selected *Trichoderma reseei* (TrEH, PDB ID: 5URO) soluble epoxide hydrolase. This enzyme belongs to the /-hydrolase superfamily and converts epoxides to diols. TrEH structure consists of /-hydrolase domain and a cap domain, and it has a high content of beta structures compared to helical structures [1]. The active site is located in the center of the structure and is connected with the environment by tunnels.

Hot-spots detection in MD simulations was carried out with AQUA- DUCT (AQ) software [2]. AQ uses water tracking approach for proteins description and investigation. It can locate entrances/exits of tunnels, and also allows to detect particular amino acids hot- spots which trap or attract water molecules. Our results show differences in the identified hot-spots in shorter and longer simulations. However, significance of observed differences needs to be investigated in other systems.

Refrences:
1. de Oliveira et al. 2016. Int. J. Biol. Macromol. 89: 569574
2. Magdziarz et al. Bioinformatics 2017. 33 (13): 2045-2046.

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[III-21] TOWARDS INTELLIGENT DRUG DESIGN SYSTEM: APPLICATION OF ARTIFICIAL DIPEPTIDE RECEPTOR LIBRARY IN QSAR-ORIENTED STUDIES

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The pharmacophore properties of a new series of potential purinoreceptor (P2X) inhibitors determined using a coupled neural network and the partial least squares method with iterative variable elimination (IVE-PLS) are presented in a ligand-based comparative study of the molecular surface by comparative molecular surface analysis (CoMSA). Moreover, we focused on the interpretation of noticeable variations in the potential interaction selectiveness of individual inhibitor-receptors due to their physicochemical properties; therefore, the library of artificial dipeptide receptors (ADP) was designed and examined. The resulting library response to individual inhibitors was arranged in the array, preprocessed and transformed by the principal component analysis (PCA) and PLS procedures. A dominant absolute contribution to PC1 of the Glu attached to heptanoic gating acid and Phe bonded to the linker *m*-phenylenediamine/triazine scaffold was revealed by the PCA. The IVE-PLS procedure indicated the receptor systems with predominant Pro bonded to the linker and Glu, Gln, Cys and Val directly attached to the gating acid. The proposed comprehensive ligand-based and simplified structure-based methodology allows the in-depth study of the performance of peptide receptors against the tested set of compounds.

[III-22] DEVELOPMENT OF DIGITAL MODEL FOR ANALYSIS OF ATHEROSCLEROSIS WITHIN CORONARY ARTERIES - LABORATORY TEST RIG FOR MODEL VALIDATION

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Medical diagnostic tools will play a major role in future quest for an effective treatment to reduce patient mortality due to cardiovascular system diseases (CVDs). The CVDs are the leading cause of death in developed countries. According to the World Health Organization, CVDs caused 31% of deaths in 2015 in the world, while in Europe 45% of all deceases are the consequence of CVDs. Taking into account the cited numbers there is plenty of room for the improvement of both diagnostic and therapeutic procedures leading to more successful therapies without increasing costs. This implies that diagnostics will remain the major factor in reducing patients mortality. One of the grand challenges for physicians is the need for accurate, robust and stable computer tool for virtual patient diagnostics and treatment. To address such a trilemma, the medical industry will have to go through profound innovations in the future, to develop new technologies which can be useful at the stage of surgery planning and during its performance. Given this , the physician involved in cardiovascular surgery faces a grand-challenge in the future, namely the development of a validated, predictive, robust, multi-scale modelling capability, to enhance cardiac diagnostic capabilities and patient treatment applying advanced computational techniques.

Development of the digital model which can be seen as numerical representation of real system is very challenging and demanding process. This is even more complicated in the case of its future medical applications. In order to ensure high reliability of numerical model it needs to be initially validated against experimental data. The aim of presented work is to develop a numerical model that can be used for modeling conditions prevailing in coronary arteries with arteriosclerotic lessons. The functionality of the digital model as well as its accuracy need be tested using data collected at the experimental test stand. Presented work describes the test rig development process, used apparatus and its possible application for various purposes. Configuration of used equipment allows to investigate the influence of different types of narrowing in the coronary arteries, caused by arteriosclerosis lesions, on the flow field as well as measured level of Frictional Flow Reserved (FFR) which represents the pressure ratio (distal to proximal) used for invasive patient diagnosis.

[III-23] PAN-CANCER ANALYSIS OF MOLECULAR DIFFERENCES FOR DIVERSE CANCER TYPES USING MALDI-MSI

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Introduction: Pan-Cancer analysis aims to examine similarities and differences across diverse cancer types. The complexity in the molecular signature of cancers is influenced not only by their location in the organism, but also by hormonal and environmental factors prevailing in some cancers in the carcinogenesis process. Therefore, current research of biological differences directly influences the accepted treatment strategies. One of the analytical techniques widely used in tissue-based study is mass spectrometry imaging, which in this case can be used to study distribution of different molecules in healthy and caner tissues. The aim of this work was to present the preliminary results of molecular differences between six different types of cancers (head and neck, testicle, intestine, thyroid, prostate, stomach) using MALDI-MSI data.

Methods: This paper presents the results of a comparative analysis of different types of cancer and normal tissue using data from MALDI-TOF- molecular imaging. Spatially distributed spectra were collected by ultrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) in positive mode within the mass range of 700-3500 m/z. Spectra preprocessing included: spectra resampling to common m/z channels, adaptive baseline correction, PAFFT alignment and TIC normalization. The common average spectrum was subjected to Gaussian mixture modelling. Redundant components were filtered out and then spectra and GMM components convolution was calculated to determine peptide abundance for comparative analysis. The comparative analysis of potential differentiating biomarkers was made using Kruskal-Wallis test, Benjamini-Hochberg correction, Dunns multiple comparison test and measure of Cohens effect size and Eta-square.

Results: The final data set included 164966 spectra (samples) and 1491 peptides (features) for the analysis of potential differentiation. Analysis of the Eta-square value between cancer and healthy tissue showed weak differentiation at the median level of 4.05e-04. The analysis conducted for hormone-based cancers together and environmental-based cancers together, showed greater ability to differentiate between healthy and diseased tissue for hormone-based cancers. In the case of paired comparisons, greater molecular heterogeneity revealed different types of cancer than different types of healthy tissue. The most differentiating peptides with at least medium Cohens effect were found between testicular and thyroid cancer (395 features) as well as between prostate and thyroid cancer (355 features).

Conclusions: This study allowed to examine heterogeneity of hormone-based and environmental-based cancers. Comparative statistical analysis identified a set of peptides which differentiate healthy and cancer tissue and different types of cancer.

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[III-24] DIGITAL MODEL FOR ANALYSIS OF ATHEROSCLEROSIS WITHIN CORONARY ARTERIES – NUMERICAL MODEL DEVELOPMENT PROCESS

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The life habits of numerous humans pose serious risk for the formation of atherosclerotic changes in the cardiovascular system. According to the World Health Organization the cardiovascular system diseases (CVDs) are the leading cause of death in developed countries. One of the effects of atherosclerosis is the reduction of the cross section of blood vessels, reduced blood flow, hypertension and, in seroius cases, stroke and death. This implies that diagnostics remains potentially a major tool lowering patient mortality. Both diagnostic and therapeutic procedures leading to more successful therapies without increasing costs are in high demand. For instance, in order to determine the degree of vessel narrowing, an invasive Frictional Flow Reserved (FFR) examination is performed. A probe inserted into blood vessel allows to measure pressure ratio before and behind the narrowing. Nowadays, enormous effort is orientated in the direction of reducing invasive diagnostic procedures which lead to certain complications in patients. In order to avoid the necessity of such invasive tests, the idea of building a virtual tool that can replace invasive procedure is constantly evolving. For that purpose a digital model developed using Computational Fluid Dynamics (CFD) can be used. CFD allows predicting flow field, pressure drop across the narrowing using geometrical model created based on the computed tomography (CT) scan.

The presented work shows the development process of digital model for modeling atherosclerotic lesions within coronary arteries. The diagnostic strategy is as follows: images of the atherosclerotic artery from CT scan are used to create a three-dimensional model as a result of segmentation using 3D Medical Image Processing Software - Mimics. The resulting 3D model is imported into the ANSYS Fluent software, where after defining boundary conditions, it is possible to simulate blood flow through the analyzed section of CV system. An important step in the numerical model development process is its validation. For that purpose a simple model should be used. Accordingly, initial digital model has been validated using simple geometry with created narrowing and data collected at the in-house experimental rig. Validated model can be then used for modeling a real system which is the main aim of the digital model application.

[III-25] TRANSLATION MODELING AS A MARKOV PROCESS

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Proteins are produced in living cells during the process of translation of mRNA, in which the executive elements are ribosomes. Many ribosomes can be attached to mRNA strand, so the rate of formation of new molecules depends on their number. This process is subject to many regulatory mechanisms, among which an important role is played by microRNA molecules (miRNAs). MiRNAs can be attached to mRNA as an appropriate complex, which leads to inhibition of protein production. Translation, as a stochastic process, can be modeled with Markov chain. In Markov chain we can distinguish different states, correlated to different factors like, eg., number of ribosomes on mRNA. Assuming that the chain is currently in state s_i (where s_i is defined as number of ribosomes on mRNA), the change of state in the next step depends on transition probability and is described as p_ij. The pij probability is not dependent on past states of chain. In this research we present the model of translation as a Markov process which is analyzed from a queuing systems perspective. This model was implemented as a cellular automaton, which allows to input regulation factors such as miRNA or interaction with another protein.

[III-26] ESTIMATION OF THE LEVEL OF CANCER ASSOCIATED FIBROBLASTS PRESENT IN PROSTATE TUMOR TISSUES USING RNA-SEQ EXPRESSION DATA

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The tumor microenvironment consists of heterogeneous populations of cells that impact tumour growth and disease progression. Cancer-associated fibroblasts (CAFs) are one of the key cells within the stroma of many tumor types. Numerous previous studies have highlighted a pro-tumorigenic role for CAFs via secretion of various growth factors, cytokines, chemokines, and through remodelling of extracellular matrix. Increasing evidence suggests that that CAFs interact with tumor-infiltrating immune cells to promote cancer progression. CAFs are identified by their expression of various proteins such as alpha smooth muscle actin (-SMA), platelet-derived growth factor receptor alpha (CD140a), platelet-derived growth factor receptor beta (CD140b), fibroblast-specific protein 1 (FSP-1) and fibroblast activation protein (FAP). Gene expression profiling of tumour tissue has resulted in the identification of molecular subtypes and the development of models for prediction prognosis and has enriched our knowledge of the molecular pathways of tumorigenesis. Such studies may provide further evidence for novel drug resistance mechanisms informing novel combinatorial, adaptive and tumour immune-therapies placed within the context of the tumour microenvironment. The aim of our study was to determine a proportion of CAFs in tumour samples of prostate cancer based on RNA-seq gene expressions profiles and mutual relations between the expression profiles of different cell types in the context of clinical outcome.

The analysis was performed on gene expression profiles (HTSeq-FPKM and HTseq-FPKM-UQ workflow) from 551 samples of prostate cancers. RNA-seq gene expression profiles and clinical data were obtained from the TCGA Data Portal. We adapted the ESTIMATE algorithm, to estimate not only the stromal tissue and immune cell infiltration as well as tumour purity, but also, based on the signatures related to CAFs the percentage of CAFs in each sample. We also investigated the association between the stromal, immune and CAFs percentage and clinical characteristics**.**

Our results show that the levels of CAFs in the tumour tissue can be associated with clinical characteristics and may provide an additional information to increase our understanding of molecular phenotype.

The calculation of CAFs infiltration in tumour tissue may help in elucidating the facilitating roles of the microenvironment to cancer cell and provide new insights into the role of CAFs in tumour progression. The comprehensive understanding of CAFs in tumour tissues may provide important insights into tumour biology and aid in the development of robust prognostic and predictive models.

This study has been supported by a grant of National Science Centre in Poland (Miniatura grant 2017/01/X/NZ3/01158 to PK).

[III-27] IMPACT OF THE CELL CYCLE PHASE AT THE TIME OF IRRADIATION ON RADIOSENSITIVITY OF THE CELL POPULATION – A SIMULATION STUDY

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DNA damage detection and repair processes, which are crucial for the survival of the cell, are regulated by several signaling pathways. One of the most important mechanisms, influencing DNA damage detection and repair, is the cell cycle. Depending on the cell cycle phase DNA has different susceptibility to damage. DNA damage repair methods also differ between phases of the cell cycle. Double strand breaks, which are the most dangerous type of lesions, are repaired by non-homologous end joining and by homologous recombination repair. The latter is, however, present only in S and G2 phases of the cell cycle.

In this work we studied the impact of cell cycle phase on DNA damage detection and repair processes after treatment with ionizing irradiation. We developed a mathematical model combining cell cycle with DNA damage detection pathways. Stochastic cell cycle length and irradiation time allow to recreate conditions in the cell population. Parameters for our model were partially obtained from biological experiments performed on U2OS cell line. We took into account availability and kinetics of various repair mechanisms in different cell cycle phases.

Our results are consistent with biological findings showing that radiosensitivity of the cells differ between cell cycle phases. We found, that synchronization degree in the population of cells has strong impact on the size of surviving and apoptotic fractions. Our results may explain why cells from heterogeneous populations exhibit different responses to radiation, what is commonly observed during biological studies performed using cell cultures.

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Session IV: Biomarkers

[IV-1] THE MMP AND TIMP GENE VARIANTS ARE ASSOCIATED WITH PROGRESSION AND PROGNOSIS IN HEAD AND NECK CANCER

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Structure and composition of extracellular matrix (ECM) are important factors in angiogenesis regulation. Progression and metastasis in solid tumors, including squamous cell head and neck carcinoma (HNSCC), depend on angiogenesis and ECM remodeling. The key players in these processes are matrix metalloproteinases (MMPs) and their specific endogenous tissue inhibitors, TIMPs. MMPs are peptidases directly involved in degradation of basement membrane and ECM components. By releasing growth factors, MMP2 and MMP9 gelatinases play a special role in angiogenesis and metastasis. Both, MMP2 and MMP9 are critical in vascular remodeling, cellular migration and sprout formation. In addition to the inhibitory role against MMPs, TIMP2 is able to suppress cell proliferation in response to angiogenic factors, while TIMP3 has the ability to block the binding of VEGF to its receptor that inhibits neovascularization. In locally advanced HNC, radiotherapy (RT) and cisplatin-based chemotherapy (CT) are commonly used treatment strategies, the efficacy of which is affected to a large extent by hypoxia and deregulated angiogenesis. In this study, we assume that genetically determined differences, caused by gene polymorphism, in levels and activity of the above-mentioned proteins may influence individual angiogenic and metastatic potential, as well as impair treatment effectiveness and survival. In HNSCC, polymorphism in *MMP* and *TIMP* genes has not been investigated in the context of progression and prognosis.

We aimed to evaluate the effect of 8 functional polymorphisms in *MMP2, MMP9, TIMP2* and *TIMP3* genes on loco-regional and distant failure, overall (OS), relapse-free (RFS) and disease-free (DFS) survival in 519 patients with HNSCC treated with RT alone or in combination with cisplatin-based CT. TaqMan-MGB probes were used for genotyping. Kaplan-Meier method and Cox regression models were applied for analysis.

The *MMP2* 3UTR C and -1306 T alleles were significant predictors of poor OS and DFS in patients treated with RT and CT combined. The *MMP2* -1306 T and TIMP3 -1296 C variant carriers demonstrated shorter RFS, especially due to a higher risk of local failure.

Impact of the studied variants on clinical outcome was observed mainly in the combination therapy subgroup. Our results indicate that certain *MMP2* and *TIMP3* polymorphisms may be associated with progression and prognosis in HNSCC.

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

[IV-2] THE COMBINATIONS OF CLINICAL AND GENETIC FACTORS IN SURVIVAL PREDICTION IN BREAST CANCER PATIENTS

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Breast cancer is one of the most common cancers among women, it accounts for over 20% malignant tumors in women. The CONCODR-3 study reports that in Poland patients 5-year survival tend to improve, from 71.3% (2000-2004) to 76.5% (2010-2014) for all age groups. Unfortunately due to the breast cancers heterogeneity and patients own unique genetic make-up, the clinical resistance to treatment is often observed as incidences of disease progression, local recurrence, primary and secondary tumors at different locations, and cancer-related mortality.

In this study we combined known clinical survival-reducing factors like TNM staging, receptors status, age of patients and treatment particulars, with genetic data in order to establish a predictive models for overall, progression-free and recurrence-free survival (OS,PFS, RFS). The patients group consisted of 324 female breast cancer patients treated with first-line FAC chemotherapy (doxorubicin, 5FU, cyclophosphamide). Genetic data consisted of the genotyped 33 variants in 3UTR regions of 23 genes involved in FAC drugs transport, metabolism, DNA damage recognition, repair and cycle control, regulation of detoxification pathways and steroids binding.

