XXIst Gliwice Scientific Meetings 2017

Gliwice, November 17-18, 2017

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

Organizers of Gliwice Scientific Meetings 2017:

Association for the Support of Cancer Research
Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch
Silesian University of Technology

Patronage and Co-organizers:

Marshal's Office of the Silesian Voivodeship
Ministry of Science and Higher Education
Polish Academy of Sciences
Gliwice Municipal Office

Scientific Committee of Gliwice Scientific Meetings 2017:

Mieczysław Chorąży *(Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch)*
Artur Cieślar-Pobuda *(Silesian University of Technology, Gliwice)*
Katarzyna Lisowska *(Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch)*
Monika Pietrowska *(Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch)*
Joanna Polańska *(Silesian University of Technology, Gliwice)*
Joanna Rzeszowska *(Silesian University of Technology, Gliwice)*
Andrzej Świerniak *(Silesian University of Technology, Gliwice)*
Piotr Widłak *(Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch)*

Organizing Committee:

Iwona Domińczyk
Agnieszka Dudło
Roman Jaksik
Joanna Łanuszewska
Lucyna Ponge
Jacek Rogoliński
Magdalena Skonieczna
Aleksander Sochanik

MARIA SKŁODOWSKA-CURIE INSTITUTE
ONCOLOGY CENTER
GLIWICE BRANCH, POLAND

Seventieth Anniversary

1947 - 2017

21st Gliwice Scientific Meetings 17-18 November 2017

Friday 17th November

9.00 - 9.15 Opening Ceremony

9.15 - 11.30 **Session 1. Exosomes and Intracellular Communication**
(Chairperson - Monika Pietrowska)

Theresa Whiteside *(University of Pittsburgh, Pittsburgh)*: Exosomes in cancer: information transfer, re-programming and pro-tumor activities (30')

Katalin Lumniczky *(OSKKI/NRIRR, Budapest)*: The role of extracellular vesicles in mediating radiation-induced bystander effects (30')

Marcus Duechler *(Centre of Molecular and Macromolecular Studies, PAS, Łódź)*: Melanoma-derived exosomes contribute to cancer immunosuppression (30')

Sonja Ludwig *(University of Duisburg-Essen, Essen)*: Immunoregulatory and proteomic profiles of exosomes produced by HPV+ and HPV- Head and Neck Cancer (HNC) cell lines (30')

Agata Abramowicz *(MSC Institute, Gliwice)*: Proteome of exosomes relased from head and neck cancer cells in response to genotoxic agents (15')

11.30 – 12.00 Coffee break

12.00 – 14.20 **Session 2. Stem Cells and Regenerative Medicine**
(Chairperson – Artur Cieślar-Pobuda) [EACR Session]

Philippe Collas *(Universtity of Oslo, Oslo)*: Going 4D: a dynamic chromatin architecture during differentiation of adipose stem cells

Hirak Patra *(University of Cambridge, Cambridge)*: Programmable Nano architecture: Quest for Guiding the Stem Cell Fate (25')

Adam Filipczyk *(Oslo University Hospital, Oslo)*: An RNA regulatory circuit key to Pluripotent Cell State Traverse (25')

Alicja Józkowicz *(Jagiellonian University, Kraków):* Heme oxygenase-1 deficiency in the niche triggers premature aging of hematopoietic stem cells (25')

Piotr Rieske *(Medical University of Lodz, Lodz):* Do glioblastoma stem cells really exist? Heterogeneous glioblastoma cells cooperation model (25')

Joanna Gola (*Medical University of Silesia, Katowice*): Factors influencing *ex vivo* differentiation of adipose derived mesenchymal stem cells (15')

14.20 – 15.00 Lunch break

15.00 – 16.00 Poster Session and Coffee

16.00 – 18.10 **Session 3. Bioinformatics in Omics**
(Chairperson – Joanna Polańska)

Maciej Lalowski *(University of Helsinki, Helsinki)*: Targeting molecular dynamics of the tissue: a quantitative -omics approach (30')

Angeles Sierra Jimenez *(IDIBAPS & UVic, Barcelona)*: Spectroscopy towards the metabolic characterization of metastatic cells (30')

Fady Mohareb *(Cranfield University, Cranfield)*: A global transcriptome profiling for Potato (*Solanum tuberosum* L.) during dormancy break using RNA-Seq (30')