The multifactorial models were possible to establish for all three survival analyses. For OS, six factors were linked to high risk of death- three components of TNM staging combined with the variants in doxorubicin importer gene *SLC22A16* (2 SNPs) and in pregnane X receptor NR1/2- the switch of detoxification pathways. The median survival for the carriers of 5 and all six unfavorable factors was drastically shortened (36.3 vs 92.2 months, log rank p0.00001), with the risk of death of HR 6.34, p0.00001). For the PFS four risk factors were established, with the strongest being the preexisting metastases, followed by weaker variants in *SLC22A16* and in progesterone receptor gene (2 SNPs) The chance of progression for the accumulation of all four risk factors resulted in risk of HR 19.18 (p0,00001) with the median PFS shortage from 89.82 to 16.03 months (log rank p=0.00004). Lastly, for the RFS there were three high-risk factors, namely the PR status and two SNPS in 5FU deactivator *DPYD* and in the gene *AKR1C3* encoding the enzyme of the main metabolic pathway of doxorubicin. In this model, the median survival of the carriers of all three factors was 52.34 months vs 83.3 for non-carriers (log rank p0.00001), and the risk of recurrence reached HR 10.60 (p=0.00009).

The results suggest the usefulness of established models in selection of breast cancer patients into groups with different predicted prognosis in regard of death, progression or recurrence. This study also proves that while the clinical prognostic factors still have the strongest impact on prognosis, the patients genetic characteristics have also strong influence on survival.

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[IV-3] WHOLE-BODY RESPONSE TO RADIOTHERAPY DETERMINED BY SERUM METABOLOME OF HEAD AND NECK CANCER PATIENTS

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Introduction: Serum is known to carry molecules associated with various disease states such as cancer. Searching for new biomarkers which could correlate with treatment response in cancer patients is of particular importance. Cancer radiotherapy (RT) induces a response of the whole patients body that could be detected at the blood level. Radiation-induced changes in the proteome and transcriptome of serum have been widely described and discussed, however, metabolites present in blood have not been given as much attention. Metabolomics of serum of cancer patients could also provide a valuable insight into the response of both the tumor and the whole organism to the treatment. The aim of the study was to compare serum metabolome profiles in head and neck cancer patients before and after radiotherapy.

Methods: Serum samples from patients with pharynx region carcinoma (10 patients) were taken before (A) and after (B) radiotherapy. Healthy volunteers (10 individuals) were used as a control group. A mixture of MeOH/H2O was used for extraction of low-molecular weight metabolites. Samples were analyzed by gas chromatography-mass spectrometry (GC-MS).

Results: Changes in the level of several compounds were observed between samples collected before and after irradiation. Amino acids were predominant in the group of compounds the level of which significantly decreased in post-treatment samples. Up-regulation of mainly ketone bodies derivatives was observed in patients after RT, which is in accordance with previous studies.

Conclusions: Radiotherapy caused significant changes in levels of several serum metabolites. Amino acids deficiency and ketone bodies increase in post-RT patients is caused by weight loss either as a result of anti-hypoxia cell protection or by dysphagia disorders.

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[IV-4] NGS ANALYSIS OF MELANOMA SAMPLES USING ION TORRENT PLATFORM AND CANCER HOTSPOT PANEL – PRELIMINARY RESULTS AND TECHNICAL OVERVIEW

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Introduction: Next Generation Sequencing (NGS) is a very robust, efficient and precise technology which has already reached diagnostic laboratories. It is a convenient tool for simultaneous analysis of many genes in many samples. One of research implementations of this technology is searching for genetic variants responsible for resistance to therapies e.g. targeted therapy in melanoma. Here, we show the preliminary results of Ion Torrent NGS analysis of melanoma samples using Cancer HotSpot Panel.

Methods and Materials: We performed NGS analysis of 9 FFPE melanoma samples derived from advanced melanoma patients treated with targeted therapy. AmpliSeq Cancer Hotspot Panel v2 was used for library synthesis and Ion Torrent PGM Chef system and 316TM Chip v 2 (Thermo Fisher Scientific) for sequencing. The data was analyzed using Ion Torrent Reporter programme and available tools e.g. VarSome, wANNOVAR. Selected variants in some samples were additionally validated using Somatic 1 Multiplico Kit (Agilent).

Results: Seven out of 9 samples showed very good NGS quality with more than 75% amplicons covered 500 times. The NGS analysis revealed known BRAF mutations and several other (pathogenic or of unknown significance) mutations which may influence response of melanoma to targeted therapy. The most interesting ones are: CTNNB1_S45F, CDKN2A_P114L, ERBB4_S184L, NRAS_G13S, and KRAS_Q22R.

Conclusions:

1. Most DNA from FFPE melanoma samples collected in our biobank is of good quality for NGS analysis.

2. Cancer HotSpot panel has only limited applicability in prediction of melanoma resistance. It enabled identification of genetic variants potentially responsible for primary resistance of melanoma to targeted therapy in 4 out of 9 cases.

3. Ion Torrent NGS may generate artefacts in melanoma samples concerning V600M mutation.

4. It may be difficult to distinguish between formalin artefacts and UV mutations in melanoma samples.

[IV-5] COMPLEX ARTIFICIAL NEURAL NETWORK MODEL FOR DIAGNOSIS OF PANCREATIC CANCER BASED ON MULTIPLE CIRCULATING MIRNAS – A PILOT STUDY

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Introduction/Rationale: Despite extensive effort and progress in scientific research, mortality due to pancreatic cancer is still increasing. Since the diagnosis of pancreatic cancer at the late stage is associated with a dismal prognosis, the discovery of biomarkers that would enable the diagnosis in early stages of the disease is one of the most urging aims of oncological research. The aim of our study was to assess the potential of identifying of a set of circulating miRNAs that could be used to create an efficient diagnostic and/or prognostic model for diagnosis of pancreatic cancer using state-of-the-art tools of artificial intelligence.

Methods: We analyzed the expression of circulating miRNAs in the serum of patients with a diagnosis of pancreatic cancer as well as in age- and sex-matched controls. Next generation sequencing was used to quantify the number of miRNA transcripts in the samples. Firstly, feature selection (dimensionality reduction) was performed using 3 distinct methods: (1) fold change-based (including miRNAs with fold change 2 or 0.5 between cancer and control cases), (2) p-value-based (including miRNAs with false detection rate 0.005) and correlation-based feature selection combined with 10-fold cross-validation. Reduced datasets (with feature selection) were divided into training set (46 cases) and test set (16 cases). Multiple data mining modelling methods were used for models induction (random forest, classification and regression tree (CART), nave Bayes, logistic regression, support vector machine with linear kernel as well as artificial neural networks with one hidden layer). Finally, the performance of the models was assessed by comparing areas under the receiver operating characteristic curves (AUROC) in the test set.

Results: A group of 30 patients with pancreatic cancer and 30 controls was included in the analysis. Fold change-based feature identified 15 miRNAs in reduced dataset for model development, while p-value-based and correlation-based feature selection yielded in selection of 9 and 6 miRNAs, respectively. The validation on test sets of artificial neural networks (mean AUROC 0.95; 95%CI: 0.88-1.00) outperformed other models: random forest (0.90, 95%CI: 0.86-0.93); CART (0.85, 95%CI: 0.80-0.91); nave Bayes (0.89, 95%CI: 0.85-0.93); logistic regression (0.89, 95%CI: 0.86-0.92); and support vector machines (0.85, 95%CI: 0.78-0.91). This method of modelling showed also one of the best-achieved sensitivity and specificity across all reduced datasets in detecting pancreatic cancer (mean sensitivity 91% and specificity 88%).

Conclusions/Novel aspect: Our pilot study shows that development of an overfitting-resistant artificial neural network model for the diagnosis of pancreatic cancer based on the expression of selected circulating miRNAs is a promising concept requiring further extensive research.

[IV-6] DISCRIMINATION OF NORMAL ORAL MUCOSA FROM ORAL CANCER BY MASS SPECTROMETRY IMAGING OF PROTEINS AND LIPIDS

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Introduction: Identification of biomarkers for molecular classification of cancer and for differentiation between cancerous and normal epithelium remains a vital issue in the field of head and neck cancer management. Here we aimed to compare the ability of proteome and lipidome components to discriminate oral cancer from normal mucosa.

Methods: Tissue specimens including squamous cell cancer and normal epithelium were analyzed by MALDI mass spectrometry imaging (MALDI-MSI). Two molecular domains of tissue components were imaged in serial sections peptides (resulting from trypsin-processed proteins) and lipids (primarily zwitterionic phospholipids), then regions of interest corresponding to cancer and normal epithelium were compared.

Results: Heterogeneity of cancer regions was higher than the heterogeneity of normal epithelium, and the distribution of peptide components was more heterogeneous than the distribution of lipid components. Moreover, there were more peptide components than lipid components that showed significantly different abundance between cancer and normal epithelium (median of the Cohens effect was 0.49 and 0.31 in case of peptide and lipid components, respectively). Multicomponent cancer classifier was tested (vs. normal epithelium) using tissue specimens from three patients and then validated with a tissue specimen from the fourth patient. Peptide-based signature and lipid-based signature allowed cancer classification with a weighted accuracy of 0.85 and 0.69, respectively. Nevertheless, both classifiers had very high precision (0.98 and 0.94, respectively).

Conclusions: We concluded that although molecular differences between cancerous and normal mucosa were higher in the proteome domain than in the analyzed lipidome subdomain, imaging of lipidome components also enabled discrimination of oral cancer from normal epithelium. Therefore, both cancer proteome and lipidome are promising sources of biomarkers of oral malignancies.

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[IV-7] GENETIC VARIATION IN ANGIOGENIC GROWTH FACTORS AND THEIR RECEPTORS MAY AFFECT THERAPY RESULTS IN PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CANCER (HNSCC)

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Anticancer therapies increasingly take into consideration genetic profile of the patient. Research on new therapeutic strategies has led to development of individual treatment based, among others, on status of growth factors (GFs) and their corresponding receptors (GFRs). GFs and GFRs are proteins regulating many cellular processes, e.g. proliferation, differentiation, survival, migration, apoptosis and angiogenesis. Change in their activity, resulting from mutations or polymorphism, may lead to cancer transformation, acquisition of metastatic potential or therapy resistance. Angiogenesis has a pivotal role in tumor growth, progression, and response to treatment such as radiotherapy (RT) and chemotherapy (CT). Hypoxia and poor vascularization hinder the transport and action of anticancer drugs and decrease radiosensitivity, which leads to elevated risk of progression. Besides VEGF, other GFs, such as fibroblast growth factor FGF2 (bFGF) and platelet-derived growth factor PDGF, are potent angiogenesis stimulators. FGF2/FGFR2 axis is a signaling system for proliferation of endothelial cells. PDGF and its receptors (PDGF and PDGF), are critical in the recruitment and proliferation of vascular smooth muscle cells and pericytes. Functional polymorphisms in angiogenesis-related genes, e.g. GFs and GFRs genes, by modulation of individual angiogenic and metastatic potential, may affect tumor aggressiveness, treatment results and prognosis in many solid tumors, including head and neck cancer (HNC).

In this study, we analyzed 9 polymorphisms in 5 genes coding for pro-angiogenic GFs, *PDGFB* and *FGF2*, and their receptors, *PDGFRA*, *PDGFRB* and *FGFR2*, that have not been studied before in HNC. Our aim was to assess whether these variants were associated with local, nodal and distant recurrence, overall (OS) and disease-free (DFS) survival in 519 patients with squamous cell HNC (HNSCC) treated with RT alone or in combination with cisplatin-based CT. Variants were identified using TaqMan probes. Kaplan-Meier method and Cox proportional hazards regression were used for data analysis.

The *PDGFRB* 5UTR A allele was a significant and independent predictor of better OS and DFS in the whole group. The effect of studied polymorphisms on loco-regional and distant failure was observed only in patients treated with RT combined with CT. Namely, the *PDGFRA* 824 T carriers were at higher risk of loco-regional recurrence, especially due to local failure. The *FGFR2* T variant was associated with lower risk of nodal and distant failure, while th *PDGFB* intron 6 C carriers showed favorable MFS.

Our data suggest that pattern of treatment failure in the combination therapy subgroup may be dependent upon specific *PDGFRA*, *PDGFB* and *FGFR2* gene variants. These results show that polymorphism in PDGF/PDGFR and FGF/FGFR pathways contributes to clinical outcome of HNSCC patients treated with standard therapies.

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[IV-8] NUCLEUS-DIRECTED MASPIN AS A TUMOUR SUPPRESSOR IN BREAST CANCER

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Maspin (Mammary Serine Protease Inhibitor) is a noninhibitory serpin and it is classified as a tumour suppressor protein. Maspin expression is downregulated in malignant breast cancer and in most breast cancer cell lines. The function of maspin is not fully understood so far and reports on biological role of maspin, both in normal cells and cancer cells seem to be inconsistent. It is also currently under debate what may be a prognostic value of maspin location as a proliferation or invasion marker. We demonstrated the strong positive correlation between high nuclear maspin level and the survival of patients. We have found that in breast cancer cell lines, only nuclear fraction of maspin is responsible for antiproliferative effect. However, some of the studies have shown contradictory reports about the role of maspin in cancer cells. We imply that these discrepancies may depend on the way of delivery of maspin cDNA. We analysed the effect of the cDNA encoding nuclear maspin delivered by various systems on breast cancer cells proliferation and migration. The transfection with expression plasmids, viral plasmids and the transduction with adenoviruses encoding nucleus-directed maspin caused the inhibition of proliferation of breast cancer cells in contrast to cytoplasmic maspin. We observed significant negative correlation between nuclear maspin and the presence of proliferation marker Ki-67. However, transduction with lentiviruses also encoding nuclear maspin did not result in the inhibition of neither proliferation nor migration. Moreover, we have shown that nuclear maspin interacts with HDAC1 in breast cancer cells, so probably maspin affects cells proliferation by inhibition of HDAC1 activity.

Nucleus-directed maspin has an inhibitory effect on breast cancer cells proliferation and its nuclear location may be considered as a prognostic marker. The effect of exogenous maspin on breast cancer cells depends on the carrier used for delivering the genetic drug. Moreover, the use of adenoviral vectors for delivering sequence coding for maspin as a potent anticancer genetic drug may be potentially applied in breast cancer gene therapy.

[IV-9] CIRCULATING MICRORNAS AS BIOMARKERS OF RADIATION EXPOSURE – A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: MicroRNAs (miRNAs) were hypothesized to be robust and easy to measure biomarkers of radiation exposure which led to multiple studies in various clinical and experimental scenarios. We aimed at the identification of evolutionary conserved, radiation-induced circulating miRNAs through a multi-species, integrative systematic review and meta-analysis of miRNAs in radiation.

Methods: The systematic review and meta-analysis was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and was registered in the PROSPERO database (ID: 81701). Three databases were searched: MEDLINE, Embase and Cochrane Database of Systematic Reviews from its origins until December 5th 2017. We downloaded a list of studies with the query: (circulating OR plasma OR serum) AND (miRNA or microRNA) AND (radiat* OR radiotherapy OR irradiati*). Inclusion criteria were: experimental study; human, mice, rat or non-human primates study; serum or plasma miRNA expression measured before and after radiation exposure. Given the complexity of research designs of the full-text analysis we decided to split each article into single comparison (record) for which we could write down data considering number of exposed and unexposed participants, p value, variation measure (standard deviation, standard error or 95% confidence interval), total and fraction dose, time of radiation exposure and time elapsed between the end of radiation exposure and sample collection.

Results: We identified 467 studies - 103 from MEDLINE, 364 from EMBASE and 0 from Cochrane Database of Systematic Reviews. After deleting 116 duplicates, remaining 351 abstracts were then reviewed for eligibility criteria. Eventually, 30 articles were included in which we found 131 circulating miRNAs that significantly changed their expression after irradiation in 96 records (experimental settings) giving 2508 fold changes of circulating miRNAs. Median number of records per miRNA equalled 23 (interquartile range 17.5-25). Meta-analysis showed 28 circulating miRNAs to have significant, radiation-induced changes of expression. In meta-regression analysis 7 miRNAs miR-150 (FC=0.40, 95%CI:0.35-0.45), miR-29a (FC=0.87, 95%CI:0.79-0.96), miR-29b (FC=0.85, 95%CI:0.76-0.96), miR-30c (FC=1.19, 95%CI:1.09-1.30), miR-200b (FC=1.34, 95%CI:1.21-1.48), miR-320a (FC=1.13, 95%CI:1.05-1.23) and miR-30a (FC=1.18, 95%CI:1.07-1.30) - significantly correlated with either total or fraction dose of radiation. Additionally, miR-150, miR-320a, miR-200b and miR-30c correlated significantly with time elapsed since irradiation.

Conclusions: Circulating miRNAs reflect the impact of ionizing radiation irrespectively of the studied species. This makes the expression of extracellular miRNAs in the serum a promising class of biomarkers of radiation exposure and/or biodosimetry.

[IV-10] DYNAMIC MONITORING OF SERUM CIRCULATING MICRORNAS AS A BIOMARKER OF XEROSTOMIA IN OROPHARYNGEAL CANCER PATIENTS UNDERGOING RADIOTHERAPY

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Introduction: Severe xerostomia is noted in up to 75% of patients irradiated for head and neck cancer. Parotid-sparing radiotherapy (RT) reduces the incidence of xerostomia but may compromise the outcome of treatment. That is why, careful selection of patients whose normal tissues are particularly sensitive to radiation remains a key challenge. To tackle this problem we evaluated the potential of miRNAs expression changes as a biomarker of xerostomia in oropharyngeal cancer (OPC) patients undergoing RT.

Aim: The aim of this study was to analyze temporal changes in expression levels of miRNAs circulating in the serum and to create an efficient test for early xerostomia in patients treated for OPC.

Methods: Our study was designed as a prospective cohort study which enrolled OPC patients treated with IMRT (total dose of 70Gy or equivalent) from June 2016. We randomly selected a group of 10 patients with grade3 xerostomia and matched a comparative group of 10 patients without severe xerostomia. We collected serum samples before RT, after receiving 20Gy and within 24 hours after 70Gy. qPCR arrays (miRCURY LNA, Human panels I II, Exiqon, Copenhagen, Denmark) were used to quantify miRNA expression levels. Data were normalized toward the average expression of microRNAs detectable in all samples. Acute side effects were prospectively assessed using EORTC QLQ-C30 and EORTC HN-35 questionnaires. MiRNAs were shortlisted on the basis of univariate, Benjamini-Hochberg-adjusted, p values. The classifier for xerostomia was created using a stepwise, 5-fold cross-validated logistic regression model.

Results: One hundred thirty-eight miRNAs were detected in more than half of the samples and used in the analysis. We observed that after receiving 20Gy, 14 miRNAs differed significantly between the compared groups and 3 of them (miR-425-5p, miR-18b-5p and miR-345-5p) maintained their differences after the final RT fraction. Interestingly, all three miRNAs presented varying temporal patterns depending on the presence of xerostomia. MiR-425-5p increased throughout RT in patients with severe xerostomia and decreased in those without it. MiR-345-5p showed no changes in the severe xerostomia group while a decline of expression levels was present in the remainder. MiR-18b-5p showed opposite changes in expression between 20Gy and 70Gy timepoints (p for interaction 0.007). The logistic regression model based on miR-425-5p and miR-345-5p expressions (after 20Gy), showed nearly perfect separation of the groups with an AUC 0.95 (95%CI: 0.911.00) and maintained its performance in 5-fold cross validation (AUC=0.88, 95%CI: 0.840.93).

Conclusions: Dynamic monitoring of circulating miRNAs may serve as a non-invasive early biomarker of severe xerostomia. Changes in the expression levels of selected miRNAs can be already detected after 2 weeks of treatment which means that they may reflect the tissues early response to ionizing radiation allowing for potential RT adaptation.

[IV-11] EVALUATION OF USEFULNESS OF THE MINI-SEC METHOD FOR PURIFICATION OF EXOSOMES FOR MASS SPECTROMETRY PROTEOMIC STUDIES

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Introduction: Biological properties of exosomes in the context of cancer development and progression are the subject of numerous scientific studies. Exosomes can be isolated from various types of biological material, e.g. blood and its derivatives, urine, saliva, cerebrospinal fluid, as well as from a culture medium for different cell lines. An important issue in conducting research on exosomes is their isolation from a research material. Techniques of exosome isolation and purification are the basis for a good sample preparation for mass spectrometry analyses. Mini-SEC technique separates a solution components in terms of their mass. Therefore, exosomes get purified from proteins derived from the material they are isolated from.

Methods: We used four isolation variants and two types of research material: (i) healthy donor serum and (ii) medium from a cell culture (FaDu cell line). In addition, as a negative control, commercial exosome-free serum was used. The prepared material (serum or concentrated medium) was loaded onto columns and fractionated in terms of size from high to low mass component. The presence of exosomes was evaluated using transmission electron microscopy (TEM) and Western blot. For all fractions, MS analysis was performed for each of the conducted isolations.

Results: In the fractionated mini-SEC preparations we detected the presence of exosomes using frequently used exosome markers. However, we did not detect proteins that constitute their content (e.g. GAPDH, actin) in fractions containing exosomes. We determined that some proteins present in databases (e.g. ExoCarta) as of exosomal origin are in fact derived from the material the exosomes are isolated from.

Summary: The use of mini-SEC technique removed contamination with high-abundant proteins present in a sample (serum, plasma or cell culture medium) and also increased the number of exosomal protein identifications in a sample.

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[IV-12] SIGNIFICANCE OF HPV16 VIRAL LOAD TESTING IN ANAL CANCER

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Introduction: Anal carcinoma represents one of the highest frequency of HPV-dependent cases (~90%). In our previous study we assessed relationship between expression of some viral as well as cellular proteins and presence of HPV. Now we want to extend our analysis to examination of HPV16 copy number; we choose this HPV subtype since it was the most frequently found in anal cancer.

Methods: Expression of proteins p16INK4A, p53 and ER was tested by immunohistochemistry. HPV16 copy number was analyzed by qPCR. Analysis was performed on FFPE cancer samples. Analyzed group consisted of 30 cases of anal squamous cancer, 16 rectal adenocarcinomas and 4 benign lesions.

Results: There were 70% of HPV16-positive cases in the whole group, but in squamous anal cancer this frequency was markedly higher (96%). In rectal adenocarcinomas and benign lesions only single HPV16-positive cases were found, therefore our study was confined to anal cancer.

Analysis of relationship between HPV16 and patients characteristic showed marked differences between sexes. There was preponderance of women in HPV16-positive group (82%), whereas in HPV16-negative group the frequency of men/women was similar. Women had also higher HPV16 viral load as compared to men (307 vs. 223 copies/genome). When smoking was considered, it was found that nonsmokers were more frequently found in HPV16-negative group, on the opposite, smokers prevailed in HPV16- negative group. Nonsmokers had also markedly higher HPV16 viral load (1080 vs. 159 copies/genome). When these two data (smoking, sex) were combined it was found that majority of HPV16-positive women were also nonsmokers (62,5%). Nonsmoking women had also the highest mean copy number among all groups. As this observation was sex-dependent we asked if there was a correlation with expression of estrogen receptor (ER). However, ER positivity did not explain these differences since proportion of HPV16-positive cases was similar in both ER groups.

In majority of HPV16-positive anal cancer cases positive p16INK4A immunoreaction (86%) was found. When HPV16 viral load was analyzed it was found that p16INK4A-positive cases had 4.5 times higher mean HPV16 copy number than p16INK4A-negative ones.

Immunohistochemically assessed p53 expression was not connected with number of HPV16-positive/negative cases. In both groups defined by p53 positivity the frequency of HPV16-positive cases was similar. But when the number of HPV16 copies was analyzed, mean HPV16 viral load was doubled in cases with normal p53 expression as compared to cases with abnormal p53 (380 vs. 207 copies/genome).

Conclusions: Our study shows significance of HPV16 viral load measurement, apart from simple HPV detection. For the first time we showed HPV16 high copy number in nonsmoking women considering this as a phenomenon worth further studies.

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[IV-13] INFLUENCE OF POLYMORPHIFIC VARIANTS ON THE COURSE AND EFFECTIVENESS OF CHEMOTHERAPY OF BREAST CANCER

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The systems of detoxification, transport and metabolism of chemotherapeutics are responsible for the clinical course and effectiveness of breast cancer therapy. Single nucleotide polymorphisms (SNPs) detected in the 3UTR regulatory sequences of ADME *(absorption, distribution, metabolism, and excretion)* genes can influence on the expression of gene and consequently affect the efficiency of the used medicaments. The aim of the study was to analyze a possible impact of single nucleotide polymorphisms in 3UTR of ADME genes on 5-fluorouracil, doxorubicin and cyclophosphamide chemotherapy (FAC chemotherapy) in breast cancer patients.

We analyzed SNPs in 324 women with breast cancer. All patients were treated with FAC first-line chemotherapy. 33 SNPs in 3UTR of 23 ADME genes were evaluated on DNA obtained from peripheral blood. Subjects were genotyped using polymerase chain reaction-restriction fragment length polymorphism. Adverse side effects of treatment or toxicity of chemotherapy were determined on the basic parameters of blood tests and medical history carried out before each cycle of FAC chemotherapy. Response to treatment was based on 12 symptoms of adverse side effects and toxicity including hematological symptoms, hepatotoxicity, nephrotoxicity and gastrointestinal toxicity.

In multifactorial analyses rare CC polymorphic variant in *ABCC1* gene (rs129081) and frequent CC genotype of *DPYD* gene (rs291593) were responsible for elevated risk of leucopenia [OR 1.81; p=0.013]. Heterozygote variant AG of *AKR1C3* gene (rs3209896) and rare genotype GG of *DPYD* gene (rs291583) correlated with leukopenia in first two cycles of chemotherapy [OR 2.45; p=0.0273]. In postmenopausal women simultaneous occurrence of frequent homozygote AA of *NR1/2* gene (rs3732359) and metastases (M+) increased the risk of hepatotoxicity [OR 14.22; p=0.015]. The risk of early-onset hepatotoxicity were observed in patients with homozygote AA in *AKR1C3* gene (rs32098968) with metastases [OR 5.88; p=0.016]. We observed increased risk of nephrotoxicity in postmenopausal woman with overexpression of estrogen receptor and heterozygote variants of *DPYD* (rs291593) and *AKR1C3* (rs3209896) genes [OR 10.58; 0.010].

SNPs in 3UTR that occur commonly in population can determine the different treatment response in women with breast cancer. Identification of SNPs in regulatory sites of ADME genes will help explain inter-individual response to chemotherapy. Our study reveals that polymorphisms in genes identify a subset of patients with a potentially high risk of hematological and hepatotoxic symptoms induced during breast cancer treatment.

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[IV-14] BIOMARKERS OF BREAST CANCER SUBTYPES IDENTIFIED FROM RNA-SEQ DATA

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Introduction: Breast cancer is one of the most frequently diagnosed cancer types in women as well as a second leading cause of their cancer death. Commonly used biomarkers such as estrogen and progesterone receptors, HER2, and Ki-67 protein serve as a base for cancer classification into subtypes, crucial for therapy choice and planning. The purpose of this study is to compare five breast cancer subtypes (Luminal A, Luminal B, HER2 enriched, Basal-, and Normal-like) in terms of RNA expression. Basal-like subtype, corresponding to most Triple Negative Breast Cancer (TNBC) cases, is currently a widespread research interest due to its poor clinical outcome. The aim is to identify markers characteristic for every subtype as well as the biological processes they are involved in.

Methods: RNA-Seq results for primary tumours of women suffering from breast cancer were the material for this study. Data, a part of the TCGA-BRCA project, were collected from the GDC Data Portal as HTSeq-counts. 478 patients with known cancer subtype and all stages apart from IV were selected for the study. Subtype etiquettes were obtained based on the PAM50 classifier proposed by Parker et al. [2009]. Data were normalized with DESeq2 R package. The list of markers, for which expression is significantly different in at least one subtype compared to others, was obtained with the Kruskal-Wallis test followed by the Benjamini-Hochberg multiple testing correction. Games-Howell test was performed to verify if there are differences between every combination of two cancer subtypes. Characteristic markers defined as transcripts with the expression significantly higher or lower in only one subtype were identified. Functional analysis of resulting gene set for each subtype was performed with the Reactome Database.

Results: Over 50% of all transcripts were proven not to have equal expression levels in all subtypes. The biggest set of characteristic markers corresponded to Basal-like subtype with 525 over- and 203 underexpressed markers, while for other subtypes there were approximately 34 markers with higher and 51 markers with lower expression. Genes known for their role in the breast cancer were selected as characteristic ones, especially for Basal-like (e.g. FOXCUT, EN1, AGR2, RAB27B, and HORMAD1) and HER2 enriched subtypes (GRB7, TBX10). Selected Reactome pathways included FGFR4 mutant receptor activation, GRB7 events in ERBB2 signaling, and various pathways involved into basic cellular processes like translation.

Conclusions: The performed study provided the list of genes and processes characteristic for breast cancer subtypes, being a base for future analysis of TCGA-BRCA breast tumours samples as well as for the research of potential therapy targets. The obtained results indicate that Basal-like subtype differs distinctly from other subtypes.

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[IV-15] SEARCH FOR PREDICTIVE BIOMARKERS OF SENSITIVITY/RESISTANCE AGAINST NOVEL FGFR INHIBITOR USING ARRAY-BASED COMPREATIVE GENOMIC HIBRIDIZATION (aCGH AND RNA SEQUENCING (RNAseq) – PRELIMINARY RESULTS

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Background: Our project concerns characterization of CPL-304-110, a novel selective inhibitor of Fibroblast growth factor receptor (FGFR), recently developed by Polish pharmaceutical company Celon Pharma. We analyze sensitivity of the cells derived from different cancers toward CPL-304-110 and search for signaling pathways differentially regulated in sensitive versus resistant cell lines. In this preliminary study we assessed which signaling pathways may be affected by genome rearrangements occurring in lung cancer cell line resistant to CPL-304-110, using the method of array-based comparative genomic hybridization (a-CGH). We also performed RNA sequencing to assess gene expression changes in sensitive versus resistant cell lines.
Methods: DNA was isolated (GeneJet Genomic DNA Purification Kit, Thermo Scientific) from wild type H1703 lung cancer cell line which is sensitive to CPL-304-110 inhibitor (H1703_wt) and its variant (H1703_R) which is resistant to 2,5μM concentration of inhibitor. Array comparative genomic hybridization (aCGH) was performed using Agilient Technologies SurePrint G3 Unrestricted CGH (8x60K) arrays. Obtained dataset was analyzed using gene set analysis (GSA) for identification of affected signaling pathways, using BioCarta, KEGG and Reactome data bases.
RNA was isolated (RNeasy Plus Mini kit, Qiagen) from H1703_R and H1703_wt cells. Cells were harvested for RNA isolation 24 h after culture medium change. RNAseq was performed using HiSeq4000 (Illumina).
Results: We compared both variants of H1703 cell line according to the copy number variation of their genomes. The results of aCGH indicate that genomic regions affected by copy numer changes (amplifications and deletions) contribute to signaling pathways involved in proliferation, survival, apoptosis, differentiation, cell cycle, cellular motility and metabolism. Sequencing of cellular transcriptome (RNAseq) revealed that resistant cell line H1703-R shows significantly changed expression of many genes engaged in signaling pathways related with structure and function of extracellular matrix. Interestingly, among affected pathways were also those related with FGFR signaling, ERBB and GRB2 signaling, regulation of PTEN gene transcription.
Conclusions: This preliminary study allowed to preselect genome rearrangements and gene expression changes potentially engaged in development of resistance in H1703 lung cancer cells toward an FGFR inhibitor CPL-304-110. Further studies are in progress that will enable to find molecular markers for selection of patients that will respond to therapy with this novel FGFR inhibitor.

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Keywords: FGFR inhibitor, aCGH, RNAseq, lung cancer cell line

[IV-16] DESIGN OF FLUORESCENT PROBES TARGETING ACTIVE ERBB RECEPTORS

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Introduction: Imaging of biological events helps to understand a intracellular phenomena at the molecular level. A visual projection of intracellular protein behavior may be delivered after administration of specific small-molecule fluorescent tracer. Design of fluorescent probes entangles an intrinsic challenge how to reconcile aspects of specificity and adequate Ex/Em characteristics. Conserved kinase domains as well as extracellular compartment of ErbB receptor kinases family, made design even more complicated. Here, we discuss the effect of our cumulative work that led us to create a protype of luminescent probe, which binds EGFRvIII RTK and other ErbB receptors.

Methods: Based on the EGFRvIII structural model and our previous broad studies of this truncated receptor, we designed and synthetized probe against EGFRvIII and other active ErbB receptors. Its activity and specificity was analysed based on cell stainig (cell lines: DKMGhigh EGFRvIII high level and DKMGlow EGFRvIII low level) as well as Western blot analysis.

Results: Our molecule was proved to be stable, active in cell culture conditions and well soluble. Incubation of cell with this probe showed higher affinity towards EGFRvIII, however longer incubation time led to the lost of fluorescence. This lost was due to incresed receptor degradation and cell death, what was observed on Western blot.

Conclusions/Novel aspect: To conclude, we present a protype of luminescent probes, which display binding ability toward EGFRvIII RTK and other ErbB active receptors.

[IV-17] PROGNOSTIC VALUE OF ERYTHROPOIETIN AND HEMATOLOGICAL PARAMETERS IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Introduction: In patients with lung cancer anemia is a common symptom and usually consists of two components: treatment related anemia and anemia of chronic disease. The disparity between production and elimination of erythrocytes is a direct reason of anemia which is characterized by low levels of circulating red blood cells and hemoglobin, reduced number of reticulocytes, inadequate secretion of erythropoietin (Epo) and disturbances in iron metabolism. Although the thorough mechanisms of anemia of chronic disease is not fully understood it causes tissue hypoxia that increases resistance to radiotherapy.

The aim of this study was to assess the clinical utility parameters of the erythrocytic system: reticulocytes (Ret), immature reticulocyte fraction (IRF), reticulocyte hemoglobin content (Ret-He), and the concentration of Epo as a marker of tumor hypoxia.