Przemysław Biecek *(Warsaw University of Technology, Warszawa)*: Machine Learning Genetic Signatures (MLGenSig) based on expression and methylation data with applications to The Cancer Genome Atlas project (20')

Ernst Plefka *(BIOCRATES Life Sciences AG, Innsbruck)*: Metabolomics as a useful tool in cancer research (10')

Piotr Wardega *(NanoTemper Technologies, Kraków):* Novel biophysical methods in protein-protein interaction studies (10')

19.30 – Dinner and Social Program

Saturday 18th November

8.30 – 9.00 Meeting of the Polish EACR Group

9.00 – 10.50 **Session 4. Stress Response and Cancer**
(Chairperson – Joanna Rzeszowska)

Jolanta Jura *(Jagiellonian University, Kraków)*: The link between RNA stability and cancer development (25')

Paweł Paszek *(University of Manchester, Manchester)*: Dynamics and heterogeneity of inflammatory signalling in single cells (25')

Carmel Mothersill *(McMaster University, Hamilton*): Harnessing radiation-induced bystander signalling mechanisms for radiotherapy (25')

Marek Rusin *(MSC Institute, Gliwice)*: Regulation of innate immunity genes by p53 tumor suppressor protein (20')

Małgorzata Dawidowska *(Institute of Human Genetics, Poznań):* T-cell acute lymphoblastic leukaemia from miRNA transcriptome perspective (15')

10.50 – 11.15 Coffee break

11.15 – 13.00 **Session 5. Radiation Biology and Medicine**
(Chairperson – Piotr Widłak)

Udo Gaipl *(Universitaetsklinikum Erlangen, Erlangen)*: Immune modulatory properties of radiotherapy - rationales for combination with immunotherapy (30')

Krzysztof Składowski *(MSC Institute, Gliwice)*: title pending (25'): Can tumour control probability (TCP) be preserved when standard fractionation radiation therapy is interrupted?

Marcin Kruszewski *(Institute of Nuclear Chemistry and Technology, Warszawa):* Nanoradiotherapy: Window on the world or dead end? (25')

Wojciech Fendler *(Medical University of Lodz, Łódź):* MicroRNAs as biodosimeters and biomarkers in radiation oncology (25')

13.00 – 13.45 Lunch break

13.45 – 16.00 **Session 6. Molecular Biomarkers**
(Chairperson – Katarzyna Lisowska)

Marc Baumann *(University of Helsinki, Helsinki)*: Fine-tuning clinical proteomics for biomarker identification and qualification

Piotr Ziółkowski *(Wrocław Medical University, Wrocław):* Osteopontins as biomarkers in breast cancer

Joanna Niemiec *(MSC Institute, Kraków*): The phenomenon of membranous / cytoplasmic MIB-1 immunopositivity in breast cancer

Witold Rzyman *(Medical University of Gdańsk, Gdańsk)*: Lung Cancer Screening - moving forward

Katarzyna Lisowska *(MSC Institute, Gliwice):* Personalized treatment for ovarian cancer

16.00 – 16.30 Presentations of Awarded Posters

16.30 Closing Ceremony

Lecture abstracts

Session I

Exosomes and Intracellular Communication

(Chairperson - Monika Pietrowska)