Material and Methods: The study was performed at the Center of Oncology. Hundred twenty nine patients qualified to radiotherapy alone (42) or combined with chemotherapy (75) or chemotherapy alone (12) due to non-small cell lung cancer were involved into the study. Clinical stages (cTNM) were as follows: stage IA 3%, IB 3%, IIA 3%, IIB 4%, IIIA 37%, IIIB 35% and IV 15%. The median age was 64 years (range: 41 to 70 years). Epo and parameters of the red blood cell system were estimated in serum or blood before treatment completion.

Results: Strong negative correlation has been found between initial anemia (Hb12 g/ml) and Epo (p=.0001), Ret (.003), IRF (p=.001), Ret-He (.001). Significantly longer overall survival (OS) was found for patients with lower Epo (p=.01) and IFR (p=.001).

Conclusions: In patients with non-small cell lung cancer, anemia is a chronic disease which stimulates erythropoiesis. Ret, IFR and Ret-He may indicate increased and ineffective erythropoiesis. Initial high Epo levels and high IFR correlate with increased risk of death.

Session V:
Varia

[V-1] CHANGES IN PROTEIN COMPONENTS OF LAMIN COMPLEXES AND ITS ASSOCIATION WITH CHROMATIN IN DROSOPHILA MELANOGASTER IN NORMAL CONDITIONS AND AFTER STRESS INDUCTION

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Lamins (V type intermediate proteins) provide structural functions in the cell nucleus. They are also responsible for regulation of transcription, organization of chromatin, epigenetic modifications and DNA replication. Mutation in this protein can result in many disorders such as laminophaties. We distinguished two types of laminsA- and B-type. A-type are displayed during later stages of embryonic development, while B- type are typically expressed in every cell. It is thought that lamins interact with many proteins such as topoisomerase II, histone modifying enzymes, LEM domain proteins and many others.

In this particular study we focused on identification of protein components in complexes with lamins under normal conditions and after stress induction on a model organism, *Drosophila melanogaster.* It was chosen for this study due to its easiness of maintenance, short life cycle and more importantly, the presence of only a single isoform of heat shock factor (HSF), single lamin Dm (B-type) and single lamin C (A/C- type).

Immunoprecipitation of lamin Dm on Sepharose beads was performed together with mass spectrometry analysis (LC-MS/MS). Furthermore, changes associated with chromatin of lamin Dm and potentially interacting proteins were investigated under normal (23C) conditions and after heat shock induction (37C). It was achieved by checking the levels of soluble fractions of protein by differential salt fractionation of fruit fly embryos extracts.

We compared protein associated lamins under normal conditions and after stress induction and showed that there is a difference in protein pattern after stress. Moreover, we showed that there is a significant difference in association with chromatin of lamin Dm before and after heat shock response.

This finding allows us to hypothesize that there might be changes in phosporylation of lamin Dm after heat shock induction or there might be some co-localization changes after heat shock. This aspect has to be investigated more thoroughly along with identification of protein complexes with lamin Dm using denaturating immunoprecipitation (with cross-linking).

[V-2] ESTABLISHMENT AND CHARACTERIZATION OF THE NOVEL HIGH-GRADE SEROUS OVARIAN CANCER CELL LINE OVPA8

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High-grade serous ovarian cancer (HGSOC) is the most frequent and most deadly histological type of ovarian cancer. Unfortunately, majority of established ovarian cancer cell lines used in the research, including most frequently cited SKOV3 and A2780 lines, are poorly characterized and probably do not represent HGSOC. We have recently established new cell line, OVPA8, which is derived from histologically-confirmed HGSOC. Here we present detailed characteristics of this cell line.

STR (short tandem repeats) profile was generated using AmpFLSTR NGM PCR Kit. Karyotype analysis was performed using classical G-banding technique. *TP53* mutations were analyzed by Sanger sequencing. Genotyping for founder *BRCA1* mutations was done with allele-specific PCR. Automated library construction was done with Oncomine BRCA Research Assay and libraries were used for automated NGS template preparation on Ion Chef Instrument and sequencing with Ion Torrent Personal Genome Machine. Genetic profile of OVPA8 was analyzed using the Ion AmpliSeqTMCancer HotSpot Panel v2. Immunophenotypic characterization was done using antibodies against markers: WT1, PAX8, HBME-1, calretinin, CD68, CD44, CK19 and EpCAM. Functional analysis included migration, invasion and clonogenic assays. Cytotoxicity assay was performed for cisplatin, paclitaxel and fibroblast growth factor receptor (FGFR) inhibitors: CPL304-110-01 (CelonPharma) and AZD454 (AstraZeneca). All analyses were carried out in comparison to five commercially available ovarian cancer cell lines: SKOV3, OVCAR3, OAW42, ES2 and A2780.

STR profiling showed unique and stable identity of the OVPA8 cell line. Cytogenetic analysis showed chromosome count between 57 and 67, with multiple structural aberrations. Analysis of *TP53* gene showed homozygous mutation c.733GA (p.Gly245Ser) described as pathological. ASO-PCR test was negative according to three major Polish founder mutations in *BRCA1* gene (4153delA, 5382insC, C61G), but NGS analysis resulted in detection of *BRCA1* pathological mutation c.3700_3704delGTAAA (p.Val1234Glnf) and indicated loss of heterozygosity in *BRCA2* gene. Analysis of NGS HotSpot panel revealed molecular profile typical for HGSOC. OVPA8 cells were positive for EpCAM, CK19, and CD44. The cells had relatively low plating efficiency/ability to form spheroids, a low migration rate, and intermediate invasiveness in matrigel, as compared to other ovarian cancer lines. OVPA8 cells were sensitive to paclitaxel, weakly sensitive to CPL304-110-01 (CelonPharma) and resistant to cisplatin and AZD454.

The results of our detailed *in vitro* and molecular analyses confirmed that the OVPA8 cells have typical features of HGSOC. We believe that OVPA8 cell line will become a valuable preclinical model for studies on high-grade serous ovarian cancer.

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[V-3] ALTERNATIVE INJECTABLE BONE CEMENT FORMULATIONS BASED ON ISOSORBIDE FOR USE IN VERTEBROPLASTY

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Vertebral compression fractures are the most common injuries among the elderly due to osteoporosis. Although some vertebral compression fractures can be asymptomatic, most patients suffer substantial pain, which can become chronic. Percutaneous vertebroplasty is a minimally invasive procedure efficient in the treatment of painful vertebral fractures. Liquid bone cement is injected into an osteoporotic fractured vertebra under continuous fluoroscopic guidance, and hardens through free radical polymerization in several minutes after application. Poly(methyl methacrylate) (PMMA) bone cements are widely used in clinical practice for vertebral augmentation. The mechanical properties, especially Youngs modulus of the cured material, are far in excess of the osteoporotic trabecular bone, leading to possible fractures caused by overload of adjacent vertebrae [1].

The aim of presented work was to prepare alternative bone cement formulations useful in vertebral compression fractures treatment. The mixture of two novel resins based on isosorbide: oligo(isosorbide maleate) (OMIS) and 2,5-bis(2-hydroxy-3-methacryloyl-oxypropoxy)-1,4:3,6-dianhydro-sorbitol with methyl methacrylate was proposed as a polymer matrix of *semi*-biodegradable, injectable bone cements. OMIS as a hydrolyzable polyester resin in combination with one of bioactive fillers such as nanosized hydroxyapatite (nHA) or microsized calcium carbonate (mCC) can facilitate bone ingrowth and bonding. It is believed that bone could grow into the cement in the space left by the degraded material resulting in improved osseointegration [2]. In this study selected properties of tested materials were compared with the results obtained for commercial BIOMET Bone Cement V. The compressive modulus (Ec) of tested cured formulations was very high in comparison to commercial bone cement, but after water uptake a significant drop of Ec values was observed for all materials as they were more close to modulus of trabecular bone. The morphology of cured materials, before and after hydrolytic degradation, the release profile of bactericidal agent - ofloxacin (OFX) and its activity against *Staphylococcus aureus* and *Escherichia coli* were examined.

The results of the presented study show that new cement formulations prepared from isosorbide-based resins could be an interesting alternative to the commercial bone cements in the treatment of vertebral compression fractures.

References:
[1] Heini P.F., Berlemann U., *Eur. Spine J.* 2001, **10**, S205.
[2] Boesel L.F., Mano J.F., Reis R.L., *J. Mater. Sci. Mater. Med.* 2004, **15**, 73.

[V-4] THE CHARACTERIZATION OF THE HYBRID MEMBRANES FILLED WITH IRON-ENCAPSULATED CARBON NANOTUBES (FE@MWCNTS) AND ZEOLITE 4A AND THEIR POTENTIAL USAGE IN CO_2 SEPARATION

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The main problems with anthropogenic climate change and global warming are caused nowadays by a drastic increase of atmospheric level of various greenhouse gases, like, for instance, CO2. These issues can be resolved to a great extent by controlling CO2 emissions by its separation from post-combustion flue gases or biogas, thus protecting the environment. One of the most promising methods of CO2 capture is the gas separation using inorganic-organic hybrid membranes. The key parameters of a gas separation membrane (high permeability, selectivity and resistance) could be improved by chemical and/or physical modification or incorporation of inorganic fillers (zeolites, CNTs, silica, MOFs, CMS, etc.) into the polymer matrix. To improve their dispersion in the polymer matrix one could use few methods like functionalization/modification of filler particles and/or polymer matrix and application of electric or magnetic field.

This work concerns the study of the inorganic-organic hybrid membranes based on few modified polymer matrices and various additions of Fe@MWCNTs and zeolite 4A as fillers. The membranes were prepared from the fillers dispersion in the polymer solution, using the casting method without or with applied magnetic field for the Fe@MWCNTs arrangement. The permeation of CO2 and N2 through hybrid membranes with different filler contents have been investigated. The membranes were also characterized by X-ray diffraction, static mechanical method and vibrating sample magnetometry.

It was found that incorporation of zeolite 4A and Fe@MWCNTs into the polymer matrix and application of magnetic casting had significantly changed the gas transport parameters (D, P, S and). It was demonstrated that application of magnetic field during the Fe@MWCNT/PPO membrane preparation has enhanced the alignment of Fe@MWCNTs in the polymer matrix and improved their separation performance. In turn, the sulfonation of PEEK matrix enhanced the zeolite-polymer interphase via hydrogen bonding and allowed to obtain membranes with higher permeability and permselectivity. Mechanical properties (Rm and E), as well as magnetic membranes parameters were improved by the increase of filler content and the presence of magnetic field. The authors also propose a modern computer application *MOT* 2.0 for modelling of gas transport processes through hybrid membranes, based on the next-generation LewisNielsen model.

[V-5] "CLICK" REACTIONS IN SYNTHESIS OF AMPHIPHILIC COPOLYMERS AS SYSTEMS FOR DELIVERING ARBUTIN

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Click chemistry is often used in drug delivery systems (DDS) due to high chemical yields, possibility to obtain no toxic and physiologically stable products at mild conditions. Many examples of click procedures have been reported for synthesis and functionalization of nanosized DDS, including nanoparticles, polymeric micelles, liposomes and capsules. 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) and then the resulted HEMA-Al/MMA copolymer was clicked with monoazide-functionalized poly(ethylene glycol) (PEG-N3). The obtained graft copolymers as the self-assembling systems were tested for carriers of active substances used in cosmetology, conducting studies on their micellization, encapsulation and release process of arbutin (ARB) as model compound.

The new HEMA-Al monomer was obtained by the esterification reaction of the standard HEMA monomer with hexynoic acid. Terminal bromine of PEG-Br has been converted to the azide group via reaction with NaN3. HEMA-Al/MMA polymers were synthesized by atom transfer radical polymerization (ATRP) method and the click reaction with PEG-N3 was carried out in the presence of CuBr. Both the monomer as well as the modified and synthesized polymers were characterized at each step by 1H NMR in DMSO and also by additional methods, such as 13C NMR, ESI-MS and GPC. The encapsulation and release process was investigated using UV-Vis spectroscopy. The hydrodynamic diameters of micelles were determined by DLS method.

Successful modifications resulted in new monomer HEMA-Al (ESI-MS (m/z): calculated for C12H16O4 224.0; found for [M+Na]+ 247.0) and PEG-N3 (1H NMR: -C(CH3)2N3 at 1.4 ppm). The formation of triazole ring was identified as a result of the click reaction (1H NMR: C2HN3 at 8.0 ppm; 13C NMR: C2HN3 at 130 ppm, 140 ppm). The obtained copolymers showed the ability to encapsulate ARB at a satisfactory level (DLE = 49-64%). Additionally, the kinetics of ARB release was investigated in PBS, pH=7.4 at 36oC showing that ARB is released almost entirely up to 3h (depending on the grafting degree).

The studied copolymers, which were obtained by the click reaction between P(HEMA-Al*co*MMA) and PEG-N3, are potential candidates for micellar carriers with adjustable characteristics of active substance delivery in cosmetology. The planned studies on cytotoxicity and permeability through artificial skin let to verify the possibility of applying the proposed systems in cosmetic products like masks, which have a relatively short contact time with the skin.

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[V-6] REVEALING THE RNA M6A METHYLATION PATTERN – NOVEL MICLIP TECHNIQUE IN OUR HANDS.

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There are about 100 of different chemical modifications of nucleotides in ribonucleic acid. Among them methylation of RNA has been known since the 1970s The most prevalent one in polyadenylated mRNA and long non-coding RNA (lncRNA) is the methylation of the adenosine base at the nitrogen-6 position (m6A). This abundant modification across RNA types plays an important role in gene expression and splicing patterns. Moreover, it has been proven that there is a link between m6A modification and human diseases, especially numerous cancer types. Despite the fact that RNA methylation is a well-known phenomenon, there was a lack of suitable methods to analyze it until high-throughput techniques have been developed. However, the RNA-sequencing (RNA-Seq) carried out after enrichment utilizing immunoprecipitation of RNA fragments containing m6A modification allows for an approximate analysis of the methylation. In this classic approach, no data about exact methylation pattern can be obtained, but the percentage of methylated sites in analyzed RNA fragments only. CLIP (crosslinking immunoprecipitation) is a group of methods used in molecular biology that combines UV cross-linking with immunoprecipitation in order to analyze protein interactions with RNA. In the miCLIP variant, cross-link is made between N6-methyladenosine and anti-m6A antibody, that allows generating a pool of RNA fragments for RNA-Seq, where each fragment is terminated in exact m6A methylation site. Thus, the miCLIP allows revealing the RNA methylation sites precisely in contrast to RNA-Seq after m6A immunoprecipitation enrichment.

The aim of this study was to learn and introduce miCLIP technique into our laboratory. Samples of total RNA extracted from colorectal adenocarcinoma LoVo cells have been incubated with m6A antibody and subsequently UV cross-linked. Following miCLIP protocol, NGS libraries were obtained and, after quantification, sequenced using MiSeq System. Collected data were quality checked and analyzed using in-house bioinformatics pipeline.

In conclusion, the miCLIP method was successfully introduced into our laboratory and will be used extensively for RNA methylation analyses in future experiments.

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[V-7] INVESTIGATION ON ENZYMATIC DEGRADATION OF SEMI-DEGRADABLE PDMAEMA-B-POLYESTER: INFLUENCE OF COMPOSITION AND CONTENT

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Aliphatic polyesters, such as poly(-caprolactone) (PCL), polyglycolide (PGA), polylactide (PLA) and their copolymers, belong to a wide group of biodegradable and biocompatible polymers, which undergo both chemical and enzymatic degradation. The biodegradability of the polyesters depends on molecular weight, chemical composition, crystallinity, surface area. [1,2] Combination of polyesters with polymethacrylates impacts on the physicochemical properties extending the application directions of the obtained materials, for example in a biomedical field. Amphiphilic copolymers consisting of polyester and poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMAEMA) blocks are semi-hydrolysable, pH- and thermoresponsive. These properties can be applied to obtain triple-responsive drug delivery systems. [3]

In our research, we have focused on the enzymatic degradation of selected polyesters and polyester-*b*-polymethacrylates by Novozyme 435. The influence of the hydrophilic block of PDMAEMA on the degradation rate of PCL, PLA and poly(lactide-*co*-glycolide-*co*-(-caprolactone)) (PLGCL) in diblock copolymers was investigated. Additionally, the degradation of PCL combined with poly(methyl methacrylate) (PMMA) segment was examined, in order to confront the results obtained in the presence of the hydrophilic block (PDMAEMA) to the hydrophobic one (PMMA). Degradation rates were monitored by size exclusion chromatography (SEC), which gave the opportunity to observe the progress of the molecular weight loss and the remaining quantity of the polymer in the sample.

The results from degradation showed that the change of the content of polyesters with respect to polymethacrylate fraction affected the retention time of polymer samples in the solution, hence the decrease of molecular weights had nonlinear character. Degradation of copolymers containing biodegradable (PCL, PLA, PLGCL) and non-biodegradable (PMMA, PDMAEMA) blocks, occurred only to the point at which the enzyme reached the non-biodegradable block, where no decrease of molecular weight was observed. In addition, hydrophilic block decreased the rate of the degradation in comparison with single polyesters and hydrophobic PCL-*b*-PMMA.