EXOSOMES IN CANCER: INFORMATION TRANSFER, RE-PROGRAMMING AND PRO-TUMOR ACTIVITIES

Theresa L. Whiteside, PhD MDhc

University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213, USA

Exosomes, virus-size vesicles (30-150nm) produced by all cells represent a subcellular communication system operating in unicellular and multicellular organisms. Exosomes play a key role in health and disease. In cancer, tumor-derived exosomes (TEX) mediating autocrine, juxtacrine and paracrine signaling promote tumor growth and inhibit anti-tumor immunity. TEX carry a rich assortment of suppressive molecules, including proteins and miRNAs, that inhibit functions of immune recipient cells by signaling via surface receptors on recipient cells or at the transcriptional level. Tumor cells produce an excess of TEX which carry oncogenic signals and other molecular and genetic cargo that resembles that of the parent tumor cell. For this reason, exosomes are looked upon as potential biomarkers of the tumor presence, progression and outcome. The presence of integrins on the surface of exosomes appears to determine their ability to promote metastasis. Studies of the molecular and genetic content of TEX require their isolation from plasma and separation from non-tumor-derived vesicles, which are equally abundant in patients' plasma. Methods based on capture of TEX from plasma of patients with melanoma using anti-CSPG4 mAbs have been developed in our group to isolate TEX. The detection of the TEX cargo components by on-bead flow cytometry or by mass spectrometry is in progress, and preliminary data suggest that the TEX cargo indeed provides useful information about the disease activity and outcome, at least for some patients with cancer. The presence in exosome cargos of PD-L1, for example, is shown to correlate with disease activity and progression in patients with cancer. In a separate approach, we have investigated the ability of tumor-derived exosome to interfere with immunotherapy and to mediate epithelial to mesenchymal transition (EMT). TEX re-program the recipient cells by mechanisms that are uder intense investigation. Overall, TEX emerge as a key components of the subcellular system regulating physiological and pathological immune responses in health and disease, respectively, and as potentially useful biomarkers of the tumor progression as well as immune dysfunction in cancer..

THE ROLE OF EXTRACELLULAR VESICLES IN MEDIATING RADIATION-INDUCED BYSTANDER EFFECTS

Katalin Lumniczky, Tünde Szatmári, Enikő Kis, Dávid Kis, Eszter Persa, Anett Benedek, Géza Sáfrány

Department of Radiation Medicine, Division of Radiobiology and Radiohygiene, National Public Health Institute, Budapest, Hungary

Radiation-induced bystander effects are the manifestation of radiation effects in cells not directly hit by radiation. The mechanisms governing radiation-induced bystander effects are still not entirely clarified. Extracellular vesicles (EVs) are small, membrane-coated bodies released by the cells into the extracellular medium. Due to their complex RNA, microRNA and protein cargo their major role is in intercellular communication and thus are potential mediators of radiation-induced bystander effects.

In order to investigate the role of EVs in radiation-induced bystander effects an in vivo study was designed. C57Bl/6 mice were irradiated with different doses of ionizing radiation, EVs were isolated from the bone marrow and injected into the tail vein of unirradiated mice. The effect of EV transfer was studied by comparing molecular and phenotypic changes of bone marrow cells and splenocytes of EV-recipient, bystander mice to the directly irradiated animals. Activation of the DNA damage response pathway in the spleen of the bystander animals as measured by γ-H2AX assay was comparable to the directly irradiated animals. Phenotypical changes in both the bone marrow and spleen of bystander animals were present, however they were restricted to certain cellular subpopulations. The concentration of soluble mediators of inflammation in the plasma of directly irradiated and bystander animals was similar. Total RNA was isolated from EVs derived from the bone marrow and plasma of directly irradiated mice and microRNA profiling was performed. A panel of differentially expressed miRNAs in the EVs isolated from irradiated mice was identified with predicted involvement in pathways related to DNA damage repair, hematopoietic and immune system regulation, suggesting their participation in mediating radiation-induced bystander effects.

In conclusion, we proved that EVs mediated certain radiation effects in various organs (bone marrow, spleen, blood) of the haematopoietic system in bystander mice and identified potential miRNAs carried by EVs, which might be responsible for these effects.

This work was funded by the DoReMi and OPERRA EU-FP7 projects and by the National Research, Development and Innovation Office (grant agreement number: VKSZ_14-1-2015-0021).

MELANOMA-DERIVED EXOSOMES CONTRIBUTE TO CANCER IMMUNOSUPPRESSION

Markus Düchler

Centre of Molecular and Macromolecular Studies, PAS, Łódź

The activation cytotoxic T cells, representing the most efficient immune attack against cancer, depends on antigen presenting cells (APCs) such as dendritic cells (DCs), which take up tumor associated antigens, process them into peptides and present them on major histocompatibility complex (MHC) molecules to T cells. Through the direct interaction of the peptide-MHC complexes with the cognate T cell receptors, T cells are activated.

However, tumors exhibit an astonishing variety of mechanisms to escape from immune surveillance, such as hiding from recognition, establishing physical and chemical barriers to prevent immune cell attack, expressing death receptor ligands or secreting immunosuppressive cytokines.

In addition, tumors secrete exosomes which are able to transform immune effector cells into suppressor cells. Our research focusses on the influence of melanoma-derived exosomes on antigen presentation by DCs. Exosomes transport immunosuppressive cytokines and hundreds of microRNAs which may interfere with efficient antigen presentation. The immunosuppressive activity of these exosomes was demonstrated by their influence on the expression of critical surface receptors and cytokines of APCs. Several signals that are required for an optimal stimulation of a cytotoxic T cell response were negatively affected by melanoma-derived exosomes suggesting their contribution to cancer-mediated tolerance.

IMMUNOREGULATORY AND PROTEOMIC PROFILES OF EXOSOMES PRODUCED BY HPV+ AND HPV- HEAD AND NECK CANCER (HNC) CELL LINES

Sonja Ludwig[1,2], Monika Pietrowska[3], Lukasz Marczak[4], Agata Abramowicz[3], Marta Gawin[3], Priyanka Sharma[2], Stephan Lang[1], Piotr Widlak[3], Theresa L. Whiteside[2]

[1]Department of Otorhinolaryngology, Otorhinolaryngology, University of Duisburg-Essen, Germany; [2]Department of Pathology, University of Pittsburgh, School of Medicine and University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213, USA; [3]Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Gliwice, Poland; [4]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

Introduction: Exosomes, virus-sized vesicles, are important mediators of intercellular communication. We reported that exosomes from Head and Neck Cancer (HNC) cell lines and patients' plasma carry protein cargos resembling those of parent cells and modulate functions of immune cells. Here, we ask whether exosomes derived from Human Papilloma Virus+ (HPV+) or HPV-neg HNC cell lines differ in molecular profiles and immunoregulatory activities.

Methods: Exosomes were isolated from concentrated supernatants of three HPV+-positive (SCC-2, SCC-47, SCC-90) and two HPV-neg (PCI-13 and PCI-30) HNC cell lines using mini size exclusion chromatography (mini-SEC) on Sepharose 2B columns. Exosomes recovered in fraction #4 were assessed for morphology and size by TEM and for protein content. Molecular profiles were determined by Western blots (WBs) and protein profiles by mass spectrometry. Exosome functions were measured in co-culture assays with human T cell, NK cell and dendritic cell (DC) subsets.

Results: By TEM exosome derived from all cell lines were similar in size (30-150nm) and morphology. Protein levels ranged from 2-10ug protein /mL of supernatant from 10^6 cultured cells. Exosomes derived from all three HPV+ cell lines carried E6and E7, p16 and Rb. HPV-neg exosomes showed absence of cyclin D1 and survivin and low p53 protein levels by WBs. Immunomodulating molecules (TGFb, FasL, OX40, OX49L and HSP70) were equally prominent on all exosomes, reflecting the content of parent cells. By mass spectrometry, HPV+ and HPV-neg exosomes contained 572 overlapping and 169 non-overlapping proteins. HPV+ exosomes carried 655 proteins of which 83 were not shared by HPV-neg exosomes, and the latter carried 658 proteins of which 86 were unique and not shared with HPV+ exosomes. Gene ontology analysis of the unique proteins showed that those driving energy pathways were twice more frequent in HPV+ exosomes ($p<0.0001$). In contrast, HPV-neg exosomes were significantly enriched in proteins associated with cell growth/ maintenance. The proteins modulating immune responses were less frequent in HPV+ exosomes (14 proteins, 2.1%) than in HPV-neg exosomes (18 proteins, 2.8%). The results of mass spectrometry did not always coincide with antibody-amplified results obtained by WBs. No differences were observed in the ability of HPV+ vs HPV-neg exosomes to induce apoptosis in activated T cells or suppress their activation and proliferation. HPV-neg exosomes co-incubated with human DCs induced lower levels of CD80 and CD86 co-stimulatory proteins and impaired DC maturation more than HPV+ exosomes.

Conclusions: Exosomes derived from HPV+ tumor cells carried viral antigens and had a proteomic profile that was distinct from that of HPV-neg exosomes derived from HNC cell lines. Levels of immune suppression mediated by HPV+ and HPV-neg exosomes were comparable, although HPV+ exosomes tended to promoted rather than inhibited DC maturation in vitro. Exosomes reflect the HPV status of their parent cells and thus might serve as future plasma biomarkers of HPV infection in HNC.