References:

[1] I. Vroman, L. Tighzert, *Materials*, 2009, **2**, 307344

[2] H. S. Azevedo R. L. Reis, 2005, *Biodegradable Systems in Tissue Engineering and Regenerative Medicine*, CRC Press 177201

[3] J. R. Lopes, G. Santos, P. Barata, R. Oliveira, C. M. Lopes, *Current Pharmaceutical Design*, 2013, **19**, 71697184

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[V-8] TRAPPING AND REMOVAL OF BUBBLES IN MICROFLUIDIC DEVICES

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Microfluidic systems are the devices with very small fluid capacity that handle flow at micro- or nano- scale. They originated from microelectromechanical systems (MEMS) technology where were, among others, used in production of ink print heads. Now they are used in micro heat exchange, micro propulsion and lab-on-chip technologies. These applications indicate how many science branches utilize microfluidics e.g. fluid mechanics, heat transfer, chemistry and biology. The latter is particularly interested, because microflow is one of basic processes occurring in living organisms. Microfluidic systems can be very useful in cell culture as they are a compromise between in vivo and in vitro methods. In microfluidic cell culture systems, an environment of a living organism in which the cells grow is mimicked, while being easily accessible and reproducible compared to in vivo models. Flow of medium - resembling the human circulatory system - ensures constant cell nutrition and drainage of wastes, it also allows precise dosage of medicine into cell chambers. Advantages of microfluidic systems are reduction of reagent usage, waste production and energy consumption which result in lower costs. Furthermore, due to small size, many experiments can be carried out in parallel.

As part of a larger project involving the production of microfluidic system for cell culture, purpose of this work is to design and develop gas bubble removal device. Presence of bubbles in medium flowing through a microchannel is a significant problem, because it may cause shear force variations, disrupt flow distribution or even clog the channels. In the case of contact of a bubble with cells, cells may be detached or their membrane ruptured. There are commercial solutions to this problem, using porous membranes but their disadvantage is the relatively high price. In the literature, there are several designs of bubble traps using buoyancy force or surface tension and debubblers employing diffusion, vacuum or porous membranes. This work presents the idea of bubble removing device made of PDMS. Since PDMS is gas permeable, the principle of the devices operation is based on diffusion. Main microchannel with medium containing bubbles is surrounded by spiral vacuum channel. The design is simple, the production of the device is relatively easy and requires no special equipment.

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[V-9] LIPOPHILICITY STUDY AND DETERMINATION OF PROTEIN BINDING OF ACITRETIN

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Acitretin, chemically known as (all-*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid, is an retinoid used in the treatment of psoriasis [1]. Literature review indicates that various physicochemical properties including lipophilicity can be successfully applied to plasma protein binding of drugs. Therefore, the aim of this work was to determine and estimate the utility of experimental technique like TLC in reversed phase system (RP-TLC and RP-HPTLC) as well different software packages to study lipophilicity and protein binding of acitretin [2,3]. In order to estimate lipophilic character of the studied compound that has not been well described till now by means of experimental partition coefficient, namely log *P*, various chromatographic systems with methanol-water as mobile phase and different chromatographic plates (RP-18F254, RP-18WF254 and RP-2F254) were applied. The obtained results for chromatographic lipophilicity parameter (RMW) were compared with those determined using computational methods (*e.g.*, AlogPs, AClogP, milogP, AlogP, MlogP, xlogP2 and xlogP3) [4]. Correlation of lipophilic properties and protein binding of the investigated acitretin was discussed. Molecular docking study of acitretin in the human serum albumin confirmed lipophilic mode of interaction [5].On the basis of the obtained results in can be concluded that RP-HPTLC method is a reliable, accurate and cost-effective analytical tool for the determination of lipophilic character of biologically active acitretin and related compounds.

References:
[1] https://www.drugbank.ca/drugs/DB00459 (accessed on October 6, 2018).
[2] Jźwiak K., Szumiło H., Soczewiński E. (2001). *Wiad. Chem.* ***55****, (11/12),* 1047-1074.
[3] Rutkowska E., Pająk K., Jźwiak K. (2013). *Acta Pol. Pharm.-Drug Res.* ***70,*** *(1),* 3-18.
[4] VCCLAB, Virtual Computational Chemistry Laboratory, http://www.vcclab.org. (accessed on October 6, 2018).
[5] Chen L., Chen X. (2012). J Mol. Graph. Model ***33***, 35-43.

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[V-10] THE USE OF COPOLYMERS OF DISUCCINATE BETULIN FOR THE PREPARATION OF MICROSPHERES.

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Betulin, a lupane derivative, belongs to the pentacyclic triterpenes and occurs naturally in nature. It is obtained on a large scale from the outer layer of the birch bark. Betulin and its derivatives have a broad spectrum of biological activity, such as anti-inflammatory, anti-viral and in particular anti-cancer activity. The major problem, which limits their potential pharmaceutical uses, is the poor aqueous solubility of lupane triterpenes faced when trying to formulate pharmaceutical compounds from betulin. However, this problem can be solved by obtaining polymeric form of betulin and forming it into microspheres.

Disuccinate betulin (DBB) exhibits biological activity. It is known for its anti-cancer, anti-leishmanic, hypolipidemic, fungicidal, bactericidal and antiviral effects, including Epstein-Barr virus and HIV. On the other hand, DBB containing two carboxylic groups is excellent raw material to obtain polyanhydrides. Polyanhydrides are a class of surface-degradable polymers. 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. However, in literature, there are no reports about betulin based polyanhydrides.

The aim of this work was the synthesis of a new copolymers from disuccinate betulin and sebacic acid and then the preparation of microspheres.

Polyanhydrides were obtained by two-step melt polycondensation of betulin disuccinate with sebacic acid with the use of acetic anhydride. The content of sebacic acid in obtained copolymers was from 20 to 80 wt %. The use of sebacic acid as a comonomer increases the crystallinity of polymers, which affects the characteristics of microspheres. Polymers were used for the preparation of microspheres using emulsion (O/W) solvent evaporation technique. Poly(vinyl alcohol) was used as emulsion stabilizing agent. Using homogenization rates of 3000 rpm, microspheres with diameters of 15-90 m were obtained.

Under physiological conditions copolymers undergoes hydrolytic degradation to betulin disuccinate, whose biological activity is known and confirmed and to sebacic acid approved by FDA for use in drug delivery systems. The use of this polymer in biological systems will lead to the release of DBB, controlled by the degradation rate of the polymer. The obtained particles can be easily administered by injection or inhalation and can be used in the controlled drug delivery systems.

[V-11] "GREEN" SYNTHESIS OF STAR-SHAPED HEMA/DMAEMA COPOLYMERS AS "SMART" DRUG DELIVERY SYSTEMS

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Atom transfer radical polymerization where the activators are regenerated by electron transfer (ARGET ATRP) is one of the techniques of polymerization with a high chance of being used worldwide in the future. This process uses a lower concentration (several ppm) of catalyst in comparison to the traditional ATRP and relies on a slow, but steady regeneration of Cu(I) from Cu(II) [1]. Subsequently, the control of the reaction is retained hence macromolecules obtained during the polymerization possess well-defined structures with precisely designed molecular weight and topology. The aforementioned properties are of special importance in the case of designing novel polymeric drug carriers [2].

The presented results demonstrate the advantages of the usage of ARGET ATRP for the synthesis of 2-hydroxyethyl methacrylate/ 2-(*N*,*N*-dimethylamino)ethyl methacrylate (HEMA/DMAEMA) copolymers with a different initial molar ratio of comonomers as well as their homopolymers, using ascorbic acids as a reducing agent. Additionally, the research also explored the possibility of using ionic liquids as a green reaction media for such type of polymerizations, eliminating the use of more dangerous organic solvents. The obtained polymers were characterized by size exclusion chromatography (SEC) and nuclear magnetic resonance (H1NMR). The obtained 5-armed star-shaped (co)polymers had molecular weights in the range of 18700-44400 g/mol and a low molecular weight distribution (=1.12-1.51). The (co)polymers due to their pH- and thermoresponsive behavior will be further used as carriers for selected active substances.

References:

[1] K. Min, H. Gao, K. Matyjaszewski, *Use of Ascorbic Acid as Reducing Agent for Synthesis of Well-Defined Polymers by ARGET ATRP*, Macromolecules, **2007**, 40(6): 17891791

[2] W. A. Braunecker, K. Matyjaszewski, *Controlled/living radical polymerization: Features, developments, and perspectives*, Prog. Polym. Sci., **2007**, 32(1): 93-146

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[V-12] STRESS INDUCTION EFFECTS - NUCLEAR DISTRIBUTION AND THE LEVEL OF LAMINS AND INTERACTING PROTEINS IN THE DROSOPHILA MELANOGASTER MODEL SYSTEM

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Lamins belong to V intermediate filaments proteins performing a range of structural and regulatory functions. Lamins are responsible for the shape of the nucleus and are regulating nuclear events such as transcription, DNA replication, repair and chromatin organization. It is obvious, that in order to play such a variety of functions, lamins have to interact with different nuclear proteins, which are directly responsible for a particular function. Since lamin itself as well as through interacting proteins (e.g. topoisomerases II, HDACs, otefins) is also implied to participate in reaction to stress induction, we decided to analyze their potential role that processes. The best example of the stress response and the best known so far is heat shock response (HSR). This is a very conservative mechanism functioning in the cell. In Eucaryotes it is based on activation of the heat shock transcription factor (HSF). In Drosophila melanogaster there is only one isoform of HSF. In mammals, there are at least four isoforms responsible for different functions. Therefore we decided to work on the fruit fly as a simple model system with a single HSF, one lamin Dm and one lamin C. We use Drosophila melanogaster embryos and cell lines for studying the effect of stress on protein expression and subcellular distribution of lamins and interacting proteins. Here we report our results from western blot analyses and high-resolution confocal microscopy. We confirmed that lamin Dm and topoisomerase II expression is not changed as an effect of heat shock. We also show the changes in the distribution of lamin Dm, lamin C, topoisomerase II, HSF, polymerase II RNA phosphorylated on Ser5 and Hsp70 in normal, heat shock and recovery conditions.

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[V-13] POTENTIAL REMOVAL OF CYTOSTATIC PHARMACEUTICALS BY PLEUROTUS OSTREATUS STRAIN BWPH - SORPTION STUDY

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Increased use of anticancer drugs leads to elevated levels of cytostatics released to the environment. Because of this cytostatics have become one of the great environmental concerns. These compounds are recalcitrant in natural waters and they are not effectively removed during wastewater treatment processes. In addition they can exhibit carcinogenic, mutagenic, teratogenic, genotoxic and embryotoxic effects. It is therefore necessary to conduct research about the effective elimination of these pharmaceuticals from the environment.

The aim of this work was to establish sorption ability to eliminate selected anticancer drugs (bleomycin and vincristine) by a chosen fungus *Pleurotus ostreatus* (strain BWPH). Its ability to remove selected substances, was examined by placing 0.1 g of fungal biomass (both alive - rinsed for 24 h in water, and dead - autoclaved) in phials containing 10 mL of cytostatics aqueous solutions at the concentration of 10 mg/L. The process was carried out for 4 hours and the loss was measured at regular intervals using a UV-ViS spectrophotometer .

The results show that the removal of chosen cytostatic differs significantly and depends both on drug as well as on whether biomass is alive or dead. The greatest elimination (at the level of 36%) was shown for bleomycin in samples with autoclaved fungal hyphae. Generally, vincristine loss was smaller and regardless of the type of biomass after 4 hours it was about 20%.

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[V-14] EARLY CHANGES IN ENDOCRINE PARAMETERS AFTER RADIOTHERAPY OF PATIENTS WITH OROPHARYNGEAL CANCER

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Introduction: Hypothyroidism is a common complication of radiation therapy (RT) of the oropharyngeal cancer (OPC) and typically appears after 1 to 2 years, contributing to a variety of symptoms in cancer survivors. Short-term disturbances in parathyroid glands function, resulting in abnormal levels of serum parathormone, calcium and phosphate, were also reported in patients treated with RT for cancers of the head and neck region.

Aim: We aimed to evaluate changes in thyroid and parathyroid gland function-related parameters in short term following RT in a group of patients with OPC. Secondary aim was to identify associations between radiation-induced hypothyroidism (RIHT) probability calculated from 5 normal tissue complication probability (NTCP) models available from the literature and the changes in these parameters.

Methods: We evaluated 55 OPC patients treated with intensity-modulated RT (total dose 70 Gy or equivalent). We extracted dosimetric parameters from dose-volume histogram data. Concentrations of free thyroxine (fT4), thyroid stimulating hormone (TSH) and parathormone were measured using ELISA kits, while levels of calcium, phosphate and total albumin levels were assessed using colorimetric assays. The measurements were performed at two timepoints: during 7 days before the beginning of RT and during 7 days after the end of RT. Additionally, we calculated NTCP scores corresponding to risk of RIHT development based on 5 models incorporating various dosimetric and clinical parameters, available from the literature. Afterwards, endocrine variables were compared between the two timepoints and correlated with dosimetric variables and NTCP scores.

Results: TSH levels were significantly decreased following RT; median difference 0.36 mU/L (interquartile range -0.8 to -0.04), p0.001. Albumin levels also lowered significantly over the course of therapy by a median of 0.6 g/dL (interquartile range -0.9 to -0.2), p0.001. Changes in fT4, parathormone, corrected calcium and phosphate levels were not significant (p=0.267, p=0.161, p=0.084 and p=0.234, respectively). Considerable differences between NTCP models results were observed, with varying number of patients predicted to develop hypothyroidism in long-term follow-up, based on the results from respective models: 46 (83.6%), 26 (47.3%), 51 (92.7%), 21 (38.1%) and 49 (89.0%). TSH levels from both timepoints were significantly correlated with the results from 3 NTCP models that incorporated thyroid volume into predictions (all R0.45, p0.001). However, the changes in TSH levels were not correlated with the results from NTCP models (all p values0.1).

Conclusions and future objectives: Serum TSH and albumin levels decreased over the course of RT treatment in OPC patients. Risk of RIHT predicted by different NTCP models varies widely and is not associated with early changes in TSH or fT4 levels.

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[V-15] GENOTOXIC AND PHYTOTOXIC EFFECTS OF LANDFILL LEACHATE TREATED WITH ANAMMOX PROCESS

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Landfill leachates are characterized by high toxicity, due to their composition. They include, among others, organic compounds, heavy metals and high concentration of ammonium. Consequently, biological methods for removing nitrogen from sewage, such as denitrification and nitrification generate high costs of wastewater treatment. However, anammox process application might be a solution of this problem. One of the most important purpose of wastewater treatment is to reduce its toxicity, apart from effective and cheap removal of ammonium. The aim of this study was to investigate genotoxicological and phytotoxicological effects of landfill leachate, treated in SBR (sequencing batch reactor) by anammox process.

Phytotoxicity on *Lemna minor* was investigated according to the OECD Test No. 221 (2006). PCR-RAPD (polymerase chain reaction - random amplification of polymorphic DNA) for genotoxicity testing on genomic DNA of *Allium cepa* was applied. For PCR-RAPD the primer OPA04 was used. PCR products were separated by gel electrophoresis on 1% agarose gel. Dendrograms of the genetic distance and diagrams presenting genetic similarities to negative control (H2O) were constructed using the neighbor - joining algorithm.

The obtained results show that phytotoxicity of landfill leachate decreased, probably due to the reduction of heavy metals and ammonium concentration in anammox process. However, treatment did not change the genotoxicity of landfill leachate. These results might suggest that the tested landfill leachate included non-biodegradable substances, which are much more harmful for organisms at molecular level (caused high genotoxicity for *Allium cepa* genomic DNA), but did not influence phytotoxicity in *Lemna minor* after treatment.

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[V-16] ISOLATION AND TESTING OF CELLULOLYTIC BACTERIA FOR GENERATING METHANE FERMENTATION SUBSTRATE

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Deficiency of energy sources and environmental pollution are current problems worldwide. In this situation renewable energy sources, such as biogas from post-production waste seem to be a reasonable solution. Biogas consists mainly of methane, which is obtained in microbiological process called methane fermentation. In this biotechnological process consortia of microorganisms use organic compounds in their metabolic pathways to produce, among some other by-products, methane. These processes require the usage of organic-rich substrates, for example various types of biomass. Biological sludge, a waste from a biological wastewater treatment plant (WWTP), is a good source of biomass for methane fermentation. Preliminary studies have shown that the sediments from Klimzowiec WWTP located in Chorzw, Poland contain significant amounts of cellulose, relatively difficult to decompose, but useful as methane fermentation substrate after pretreatment. This pretreatment could be performed with cellulolytic bacteria to fasten cellulose decomposition before fermentation. These microorganisms can be obtained from the environmental samples, such as sludge itself. In this project it is planned to isolate environmental bacteria able to degrade cellulose and use them for preliminary biomass decomposition for methane fermentation. The experiment is planned to be performed at lab scale with a newly constructed bioreactor with automatic parameters control. During the experiment the methane production efficacy will be checked with and without pretreatment with cellulolytic bacteria in order to verify the possibility of using such biopreparation for enhanced biogas production.