METHODOLOGICAL ASPECTS OF MASS SPECTROMETRY-BASED EXOSOMAL STUDIES

Agata Abramowicz[1], Anna Wojakowska[1], Łukasz Marczak[2], Piotr Widłak[1], Monika Pietrowska[1]

[1]Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Gliwice, Poland; [2]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

Exosomes are small extracellular vesicles with phospholipid bilayer membrane and diameter around 100 nm. They are formed by inward budding of late endosomes and secreted after their fusion with plasma membrane. Besides nucleic acids, exosomal cargo includes also proteins which makes these nanovesicles an appreciated object of interest for proteomics. Exosomes can be isolated from different body fluids like blood (plasma/serum) or urine as well as from *in vitro* cell culture media. Therefore, standardization of sample preparation is necessary. For proteomic analysis, a particularly important aspect is significant reduction of contaminants like protein components of intercellular compartments or high abundant proteins, for example from cell culture media supplements, like albumin delivered with FBS. Since all prevalent methods of exosome isolation are based rather on exosome enrichment than on highly selective separation, it is necessary to minimalize the influence of background molecules on mass spectrometry measurement.

In our studies, we identified several issues potentially influencing results of mass spectrometry analysis of exosomal proteins. We show that cell culture media-derived protein contaminants significantly reduce the number of identifications of exosomal-specific proteins and we suggest size exclusion chromatography as an effective tool for exosomal sample purification. Moreover, we indicate that apart from the properties of the sample itself and the selected method of vesicle isolation/purification, also the method of exosomal proteins extraction and further sample processing can determine the final list of identified proteins. Therefore, we compared four different methods of exosomal sample preparation for mass spectrometry analysis, especially focusing on the range of obtained identifications.

These studies allow us to present an alternative method of effective exosomal protein extraction based on an organic solvent, fully compatible with mass spectrometry requirements.

Session II:

Stem Cells and Regenerative Medicine

(Chairperson – Artur Cieślar-Pobuda)

[EACR Session]

GOING 4D: A DYNAMIC CHROMATIN ARCHITECTURE DURING DIFFERENTIATION OF ADIPOSE STEM CELLS

Philippe Collas

Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

Dynamic changes in the 4-dimensional (4D) conformation of the genome, where the 4th dimension is time, establish blueprints of developmental gene expression. We report alterations in 4D genome organization during early stages of differentiation of human adipose tissue stem cells into adipocytes. We combine genome-wide maps of topologically-associated domains (TADs) and lamina-associated domains (LADs), together with a new computational 3D genome modeling tool (Chrom3D) [1], to infer TAD position in space during differentiation. Our data reveal the formation of 3D TAD-TAD hubs drawing genic regions towards a repressive environment at the nuclear periphery. Independently of TADs, local gains and losses ("switches") of chromosomal contacts reflect transient opening and closing of chromatin regions. We highlight transient TAD-LAD regulatory interactions around a key adipogenic locus. LADs also appear as facilitators of long-range TAD repositioning in the nucleus space that establishes new patterns of TAD-TAD interactions. We have also investigated the impact of a lipodystrophy-causing lamin A hotspot mutation on spatial chromatin organization [2]. The mutation inhibits adipogenic gene induction by deregulating the epigenetics of enhancers of the anti-adipogenic microRNA *MIR335* gene, and *MIR335* enhancer-promoter interactions. Our data indicate that genome confirmation in adipose stem cells is dynamic and affected by mutations in structural proteins of the nucleus that cause developmental disorders.

[1] Paulsen et al. 2017. Genome Biol 18, 21.
[2] Oldenburg et al. 2017. J Cell Biol 216

PROGRAMMABLE NANO ARCHITECTURE: QUEST FOR GUIDING THE STEM CELL FATE

Hirak K. Patra

Bioscience Engineering Group, Dept. of Chemical Engineering and Biotechnology, Cambridge University, UK; Translational Theranostics Group, Dept. of Experimental and Clinical Medicine, Linköping University, Sweden

Stem cells are progressively under investigation due to their emerging potential to reinforce wide spectrum of innovative therapeutic procedures such as cell-selective therapy, regenerative stratagems, multicomponent tissue regeneration to name a few to address complex heterogeneous diseases conditions. The phenomenal speciality of the unspecialized stem cells is that they are very tightly regulated by manifold cues from diverse surroundings comprising of dynamic microenvironment, intrinsic and extrinsic signalling and associated loco-regional niche. Apart from biochemical and growth factors which are direct cues, there are several other important factors including architecture, size, shape, porosity, rigidity and dynamicity of the microenvironment are involved in determining the final fate of the stem cells. Although the chemical cues are being well established so far but are not sufficient to manipulate the stem cells in directing for desired therapeutic purpose. Biophysical contributors such as nano-topography, mechanical network, elasticity of the matrix, and interacting surface landscape, all influence the stemness, directed differentiation and fate of the stem cells.

The integrated architecture and surface topography at the nanoscale in matrix scaffold showed direct influence on regulating the stem cell comportment. Therefore, advanced programmable nanotechnology based approaches containing both chemical and physical cues can manipulate in directing the stem cell fate.

We have developed a series of nanotechnology based programmable on/off switchable nanosystem that can be explored in manipulating the complex stem cell differentiation process for the development of multicomponent tissue regeneration. The developed insight in switchable dynamic crosstalk at the nanoscale can be translated into potential programming in dictating specific stem cell fate for prospective therapeutic applications.

AN RNA REGULATORY CIRCUIT KEY TO PLURIPOTENT CELL STATE TRAVERSE

Kangxuan Jin, Hazal Zuo, Michelle Giller, Konstantinos Anastassiadis, Carsten Marr, Arne Klungland, Adam Filipczyk

Oslo University Hospital, Oslo

RNA layer control mechanisms involved in pluripotent cell state traverse are poorly understood. The RNA methyltransferase-like 3 and 14 (Mettl3 and Mettl14) are critical components of a methyltransferase complex which deposits the dynamic 6-Methyladenosine (m^6A) modification unto RNA. In the naïve pluripotent state, Mettl3 loss and complex disruption was shown to promote a hyper-pluripotent cancer-like phenotype. In primed pluripotency and epiblast stem cells (EpiSCs), Mettl3 depletion resulted in cell differentiation or death. We examined the effects of Mettl3 or Mettl14 depletion on pluripotency heterogeneity in Serum+LIF conditions using protein fusion double knock-in mESC reporters (NanogKATUSHKA/Oct4VENUS). By transcription factor, quantitative live-cell imaging over many cell generations, we show for the first time, the emergence of a NanogKATUSHKA-/Oct4VENUS+ epiblast like cell (EpiLC) state, at single cell resolution. Mettl3 depletion culminates in the upregulation of epiblast transcription factors (TFs) Otx2/Oct6 and elevation of pAKT and pErk signaling pathways. We define how these downstream changes co-operatively promote the EpiLC cell state traverse and maintenance. Furthermore, we assess the propensities of Mettl3 depleted EpiLC cell fractions for differentiation into key downstream cell lineages.

HEME OXYGENASE-1 DEFICIENCY IN THE NICHE TRIGGERS PREMATURE AGING OF HEMATOPOIETIC STEM CELLS

Krzysztof Szade, Monika Żukowska, Witold Nowak, Agata Szade, Maciej Cieśla, Karolina Bukowska-Strakova, Neli Kachamakowa-Trojanowska, Anna Kusienicka, Szymon Czauderna, Józef Dulak, Alicja Józkowicz

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Hematopoietic stem cells (HSCs) sustain production of blood cells throughout lifetime, however their regenerative potential declines with aging. While HSC-intrinsic changes acquired during aging are well characterized, it remains unclear how aging of the HSC niche affects hematopoiesis. Here we demonstrate that cells that compose the HSC niche – endothelial cells (ECs) and CXCL12-abudant reticular cells (CARs) – express high levels of heme oxygenase 1 (HO-1), but its expression decreases in old mice. RNA–sequencing shows that ECs and CARs from HO-1 deficient mice (HO-$1^{-/-}$) produce smaller amounts of key hematopoietic factors. Consequently, HSCs from young HO-$1^{-/-}$ mice are loosing quiescence and have impaired regenerative potential. We demonstrate that young HO-$1^{-/-}$ HSCs already show features of premature aging on whole-transcriptome and functional level. HO-$1^{+/+}$ HSCs transplanted to the HO-$1^{-/-}$ recipients exhaust their regenerative potential early and do not reconstitute secondary recipients. In turn, transplantation of HO-$1^{-/-}$ HSCs to the HO-$1^{+/+}$ recipients reinstates the regenerative potential of HO-$1^{-/-}$ HSCs and reverses part of their transcriptional alterations.