[V-17] THE INFLUENCE OF INTERLEUKIN-6 ON THE REGENERATION OF DAMAGED MUSCLE IN MURINE MODEL OF HINDLIMB ISCHEMIA

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Adipose derived stromal cells (ADSCs) secrete significant amounts of interleukin 6 (IL-6), which is a pleiotropic cytokine. It has been reported that IL-6 may play a role in polarization of macrophages with proinflammatory phenotype (M1) to macrophages with antiinflammatory phenotype (M2). M2 macrophages are involved in inhibition of the inflammatory response as they initiate tissue repair processes. They secrete and release many proangiogenic factors (chemokines, cytokines, growth factors) that stimulate endothelial cells to migrate and proliferate. The aim of our work was to examine the influence of Interleukin-6 (recombinant and secreted by ADSCs) on the regeneration of damaged muscle in a murine model of hindlimb ischemia.

Unilateral femoral artery ligation was performed on male mice of the C57BL/6NCrl strain (8-10 week-old). One hour after ligation, 10e6 hADSCs and mADSCs or 150ng of recombinant murine or human IL-6 (Abcam) were administered (in 100 L PBS) into the femurs of the studied mice. On 7th day of the experiment the muscles were excised, fixed in liquid nitrogen and stained by immunohistochemistry for the presence of F4/80 (mature macrophages) and CD206 (the M2 macrophage marker). To confirm the effect of macrophage presence in the muscle on the formation of new blood vessels the muscle specimens were stained by immunohistochemistry for endothelial markers (CD31).

Muscle regeneration 7 days after surgery was observed only in hADSCs group. Immunohistological assessment of the gastrocnemius muscle extracted on day 7th showed that capillary density was significantly increased in ADSC (both murine and human) -treated group compared to untreated control (PBS) and recombinant IL-6 group. The largest influx of F4/80+CD206+ macrophages was observed in muscle after injection of human ADSCs.

More new blood vessels formed in the limbs of mice receiving xenographs (hADSCs) than in limbs receiving mouse ADSCs and recombinant IL-6. Human ADSCs also increased the infiltration of macrophages with the M2 phenotype (F4/80+/CD206+) to the ischemic limbs compared to mouse ADSCs. Administration of Interleukin 6 alone had no therapeutic effect in mouse model of hindlimb ischemia.

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[V-18] INFLUENCE OF COMMONLY USED SYNTHETIC SUBSTANCES ON THE GROWTH AND DEVELOPMENT OF LIVING ORGANISMS

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The environmental pollution is on the rise also because of increasing societal consumption of pharmaceuticals. These substances, as a result of (inter alia) improper disposal of medicines by producers, excretion by humans and animals, often end up in soil or surface waters and sewage treatment plants. They might also accumulate in water organisms, penetrate into groundwater and end up in drinking water.

The toxicity test for pharmaceuticals was carried out using *Aspergillus ochraceus*, because previous research on the decolorization of synthetic dyes showed that this fungus has high potential to remove dyes with complicated aromatic structure (8 out of 10 dyes tested).

Due to the wide range of decolorization activity *A. ochraceus* could be used as a biopreparation for wastewater treatment. It was checked, therefore, whether other substances (pharmaceuticals) could inhibit growth of this species.

Toxicity tests were carried out using *A. ochraceus* isolated from wetlands at the Silesian University of Technology and four pharmaceuticals: Solpadeine (painkiller), Arechin (a drug against malaria), Verhistr (a medicine for dizziness) and Lesinelle (contraceptive pills).

The tests were carried out using two different media: Sabouraud (Biomerieux) and MSB (Mineral Salt Broth). The MSB medium contained: 10 g glucose, 0.2 g ammonium tartrate, 10 mg thiamine, 2 g KH2PO4, 0.5 g MgSO4*7H2O, 0.1 g CaCl2, 20 g agar per liter.

Pharmaceuticals were added to the prepared hot medium. For Sabouraud medium the concentrations of pharmaceuticals wereŁ 0.01 mg, 0.02 mg, 0.04 mg, 0.08 mg and 0.1 mg per liter. The exception were hormones which were tested at 0.1 mg, 0.2 mg, 0.4 mg, 0.8 mg and 1 mg per liter.

For MSB medium the concentrations of all substances tested were: 0.001 mg, 0.01 mg, 0.1 mg, 1 mg and 10 mg per liter. Controls were used for both media. After 7 days of incubation at 25degC colonies in the medium were counted. At the next step, to designate EC50 (effect concentration) logarithmic-probit method was used.

The difference in toxic effect was observed for both media. With Sabouraud medium, the most toxic substnce was solpadeine (EC50 = 0.017 mg/l), the least toxic was velhistr (EC50 = 0.317 mg/l). For other substances, the EC50 was: hormones - 0.024 mg/l, arechin 0.019 mg/l. In the case of mineral medium, the most toxic substance was velhistr (EC50 = 0.0053 mg/l) and the least arechin (EC50 = 0.16 mg/l). For other substances, the EC50 was: hormones - 0.017 mg/l, solpadeine - 0.0091 mg/l.

The conducted studies have shown that the selected pharmaceuticals have strong influence on growth of the tested fungus. The effect was stronger for medium with low concentration of carbon and nitrogen even when concentration of pharmaceuticals was low.

[V-19] pH AND TEMPERATURE EFFECT ON THE ABSORPTION CAPACITY OF THE SODIUM ALGINATE-POLYVINYL ALCOHOL CARRIERS

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Hydrogel polymers are increasingly investigated for their use in immobilization techniques. Immobilization via substances as among others polysaccharides, alginates, agar or polyacrylates is commonly used in multiple aspects of life, such as wastewater treatment, medicine and biotechnology. Selection of the best carrier for immobilization is crucial for each process efficiency. Knowledge about advantages and disadvantages of different types of immobilizers enables to combine them with each other to enhance mechanical properties and chemical stability of the carriers. In case of presented study we decided to use combination of sodium alginate (SA) and polyvinyl alcohol (PVA) to strengthen the effect of crosslinking, and thus enhance mechanical and chemical properties of the prepared carriers. Purpose of this work was gathering information about the mass transfer rate and the ability of the absorption in the case of different concentration of PVA in SA-PVA carriers at different temperature and pH range.

Carriers were prepared as a combination of 2% SA with: 2,5% PVA, 5% PVA, 10% PVA and 15% PVA. The experiment was carried out in 50 ml methylene blue (MB) solutions (20 mg/L) in three different temperatures: 15C, 23C and 30C. In the case of each temperature carriers were exposed to a neutral, acidic and alkaline conditions. SA-PVA pellets were weighted, then put into MB solutions. Measurement of the changes in MB concentration in time allowed to estimate the absorption capacity of the SA-PVA carriers in every investigated conditions. Samples of upper layer liquid were collected during the experiment at regular intervals, then the absorption was measured at wavelength of 665 nm using a UV-VIS spectrophotometer. The absorption capacity and mass transfer rate were calculated both according to linear regression of the measurements in time and according to difference between initial and equilibrium MB concentration.

The best absorption of MB by the carriers was noticed in pH=14 in temperature 23C. In the same condition we noticed the higher mass transfer rate.

There were no significant differences in absorption in case of each temperature at neutral conditions (pH = 7 0.4).

The lowest absorption was observed in acidic environment, capsules were able to absorb the lower rate of the dye.

Based on the results, it can be concluded that the absorption capacity and mass transfer rate of SA- PVA carriers depends on pH and temperature. Higher absorption capacity was observed in neutral conditions (each temperature) and alkaline conditions in 23C. The lowest degree of absorption was observed in the acidic environment. These results may help to optimize processes of immobilization via SA and PVA polymers. Moreover, in appropriate conditions, SA PVA pellets may be an alternative for a dyes removal processes, because of their absorption capacity and possibility to regeneration.

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[V-20] IMPACT OF OPERATIONAL EVENTS ON ANAMMOX PROCESS AND PARTIAL NITRIFICATION

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High concentration of ammonium and deficiency of organic carbon in reject water after dewatering of digested sludge is problematic for contemporary wastewater treatment plant (WWTP). Such stream has to be recirculated to the main line of the treatment plant what cause increase of the nitrogen load and as a consequence increased electricity consumption for aeration and increased demand for organic carbon needed in denitrification. In case of nitrogen reach streams an Anammox (ANaerobic AMMonium OXidation) process is alternative for nitrification and denitrification. The anammox process is especially suitable for ammonium-rich wastewater with lack of organic carbon and with relatively high temperature (above 30C). The main advantages of this process compared to the conventional nitrification/denitrification are: low sludge production, decrease of the aeration costs by almost 60% (only half of the ammonia is oxidized to nitrite in the nitritation process without further oxidation to nitrate), and no need for external organic carbon source addition (Anammox process). The low sludge production is another factor that contributes to the substantially lower operation costs compared to conventional denitrification systems. The goal of this study is investigate impact of operational events on the stability of the partial nitritation and anammox processes. The tests are performed in the SBR reactor with working volume equal to 25 litres. The reactor works in 4 cycles and time of one cycle is 360 min. The intermittent aeration with total aeration time equal to 126 min. was performer in order to promote first step of nitrification process. . The flow is 4 liters per cycle, Hydraulic Retention Time is 1,52 d and the average sludge retention time is 40 days. In the first part of the experiment reactor worked on real wastewater from municipal WWTP in Gliwice. Tests were focusing on controling stable reactor opeartion. Concentration of ammonium in incoming sewage was about 618,7 48,8 mg/l, average reactor load is 0,2 gN/L*d and average rate of contaminant removal is 0,15 gN/L*d. Investigation showed that first phase of nitrification combined with anammox process can remove nitrogen with 60% - 70% efficiency and ammonium about 70%. Avarage concentration of ammonium in outgoing sewage about 132,7 mg/l. But the lowest noted concentration in outgoing sewage was 28 mg/l where in ingoing sewage concentration of ammonium was 620 mg/l. In the second part of the experiment the operational events as change of pH, heating cut-off, overloading and underloading of activated sludge will be investigate.

[V-21] PRELIMINARY INVESTIGATION OF THE CYTOXICITY OF CHOSEN PHARMACEUTICAL PRODUCTS PRESENT IN ENVIRONMENTAL WATERS

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A lot of studies have revealed that unmetabolised pharmaceutical products (PPs) and their by-products enter the sewage system and, later, appear in the wastewater treatment plants. The treatment process in the plant usually fails to completely eliminate the drugs from wastewater. In consequence, the drugs, together with treated wastewater, are released into environmental waters. The impact of PPs and, especially, their degradation products on organisms is not yet fully understood, however there are reports that it can significantly affect the functioning of cells, including human ones.

In this study, the cytotoxicity of four chosen pharmaceutical products (sulfadiazine (SDA), sulfamethazine (SMA), diatrizoate (DTZ), iodipamide (IDP)) was investigated by MTS assay.

SDA and SMA are synthetic antimicrobials, which were developed in the beginning of he 20th Century and they are still in use. They create a bacteriostatic effect by delaying reproduction of cells and inhibiting the production of folic acid. DTZ and IDP belong to widely-used iodinated contrast media. They are aministered to the patients in radiology departments to improve the visibility of body tisues during imaging. This class of substances have been identified as the major source of the AOX in the hospital effluents.

A MTS is a tetrazolium salt, which is converted into a coloured soluble formazan product by viable cells at 37C. The MTS assay has been reported as a convenient method for assessing in vitro cytotoxicty of chemicals.

For the MTS assay human colon cancer cell line HCT 116 was used and the incubation time was set to 6 h. The results sugest that low concentration (3 g/ml) of chosen pharmacetics could promote (IDP) or inhibit (SDA) proliferation of HCT116 cells. However, the results showed that the higher concentration (1 mg/ml) of these pharmaceutics had only a slight effect on HCT116 cell viability.

The results presented here are only preliminary; our investigation aims to assess toxicity not only of parent compounds, but also mixtures of chosen PPs, their metabolites and transformation products, which are formed during conventional wastewater treatment and advanced oxidation processes.

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[V-22] INDIVIDUAL-NUCLEOTIDE RESOLUTION CROSS-LINKING AND IMMUNOPRECIPITATION (iCLIP) FOR ANALYSIS OF HNRNPA2/B1 PROTEIN-BOUND RNA FRAGMENTS

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Recently introduced method of individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP, Huppertz et al, 2014) allows identification of the RNA-protein interaction sites at the genome-wide scale. iCLIP protocol, utilizes an intramolecular cDNA circularization step that enables analysis of cDNAs truncated at the protein-RNA crosslinking sites, without the need to use 5 adaptor required in previously described photoactivatable ribonucleoside enhanced CLIP (PAR-CLIP) protocol. The iCLIP method, that was up to date successfully used to study RNA modifications, RNA-binding proteins (RBPs) function in alternative splicing, mRNA stability, etc., have now been fully implemented at the Center for Medical Genomics OMICRON and will be extensively used further in various research projects.

hnRNPs heterogeneous nuclear ribonucleoproteins, are a family of RNA-binding proteins that form complexes with heterogeneous nuclear RNA (hnRNA) that is subsequently transformed into mature mRNA. hnRNPA2/B1, a member of this family is a miRNA-binding protein with a few known miRNA-binding motifs. Its also related to RNA sorting into exosomes whch play an important role in cellular micro- and macro-environment, as well as in cancer progression and metastasis.

The goal of the presented study was to introduce the iCLIP methodology at the CGM OMICRON and to use it to analyze the various RNAs bound by the hnRNPA2/B1 protein. To this aim the liver hepatocellular carcinoma cell line (HepG2), known to have functional hnRNPA2/B1 protein, was used. The collected cells were UV crosslinked and, following the iCLIP protocol and NGS sequencing on the MiSeq platform, the sequences of bound RNAs were obtained. Collected data were quality controlled, normalized and analyzed with in-house bioinformatics pipeline. Additionally, the results were compared with the data from the Villarroya-Beltri et al, 2013 study.

References:

Huppertz I, Attig J, DAmbrogio A, Easton LE, Sibley CR, Sugimoto Y, Tajnik M, Knig J, Ule J. iCLIP: protein-RNA interactions at nucleotide resolution. Methods. 2014 Feb;65(3):274-87

Villarroya-Beltri C, Gutirrez-Vzquez C, Snchez-Cabo F, Prez-Hernndez D, Vzquez J, Martin-Cofreces N, Martinez-Herrera DJ, Pascual-Montano A, Mittelbrunn M, Snchez-Madrid F. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun. 2013;4:2980.

[V-23] NOVEL IN SITU FORMING DRUG DELIVERY SYSTEMS BASED ON POLY(ISOSORBIDE SUCCINATE) RESIN

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In situ forming systems are liquid formulations which, after injection into the tissue or body cavity in a minimally invasive manner, transform into a semi-solid depot. This kind of materials, apart from structural function, may also serve as carrier of bioactive compounds delivered directly to the site of treatment. *In situ* forming implants (ISFI) can be obtained either by reaction of functional groups within system components or as a result of physical processes [1,2]. As a type of parenteral controlled drug release systems, ISFI solidifying by solid phase separation are currently commercially available and used in clinical practice. These systems are based on aliphatic polyesters such as polylactide or poly(lactide-*co*-glicolide) accepted by U.S. Food and Drug Administration for biomedical applications.

The goal of this work was to prepare a new type of ISFI formulation based on poly(isosorbide succinate) (PISU) and evaluation of its selected properties, which are crucial regarding possible usage as an injectable drug delivery system.

The *in vitro* Alamar Blue cytotoxicity assay was carried out for PISU using human skin fibroblasts (FK-1) and aortic smooth muscle cells (AoSMC). PISU resin was found to be non-toxic in a wide range of concentrations. Dynamic viscosity of liquid formulations, dependent on shear rate, facilitates their easy injection into required site where solid depot is formed immediately after injection. Doxycycline hyclate (DOXY), incorporated into this formulation, was released *in vitro* within 21 days, during which collected solutions exhibited antibacterial activity against gram-positive and gram-negative bacteria *Staphylococcus aureus* and *Escherichia coli*, respectively. The morphology of the precipitated depots at different stages of DOXY release was characterized by scanning electron microscopy. The *in vitro* studies of the depot behavior in phosphate buffered solution revealed deposition of crystals with the cauliflower-like morphology.

On the basis of these results, it can be concluded that PISU-based depots demonstrate bioactivity, revealed as formation of bone-like apatite layer at their surface. This feature of the reported depots should be beneficial for its application in bone tissue local treatment.

References:
[1] Kempe S., Mder K., *J. Control. Release* 2012, **161**, 668-679.
[2] Śmiga-Matuszowicz M., Korytkowska-Wałach A., Łukaszczyk J., *Polimery* 2015, **60**, 149-159.