Concluding, we identified HO-1 as a new factor in bone marrow niche that regulates aging of HSCs. Our results suggest that modulation of HO-1 activity might be used to restore impaired function of aged HSCs.

DO GLIOBLASTOMA STEM CELLS REALLY EXIST? HETEROGENEOUS GLIOBLASTOMA CELLS COOPERATION MODEL.

Piotr Rieske

Department of Tumor Biology Medical University of Lodz, Poland; Research and Development Unit, Celther Polska LTD

Neural stem cells are relatively well defined. On the contrary, the term “glioblastoma stem cells” (GSCs) is still considered very vague, mostly due to the fact that analysis of putative GSCs is very problematic.

First of all, primary glioblastoma cells quickly become senescent or apoptotic *in vitro* [PLOS one, Stoczyńska-Fidelus E, 2014], whereas stable glioma cell lines do not show the majority of phenotypic features specific for glioblastoma cells isolated directly from tumours. Secondly, stable glioblastoma cell lines do not usually exhibit genetic alterations critical for these tumours, such as *IDH1* or *EGFR* mutations [BJC, Piaskowski S, 2011]. Finally, extrachromosomal amplification, extremely important for genetic and phenotypic flexibility of glioblastoma cells, is frequently observed in tumour specimens but almost always absent in glioblastoma cell lines [Nature, Turner KM, 2016].

During our analyses we used neural stem cells, primary glioblastoma cells and several stable cell lines, including unique lines showing extrachromosomal amplicons. Analyses of these cells resulted in several interesting suggestions, especially for researchers using term "glioblastoma stem cells". We propose glioblastoma stem cells model to be replaced with model of heterogeneous glioblastoma cells cooperation.

FACTORS INFLUENCING *EX VIVO* DIFFERENTIATION OF ADIPOSE DERIVED MESENCHYMAL STEM CELLS

Joanna Gola[1], Aleksandra Skubis[1], Bartosz Sikora[1], Agnieszka Witkowska[2], Urszula Mazurek[1], Marek Łos[1]

[1]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Jedności 8, 41-200 Sosnowiec, Poland; [2]Department of Internal Diseases, Diabetology and Nephrology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, 3 Maja 13-15, 41-800 Zabrze, Poland

Stem cells, due to their unique ability for unlimited division and differentiation into many cell types, are increasingly being used in tissue regeneration. There are different strategies for tissue regeneration, such as stem cell transplantation or transplantation of cells obtained by *ex vivo* stem cell differentiation. One of the possible procedures is to obtain patient's mesenchymal stem cells from adipose tissue, which, after *ex vivo* differentiation, can be implanted to regenerate a particular tissue. However, there are a number of factors that can affect both the proliferation process and the cell differentiation. These include the age and health (accompanying diseases) of the donor, the procedure of adipose tissue collection, the type and source of adipose tissue, and a number of factors influencing these processes in cell culture conditions.

Our results point to differences in the proliferative potential of mesenchymal stem cells and their ability to differentiate into osteoblasts, not only depending on the incidence of diabetes, but also on the stage of its development. We have also found that the efficiency of these processes is influenced by several factors in cell culture conditions, including combinations and concentrations of differentiation factors, as well as combinations of antibiotics and antifungal agents used. We have also studied viability of limbal epithelial stem cells in the co-culture systems, depending on the presence of pro-inflammatory factors. It has been recently shown that the differentiation of adipose-derived stem cells into corneal epithelial cells could be facilitated by co-culture with the limbal epithelial stem cells. Our results showed a number of changes at the molecular level both in cells cultured in the presence of epithelial cells and conditioned medium.

Session III:
Bioinformatics in Omics
(Chairperson – Joanna Polańska)

TARGETING MOLECULAR DYNAMICS OF THE TISSUE: A QUANTITATIVE -OMICS APPROACH

Maciej Lalowski

Medicum, Meilahti Clinical Proteomics Core Facility, Finland; Finnish Proteomics Society, President

Various –omics methods are currently in use enabling to tackle the molecular dynamics in the tissue. In this presentation various aspects of implementation of such techniques will be presented, with the main focus on quantitative aspects of such methodologies and implications on elucidation of disease mechanisms.