[V-24] ALGINATE MEMBRANES WITH CHITOSAN MICROPARTICLES IN PERVAPORATIVE ETHANOL DEHYDRATION

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Hybrid alginate membranes (ALG) with dispersed pristine chitosan particles CS, and glutaraldehyde modified chitosan particles CS-GA were prepared and their effectiveness in pervaporative dehydration of water/ethanol mixture were investigated. The influence of both types of chitosan particles as well as their content on the transport properties of the investigated membranes were discussed. It was founded that the addition of the bare chitosan particles to the alginate matrix significantly alterates its transport properties leading to the improvement of separation parameters. In case of ALG_CS membrane with 5 wt% of bare CS filler the values of flux and separation factor was about 2.0 times higher, and the *PSI* was about 3.5 times bigger in comparison with the pristine ALG membrane. The modification of chitosan particle impacts on the further improvement of experimentally estimated transport and effectiveness characteristic of such membrane (ALG_CS-GA). Filling with CS-GA particles causes decreasing of crystallinity degree and results in an enhanced flux of obtained alginate membrane. The best effectiveness of pervaporative dehydration of ethanol was founded for ALG_CS-GA membrane with 10 wt% of CS-GA particles. In this case, separation factor ***a*** and pervaporative separation index ***PSI*** were equalled to 48.7 and 87.8 kgm-2*h-1*, respectively, which is about 5 and about 1.5 times higher than of pristine ALG membrane and ALG_CS membrane with 5 wt% of bare CS filler, respectively. Additionally, physico-chemical properties of all membranes were studied by measuring the swelling characteristic, contact angle, FTIR spectra, DSC thermograms and SEM images. The mean size D50 of CS and CS-GA particles were determined using Malvern Zetasizer and were equalled to 0.25 and 3 m, respectively.

References:
[1] V.C. Souza et al., Braz. J. Chem. Eng. 30 (2013) 683 700.
[2] G. Dudek et al.., Sep. Purif. Technol. 193 (2018) 398-407.
[3] G.Dudek et al., Sep. Sci. Technol. 53 (8) (2018) 1178-1190.

[V-25] PRELIMINARY STUDIES ON THE INFLUENCE OF NEGATIVE PRESSURE ON ACTIVATED SLUDGE

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Introduction: Degassing of activated sludge (being a heart of conventional wastewater treatment plants (WWTP)) before its discharge to secondary clarifier improves the settling properties of activated sludge, and enhances the efficiency of nutrient removal. Degassing of the activated sludge occurs due to a short-term reduction of pressure according to Henrys law. The observed effect of applying a degassing unit seems to both WWTP operators and scientists to be surprisingly good to result only from simple degassing. There are only scarce literature data concerning low vacuum (or negative pressure) influence on microorganisms. The proposed research topic, just because of the paucity of references, stresses the importance of research developing knowledge on the effect of negative pressure on bacteria and structure of activated sludge.

Methods: Activated sludge taken directly from municipal wastewater treatment plant. Sludge volume index, microscopic analysis of activated sludge (size and shape of activated sludge flocs), organic carbon concentration in the extracellular polymeric substances extract and oxygen uptake rate of activated sludge bacteria before and after negative pressure exposition were analysed. In each test different negative pressures ranging from 0.1 to 0.8 bar were applied to the activated sludge for 30 to 120 seconds. Each test covered also analysis of activated sludge sample untreated with negative pressure (control sample). All experiments were carried out in triplicates at 20degC. The statistical significance was accepted when the probability of the result assuming the null hypothesis (p) was less than 0.05.

Results: A decrease of sludge volume index (SVI) of activated sludge was observed after exposition of AS to vacuum in the range of from 0.2 to 0.8 bar (duration for 30 sec). There was no change in floc structure but flocs cohesion decreased together with a value of negative pressure. The extracellular polymeric substances represented by organic carbon concentration was at a similar level as in the control, regardless of the vacuum value. The oxygen uptake rate (OUR) was the highest in samples not treated with vacuum. Vacuum exposition resulted in OUR decrease.

Conclusion: These preliminary studies show that the effect of the negative pressure on activated sludge and its flocs is higher when higher values of the pressure are applied.

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[V-26] THE INFLUENCE OF HIGH-FAT HIGH-SUGAR DIET AND BARIATRIC SURGERY ON HSP70 AND HSP90 PLASMA AND LIVER CONCENTRATIONS IN DIET-INDUCED OBESE RATS

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Background: Metabolic surgery ameliorates insulin resistance and is associated with long-term, effective weight loss, but the mechanisms involved remain unknown.

Aims and methods: Here, the duodenal-jejunal omega switch (DJOS) surgery in combination with high-fat, high-carbohydrate diet was performed on diet obese rats and joint effects of bariatric surgery and different dietary patterns on heat shock protein 70 (HSP70) and HSP90 plasma and liver concentrations using ELISA kits were measured.

Results: We found that plasma and liver levels of HSP70 were lower after DJOS surgery in comparison to the control in the groups of animals kept on control diet (CD) and high fat, high sugar diet (HFS) but the post-operative change of the diet led to the increase in HSP70 in plasma and liver concentration in DJOS operated animals. A high-calorie meal, rich in carbohydrates and fats, significantly increased circulating levels of HSP90, reducing the normalising effect of DJOS.

Conclusions: The HFS diet applied during all stages of the experiment led to the higher levels of liver HSP90 concentration. The combination of CD and DJOS surgery was the most efficient in the lowering of the HSP90 liver concentration. The normalisation of circulating levels and liver concentrations of HSP70 and HSP90 may be achieved in a combination of DJOS procedure with a proper dietary plan.

[V-27] THE USE OF ULTRASENSITIVE TARGETED ENRICHMENT AND SEQUENCING FOR NON-INVASIVE GENETIC ANALYSIS OF CELL-FREE CIRCULATING DNA OF ADVANCED TUMORS.

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Introduction: Liquid biopsy aims to detect genetic profiles and subclonal variations in the heterogeneous cancers in minimally invasive manner by means of circulating cell-free tumour DNA (ctDNA) sequencing. Therefore, the current advances in ultrasensitive targeted enrichment methods can be adapted in the analysis of ctDNA for cancer detection, risk stratification and therapy monitoring.

Aim: Development of non-invasive genetic diagnostics and cancer prevention methods, based on the liquid biopsy - analysis of ctDNA, using the new generation sequencing technology (NGS) for Polish cancer patients and people at risk. For this purpose, Agilent SureSelect XT HS technology and custom SureSelect bait library was used for targeted enrichment of cancer-related genes in ctDNA samples enabling analysis of changes in target genes.

Materials and Methods: 3-5 ml plasma samples of 9 breast cancer cases were collected. Cell-free DNAs (cfDNA) were isolated using semi-automated methods and checked for quantity/quality and size distribution using Agilent Bioanalyzer High Sensitivity DNA chips. The Agilent SureSelect XT HS Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library and custom SureSelect bait library was used to enrich targeted regions for sequencing. The data analysis was performed using Agilent SureCall 4.0 software.

Results: The cfDNA concentration, as well as fragment distribution, in plasma of cancer patients varied significantly. The sequencing metrics for the 9 samples were compared to verify the effectiveness of the enrichment depending on sample concentration and quality.

Conclusions: Results of the analysis will be disclosed and discussed during the meeting. The enrichment method of custom gene panel will be evaluated in terms of usefullness in genetic variants identification in liquid biopsy.

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DIFFERENCES IN MIRNA EXPRESSION PATTERNS BETWEEN PLASMA AND EXOSOMES IN ACUTE LYMPHOBLASTIC LEUKEMIA SURVIVORS WITH HISTORY OF ANTHRACYCLINE TREATMENT

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Micro RNAs (miRNAs) are small non-coding 18-22 nt in lenght RNAs. Beside action in the cell of origin, they are secreted to extracellular environment, and subsequently actively influence mRNA translation in recipient cells. miRNAs are circulating in blood encapsulated in the extracellular vesicles (EVs) or in the protein-bound form. It was reported that miRNAs abundance is similar between these two compartments in healthy people (Tian et al., 2017). Anthracyclines are effective and widely used antineoplastic drugs, although they may cause severe organ toxicity during the treatment as well as late long-term consequences like metabolic syndrome, dyslipidemia, cardiac disease and hypertension. At least partially, such outcomes are result of irreversible action of anthracyclines on DNA and thus could be seen on RNA expression level. Here, we aimed to compare miRNA levels in plasma and EVs in adult acute lymphoblastic leukemia (ALL) survivors, treated with doxorubicine during the course of the disease.

Methods: Samples were collected at the University Hospital in Krakow (Department of Internal Medicine and Gerontology, Collegium Medicum). Studied population consisted of adult ALL cancer survivors diagnosed before the age of 18, who completed the treatment at least 5 years prior to the study. The study was approved by the Bioethics Committee of Jagiellonian University.Libraries were prepared from total 62 RNA samples from serum and exosomes with NebNext Small RNA Library Prep and sequenced on NextSeq500 sequencer (Illumina). Reads were aligned to two different reference databases: mirBase v21 and RNAcentral (for the latter, only transcripts shorter than 200 base pairs were selected) using Bowtie and counted using Samtools. Target statistical analysis involved differential expression performed via the edgeR package, as well as co-expression based methods which rely on the Kendall coefficient and machine learning algorithms based on information-theoretical indices.

Results: We have detected a number of small RNAs differentially expressed between the two compartments under 0.05 FDR. Among them are hsa-miR-378d, hsa-miR-98-5p, hsa-miR-378c, hsa-miR-101-3p. Additionally, using a co-expression analysis, we detected several miRNAs which are proportional (hsa-miR-11401, hsa-miR-103a-3p, hsa-miR-1299) between the two compartments, and several other ones with expression profiles non-correlated between the two compartments (hsa-miR-10b-3p, hsa-miR-1185-2-3p). Given the limited samples size, we have adapted a machine-learning approach to select several features (small RNAs) which are most discriminative between serum and exosomes.

Conclusions: We have shown that there are significant differences between miRNA content in plasma and EVs from adult patients with history of anthracycline treatment in childhood.

This study has been supported by a grant of National Science Centre in Poland (SONATA 2015/17/D/NZ7/02165)

[V-28] CHARACTERIZATION OF P53 STATUS IN ME45 CELL LINE

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The p53 tumor suppressor gene is one of the most frequently altered genes in human cancer. Traditionally cancer progression and metastasis are associated with mutations of the *TP53* gene. However, in many cases cancer can be linked to changed expression level of the p53 isoforms.

The aim of this work was to identify potential mutations in the coding sequence of TP53 gene and to investigate which p53 isoforms are present in Me45 cells.

The Sanger sequencing results did not show mutation in the coding sequence of TP53 gene. The RT-qPCR and Western blot results showed that most of the p53 isoforms are present in Me45 cells, but their expression level is different. Because p53 isoforms can inhibit activity of the canonical p53, the abnormal expression of p53 isoforms can be responsible for regulating Me45 cells activities.

This work was supported by grants 02/010/BK_18/0102 (task 2) and UMO-2015/19/B/ST7/02984. The experiments were performed at the Biotechnology Center of the Silesian University of Technology using equipment financed by the Silesian Biofarma program.

[V-29] TRANSFORMATIONS OF REDUCED GRAPHENE OXIDE INDUCED BY ANAMMOX COMMUNITY

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Nowadays, the development of new technologies entails the necessity of producing significant quantities of advanced materials, useful in many fields of science and industry. Such materials include graphene-based nanomaterials, which are becoming a part of our daily life in cosmetics products or clothing. Fast development of nanotechnology and widespread use of nanomaterials reflects in greater concentrations of graphene-based nanomaterials in the wastewater treatment plants. Moreover, numerous studies have reported that these nanomaterials can be used to improve biological wastewater treatment via anammox (anaerobic ammonium oxidation) process. In this work, the long-term effects of reduced graphene oxide (RGO) and anammox microbial community interactions during synthetic wastewater treatment were studied in sequencing batch reactor (SBR).

Structure of RGO powder before addition to the bioreactor and RGO sample taken from the bioreactor after 109 days of incubation were analyzed by transmission electron microscopy (TEM), an electron energy loss spectrometry (EELS) and Raman spectrometry. Microbial community structure of anammox activated sludge samples taken from the inoculum and the bioreactor after 162 days were analyzed by metagenomic sequencing.

After incubation in anammox bioreactor, RGO showed signs of degradation and chemical changes: oxidation and calcium on its surface. Herein, it was proposed, that RGO is oxidized and oxygen is reduced by the organic mediator, involved in the enzymatic reactions. However, in the opposite to the monocultures, microorganisms in the activated sludge are a very complex communities, rich in uncultured species, what constitutes an obstacle to reveal the exact oxidation mechanisms. Structure of bacterial community of activated sludge revealed that despite the fact that RGO is regarded as antimicrobial agent, in case of activated sludge community its negative influence is visible only in case of phylum *Firmicutes.* These results underlines that bacteria living in the communities seems to be more resistant towards antimicrobial agents such as RGO. Since greater concentration of graphene-based nanomaterials is expected in the wastewater treatment plants, this observations are very promising and requires further investigations regarding biodegradation of these materials in the environment.

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[V-30] THE STUDY OF THE IMPACT OF MINE WATER ON PHYSICOCHEMICAL AND BIOLOGICAL CONDITION OF SELECTED HOLLOW RESERVOIRS

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Introduction: Mine waters are the ones flowing into mining pits, as well as technological waters pumped up from the bottom of the mine. Term of technological water often describes water contained in hydrated sand used to prevent subsidence. Waters pumped from mines are lead off towards various receivers, accordingly to obtained permits. Chemical composition of mine waters mainly depends on mining intensity and geological structure of grounds. Water maintains temperature of 13 degrees Celsius, high salinity, presence of corrosive components and radioactive elements. Numerous physicochemical along with biological changes follow inpouring of mine waters into reservoirs of surface waters. Example of such water bodies are hollow reservoirs in Czuław (Tychy). Fundamental goal of the project is execution of physicochemical studies in area of few said basins together with biological survey and determination of a gradient of chemical conditions including their influence on surrounding biological life.

Methods: Floating measuring system (catamaran) will be used for mapping of chemical condition of 4 chosen subsidence tanks. It was designed and perfected on Silesian University of Technology. The catamaran is equipped with water pumping system, leading samples towards potentiometric sensors (pH, temperature, chlorides, NH4-N, NO2-N, NO3-N). It can be controlled manually as well as remotely from computer, which allows for sampling according to net of reservoir prepared in computer program. Samples will be additionally analyzed in laboratory applying different methods (non potentiometric) of characterization in order to verify measurements obtained from catamaran. Biological study will include determining content of chlorophyll along with analysis of plankton and few selected species of mollucs.

Results and conclusions: Project is conducted in cooperation between Silesian University of Technology and University of Silesia by a group of students aside of employees of said institutions in form of Project Based Learning (Politechnika Śląska jako Centrum Nowoczesnego Kształcenia opartego o badania i innowacje). Currently the project is at the stage of checking and calibration of sensors, along with planning and optimization of field tests. Previous studies carried out within different projects showed that tanks with inflow of mine waters are highly salinitated and are inhabited by biocoenosis with species that are not native in that place (Lewin i Smoliński, 2006; Lewin, 2012). Moreover, built vehicle incomparably suits for analysis in places hard to reach form shore (Kozyra i in., 2017; Wira i in., 2017).

[V-31] pH AND TEMPERATURE EFFECT ON THE ABSORPTION CAPACITY OF THE SODIUM ALGINATE-POLYVINYL ALCOHOL CARRIERS

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Hydrogel polymers are increasingly investigated for their use in immobilization techniques. Immobilization via substances such as polysaccharides, alginates, agar or polyacrylates is commonly used in wastewater treatment, medicine and biotechnology. Selection of the best carrier for immobilization is crucial for each process efficiency. Knowing advantages and disadvantages of different types of immobilizers allows combining them with each other to enhance mechanical properties and chemical stability of the carriers. We decided to use combination of sodium alginate (SA) and polyvinyl alcohol (PVA) to strengthen the effect of crosslinking, and thus enhance mechanical and chemical properties of the prepared carriers. The purpose of this work was to gather information about mass transfer rate and ability of absorption for different concentrations of PVA in SA-PVA carriers at different temperatures and pH range.

Carriers were prepared as a combination of 2% SA with: 2.5% PVA, 5% PVA, 10% PVA and 15% PVA. The experiment was carried out in 50 ml methylene blue (MB) solutions (20 mg/L) at three different temperatures: 15°C, 23°C and 30°C. For each temperature carriers were exposed to a neutral, acidic or alkaline conditions. SA-PVA pellets were weighted, then put into MB solutions. Measurement of the changes in MB concentration in time allowed to estimate the absorption capacity of the SA-PVA carriers under all investigated conditions. Samples of upper layer liquid were collected during the experiment at regular intervals, then the absorption was measured at the wavelength of 665 nm using a UV-VIS spectrophotometer. The absorption capacity and mass transfer rate were calculated both according to linear regression of the measurements in time and according to difference between initial and equilibrium MB concentration.

The best absorption of MB by the carriers was noted at pH=14 and 23°C. Under the same conditions we noticed higher mass transfer rate. There were no significant differences in absorption for all temperatures and neutral conditions (pH = 7 ±0.4).

The lowest absorption was observed in acidic environment; capsules were able to absorb the lower rate of the dye.

Based on these results, it can be concluded that the absorption capacity and mass transfer rate of SA-PVA carriers depends on pH and temperature. Higher absorption capacity was observed under neutral conditions (for all temperatures) and alkaline conditions at 23°C. The lowest degree of absorption was observed in the acidic environment. These results may help to optimize processes of immobilization via SA and PVA polymers. Moreover, under appropriate conditions, SA – PVA pellets may be an alternative for dye removal processes, because of their absorption capacity and possibility of regeneration.

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[V-32] EPIGENETIC MODULATION OF EPITHELIAL TO MESENCHYMAL TRANSITION IN OVARIAN CANCER CELL LINES

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Cancer invasion and metastasis events depend on the third type of epithelial to mesenchymal transition (EMT). During EMT, the expression of epithelial proteins, including those that are part of cell junction complexes is downregulated and the activation of genes the protein products of which enhance mesenchymal adhesion is promoted. Epigenetic aberrations, eg. DNA hypermethylation and histone hypoacetylation silence gene expression in ovarian cancer. The strategy of synergy between DNMT (DNA methyltransferases) inhibitors and HDAC (histone deacetylases) inhibitors to improve the effects of DNMTi, which cannot reverse gene silencing because of other epigenetic marks is a promising anti-cancer therapy.

The aim of this study was to evaluate the influence of decitabine [12.5 μM, 25 μM], the DNA methyltransferase inhibitor (DNMTi), decitabine [25 μM] with trichostatin A [6.25 nM, 12.5 nM], histone deacetylase inhibitor (HDACi), and morin hydrate [150 μM, 200 μM], a natural flavonol, on metastatic and adhesive potential of ovarian cancer cells *in vitro*, in relation to changes of expression level of epithelial-to-mesenchymal transition (EMT) markers and the level of global methylation. Human ovarian cancer cell line A2780 (ECACC® 93112519) was exposed to these compounds for 6 days. After treatment adhesion to endothelial HMEC-1 cells (ATCC® CRL-3243™) and transwell migration were examined. Level of target genes' expression was quantified using PCR Array, and global DNA methylation status was determined.

The highest, statistically significant inhibitory effects on adhesion to HMEC-1 were seen, in comparison to untreated cells, with morin [150 μM] (65%), decitabine [25 μM] (56.64%) and decitabine [25 μM] with trichostatin A [6.25 nM] (53.85%). The highest influence on slowing down the migration rate was shown by decitabine in combination with trichostatin A in a dose dependent manner (25 μM and 6.25 nM – 73.9%, 25 μM and 12.5 nM – 66.42%, respectively, and decitabine [25 μM] (62.7%), in comparison to control cells. Analysis of expression of genes involved in EMT has shown a statistically significant decrease of genes involved in cell migration and motility after decitabine and decitabine with trichostatin A exposure (*ERBB3*, *MSN*, *CAV2*, *FN1*, *NODAL*, *STAT3*) and *JAG1*, *TGFB1* after exposure to all compounds used, as well as ECM molecules and those involved in cell adhesion (*BMP1*, *BMP7*, *COL1A2*, *COL5A2*, *DSC2*, *ITGA5*, *ITGAV*, and *ITGB1*) after decitabine and decitabine with trichostatin A, *SERPINE1* and *VCAN* after decitabine with trichostatin A and morin, *MMP3* after exposure to all compounds used. Morin hydrate [150 μM], decitabine [25 μM] with trichostatin A [6.25 nM], decitabine [25 μM, 12.5 μM] decrease the global methylation level in a statistically significant manner when compared to untreated cells.

Phenotypic changes have been confirmed in the decrease of expression level of genes associated, among others, with adhesion, and increase of genes downregulated during EMT in cancer cells; this may be related to different changes of the global methylation level after all used compounds in A2780.

The presented results are part of the PhD dissertation Ewa Nowak: Epigenetic modifications of expression of genes involved in epithelial to mesenchymal transformation. The study was supported by the funds of Medical University of Silesia: a) research for young scientists: founds KNW-2-B20/N/7/N, and KNW-2-B24/N/8/K; b) statutory research for 2018 and KNW-1-090/N/7/B

[V-33] INFLUENCE OF AMINOALKANOLIC XANTHONE DERIVATIVES ON THE SELECTED MELANOMA CELL LINES

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Despite significant increase in the number of therapeutic strategies developed in recent years, malignant melanoma still remains one of the most aggressive tumors. Therefore, search for the new drugs that could be applied to melanoma treatment is still necessary. Xanthones are oxygenated heterocyclic compounds that display many numerous pharmacological activities. They are biosynthesized by tropical plants or bacteria. Additionally, many synthetic derivatives have been obtained to search for the new, more effective therapeutic agents.

The aim of the study was to assess the anti-melanotic *in vitro* potential of the synthetic aminoalkanolic xanthone derivatives. Five xanthones, synthesized at the Department of Bioorganic Chemistry (CMUJ, Kraków, Poland), were included in the study due to their previously designated significant antitumor activity. All analyses were conducted on the amelanotic human cell lines: C-32 (ATCC No. CRL-1585), and Hs294T (ATCC No. THB-140).

Results demonstrate that IC50 values for all the analyzed xanthone derivatives were within a micromolar range indicating potential anti-melanotic efficiency of these compounds toward the studied cell lines. Further analyses confirmed that all compounds significantly decreased proliferation and stimulated apoptosis in the studied cell cultures. Next, the influence of the studied xanthones on the adhesion to Matrigel^{TM}-coated plates and invasion through Geltrex^{TM}-coated inserts of melanoma cells were analyzed. Results indicate that all compounds significantly decreased invasiveness and adhesion of melanoma cells. Additionally, microscopic detection of reactive oxygen species under the xanthone treatments revealed that all compounds significantly induced oxidative stress in the studied cultures.

Taken together, these results suggest that all the studied aminoalkanolic xanthone derivatives display significant cytotoxic, pro-apoptotic, antiproliferative, and anti-invasive influence on the melanoma cells. They also induce oxidative stress which might be a part of their anti-melanotic mechanism of action.

The study was supported by Medical University of Silesia funding (statutory research for 2018 and KNW-1-090/N/7/B)

[V-34] ALGINATE MEMBRANES WITH CHITOSAN MICROPARTICLES IN PERVAPORATIVE ETHANOL DEHYDRATION

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Hybrid alginate membranes (ALG) with dispersed pristine chitosan particles CS, and glutaraldehyde modified chitosan particles CS-GA were prepared and their effectiveness in pervaporative dehydration of water/ethanol mixture were investigated. The influence of both types of chitosan particles as well as their content on the transport properties of the investigated membranes were discussed. It was founded that the addition of the bare chitosan particles to the alginate matrix significantly alters its transport properties leading to the improvement of separation parameters. In case of ALG_CS membrane with 5 wt% of bare CS filler the values of flux and separation factor was about 2.0 times higher, and the *PSI* was about 3.5 times bigger in comparison with the pristine ALG membrane. The modification of chitosan particle impacts on the further improvement of experimentally estimated transport and effectiveness characteristic of such membrane (ALG_CS-GA). Filling with CS-GA particles causes decreasing of crystallinity degree and results in an enhanced flux of obtained alginate membrane. The best effectiveness of pervaporative dehydration of ethanol was founded for ALG_CS-GA membrane with 10 wt% of CS-GA particles. In this case, separation factor ***a*** and pervaporative separation index ***PSI*** were equalled to 48.7 and 87.8 *kg·m-2·h-1*, respectively, which is about 5 and about 1.5 times higher than of pristine ALG membrane and ALG_CS membrane with 5 wt% of bare CS filler, respectively. Additionally, physico-chemical properties of all membranes were studied by measuring the swelling characteristic, contact angle, FTIR spectra, DSC thermograms and SEM images. The mean size D50 of CS and CS-GA particles were determined using Malvern Zetasizer and were equalled to 0.25 and 3 μm, respectively.

References:

[1] V.C. Souza et al., Braz. J. Chem. Eng. 30 (2013) 683 – 700.
[2] G. Dudek et al.., Sep. Purif. Technol. 193 (2018) 398-407.
[3] G.Dudek et al., Sep. Sci. Technol. 53 (8) (2018) 1178-1190.

[V-35] STUDY OF DIFFUSIVE MOTION OF A TRACER PARTICLE IN HYBRID POLYMER MEMBRANE

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Curent research draws attention to the role of the structure on which the transport of penetrant takes place since the structure is the element which can be influenced. Such approach can bring scientists closer to designing membranes (the thin films) with specific and desired transport properties.

Hybrid alginate membranes containing various amount of dispersed magnetite (Alg/Fe3O4) and crosslinked using four different agents, i.e. calcium chloride (AlgCa), phosphoric acid (AlgP), glutaraldehyde (AlgGA) and citric acid (AlgC) were prepared and described in [1].

In this work, the membranes are characterized by different parameters like: the amount of polymer matrix, the fractal dimension of polymer matrix, the average size of polymer matrix domains, the average number of obstacles in the proximity of each polymer matrix pixel. Determination of the above mentioned characteristics base on the image analysis of a sufficiently large cross-sections of the membranes.

Diffusive transport is investigated by simulation of a particle motion in the membrane environment. Diffusion driven by Gaussian random walk is shown in order to check if the effective diffusion exponent at long time limit is subdiffusive and if it depends on the details of the underlying random process causing diffusion.

Thanks to such investigations the relationship between chemical composition, structure and morphology, and separation properties of the thin films, can be determined.

References:

[1] G. Dudek, M. Krasowska, R. Turczyn, M. Gnus, A. Strzelewicz, Structure, morphology and separation efficiency of hybrid Alg/Fe3O4 membranes in pervaporative dehydration of ethanol, Separation and Purification Technology 182 (2017) 101–109
[2] M. Krasowska, A. Strzelewicz, G. Dudek, M. Cieśla, Structure-diffusion relationship of polymer membranes with different texture, Physical Review E 95, 012155 (2017)
[3] A. Strzelewicz M. Krasowska, G. Dudek, A. Rybak, R. Turczyn, M. Cieśla, Anomalous diffusion on fractal structure of magnetic membranes, Acta Physica Polonica B, 44 (2013) 955-965
[4] M. Krasowska, A. Strzelewicz , A. Rybak, G. Dudek, M. Cieśla, Structure and transport properties of ethylcellulose membranes with different types and granulation of magnetic powder, Physica A: Statistical Mechanics and its Applications, 452 (2016) 241–250

[V-36] THE P53 TUMOR SUPPRESSOR PROTEIN STIMULATES THE EXPRESSION OF BLNK – GENE IMPORTANT IN HUMORAL IMMUNE SYSTEM

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Introduction: Humoral immunity is a type of immunity response, which is mediated by antibodies binding to the antigens recognized as potentially dangerous. The most important elements of this system are B cells. The *BLNK* (B cell linker) gene encodes cytoplasmic adaptor protein, which is primarily known by its critical role in B cell development and receptor signaling. The main function of BLNK is a regulation of biological outcomes of B-cell function. BLNK participates in orchestrating the pro-B cell to pre-B cell transition. The protein also plays a role in basic cell functions, for example activation of: ERK/EPHB2, NF-kappa-B kinase, JNK or intracellular calcium mobilization response. The highest expression of *BLNK* was observed in the spleen, tonsils and lymph nodes. Therefore, BLNK may be important in the pathogenesis of many diseases. Its mutations cause hypoglobulinemia, whereas its deficiency has been found in lymphoblastic leukemia. Moreover, studies show that the down-regulation of *BLNK* may be related to lymphocyte transformation mediated by Rel proteins. On the other hand, it acts as protein adapter in TREM2 pathway, which is associated with higher risk of Alzheimer's disease.
We developed a hypothesis according to which *BLNK* is regulated by tumor suppressor protein p53. We observed, that two substances: actinomycin D and nutlin-3a synergistically stimulate activation p53 in A549 cells. The analysis of transcriptome sequencing (RNA-Seq) of A549 cell line (lung cancer) exposed to actinomycin D and nutlin-3a (A+N) revealed a significant increase in the expression of over 2000 genes, including expression of 500 genes upregulated at least 10-fold. Surprisingly, the gene for BLNK was among the most strongly activated genes.
Methods: cultured cells: A549 (lung cancer), A375 (melanoma) and U-2 OS (osteosarcoma) were treated with: actinomycin D and nutlin-3a. The protein expression was examined by Western blotting. In order to confirm the hypothesis, the gene regulatory region of BLNK with a potential p53 binding site was cloned into pGL3-Basic reporter vector. Additionally, we mutated the putative p53 binding site using site-directed *in vitro* mutagenesis system.
Results: We confirmed our hypothesis that p53 affected the induction of BLNK following co-treatment with actinomycin D and nutlin-3a. Consistent with our hypothesis, A+N treatment resulted in strong up-regulation of BLNK in A549. In addition, the effect increases with the duration of cell exposure to A+N. Moreover, we observed the influence of p53 knock-down on expression of BLNK protein. The cloned promoter of BLNK contains bona fide p53 response element.
Conclusions: This recently identified new biological link between p53 and humoral immunity deserves more detailed exploration in further studies using models of B-cells.

Keywords: p53, BLNK pathway, cancer disease mechanism, humoral immune system

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[V-37] THE USE OF COPOLYMERS OF DISUCCINATE BETULIN FOR THE PREPARATION OF MICROSPHERES

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Betulin, a lupane derivative, belongs to the pentacyclic triterpenes and occurs in nature. It is obtained on a large scale from the outer layer of the birch bark. Betulin and its derivatives have a broad spectrum of biological activity, such as anti-inflammatory, anti-viral and in particular anti-cancer activity. The major problem, which limits their potential pharmaceutical uses, is the poor aqueous solubility of lupane triterpenes faced when trying to formulate pharmaceutical compounds from betulin. However, this problem can be solved by obtaining polymeric form of betulin and forming it into microspheres.

Disuccinate betulin (DBB) exhibits biological activity. It is known for its anti-cancer, anti-leishmanic, hypolipidemic, fungicidal, bactericidal and antiviral effects, including Epstein-Barr virus and HIV. On the other hand, DBB containing two carboxylic groups is excellent raw material to obtain polyanhydrides. Polyanhydrides are a class of surface-degradable polymers. 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. However, in literature, there are no reports about botulin-based polyanhydrides.

The aim of this work was the synthesis of a new copolymers from disuccinate betulin and sebacic acid and then the preparation of microspheres.

Polyanhydrides were obtained by two-step melt polycondensation of betulin disuccinate with sebacic acid with the use of acetic anhydride. The content of sebacic acid in obtained copolymers varied from 20 to 80 wt %. The use of sebacic acid as a comonomer increases the crystallinity of polymers, which affects the characteristics of microspheres. Polymers were used for the preparation of microspheres using emulsion (O/W) solvent evaporation technique. Poly(vinyl alcohol) was used as emulsion stabilizing agent. Using homogenization rates of 3000 rpm, microspheres with diameters of 15-90 μm were obtained.

Under physiological conditions copolymers undergoes hydrolytic degradation to betulin disuccinate, whose biological activity is known and confirmed and to sebacic acid approved by FDA for use in drug delivery systems. The use of this polymer in biological systems will lead to the release of DBB, controlled by the degradation rate of the polymer. The obtained particles can be easily administered by injection or inhalation and can be used in the controlled drug delivery systems.

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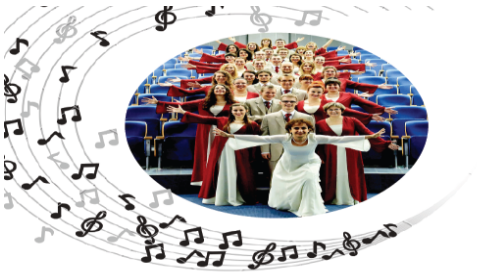

AZM

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. Hosanna - Lucjan Laprus, words: Jan Lechon
2. Ojcze Nasz (Our Father) - Lucjan Laprus, words: Kornel Ujejski
3. Anno Domini - Jakub Neske, words: St. John Paul II
4. Modlitwa o Pokoj (Prayer for Peace) - Norbert Blacha, words: Miroslawa Hanusiewicz
5. Slaska Dziewczyna (Silesian Girl) - Dariusz Janus, words: Grzegorz Poloczek
6. Wesolo nucicka (A jolly note) - Dariusz Janus, words: Anna Ruttar

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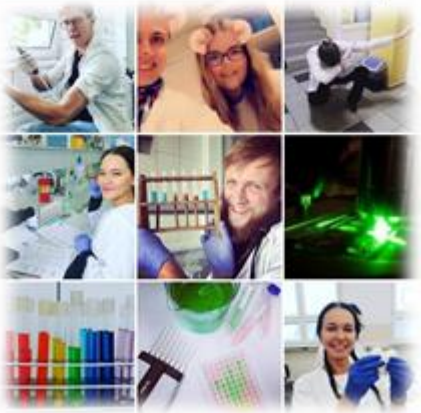

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Silesia.
Positive energy

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