The first example covers recognition of molecular signatures and functional modules connected with overexpression of human disease gene *CLN1* in a human neuronal cellular system [1]. In this study the SH-SY5Y neuroblastoma cells (differentiated into a neuronal-like phenotype), to overexpress wt*CLN1* and a selection of disease related mutations previously detected in CLN1-affected children were utilized. The differentiated cell lines underwent whole transcriptomic profiling by RNA-seq, to identify differentially expressed genes (DEGs) which are functionally related to the overexpression of wild-type or mutated PPT1. Following bioinformatic investigations, we focused on DEGs involved in palmitoylation of neuronal proteins as well as related cellular functions. Interestingly, genes coding for palmitoylated proteins assigned to neuronal functions, such as axonal growth, and to the synaptic compartment were the most significantly expressed. Moreover, to identify potential therapeutic targets for CLN1 disease, we aimed to demonstrate possible links with other NCL genes, particularly *CLN4* and *CLN10*, sharing common pathological traits with *PPT1*.

In another study, large-scale mass spectrometry-based proteomics and quantitative metabolomics were applied to gain an in-depth view of the mechanisms behind the regenerative capacity of the neonatal mouse during the first week of life [2]. Using label free quantitation of protein expression, 1937 heart proteins were quantified (366 differentially expressed), while metabolomic profiling characterized 612 metabolites (263 differentially regulated). Interlinking the *-omics* analyses demonstrated metabolic reprogramming from glycolysis to oxidative phosphorylation in seven days old mice, with increases of key enzymes and metabolites in fatty acid transport and β-oxidation, accompanied by elevated oxygen consumption. An upregulation of reactive oxygen species signalling and increases in oxidative stress markers were also visible. Furthermore, neonatal mice exhibited cellular proliferation with association to mTOR signalling and methionine and histidine pathway metabolites. These novel annotated pathways could link to the regenerative capacity of the neonatal heart.

[1] Pezzini et al. 2017. Front Mol Neurosci **in press**
[2] Lalowski et al. 2017. Scientific reports **in revision**

SPECTROSCOPY TOWARDS THE METABOLIC CHARACTERIZATION OF METASTASTATIC CELLS

Carme Bedia[1], Miriam Badia[2], Naiara Santana-Codina[3], Mónica Marro[4], Angels Sierra[2,5]*

[1]Department of Environmental Chemistry-IDAEA-CSIC, 08034 Barcelona; [2]IDIBAPS-Institut de Investigació Biomèdica August Pi i Sunyer, 08036 Barcelona; [3]IDIBELL-Institut d'Investigació Biomèdica de Bellbitge, 08907 L'Hospitalet de Llobregat; [4]ICFO-Institut de Ciencies Fotòniques, 08860 Castelldefels; [5]Physiological Sciences, Faculty of Medicine, Universitat de Vic-Universitat Central de Catalunya, 08500, Vic, Spain

One of the main obstacles for breast cancer treatment and long term survival is metastatic spread. The challenge is to identify biomarkers for predicting who will benefit from a particular targeted therapy [1]. Our purpose was to characterize biomarkers for the prediction of metastasis in newly diagnosed breast cancer patients and at early diagnosis of relapse.

Previous work: We performed transcriptomic analysis of breast cancer metastasis from patients and analysed the differential expression of organ-specific genes [2]. A network approach using these lists to search for network parameters based on the whole human interactome (topology information) was used and looked for internal connections between the genes (functional interpretation) to identify organ-specific de-regulated functions [3]. The resulted interaction networks were analysed to find de-regulated functions and key proteins in brain metastasis [4]. Three proteins related to fatty-acid metabolism were up-regulated in the brain metastasis network: The cytosolic acyl-coenzime A thioester hydrolase, ethanolamine-phosphate cytidylyltransferase, and citrate transport protein, an exchanger of citrate by malate involved in the production of a carbon source for the biosynthesis of fatty acids. The over-expression of these proteins suggested a possible increase in the synthesis of unsatured fatty acid.

Material and Methods: We used Raman spectroscopy (RS), a high-sensitive non-invading technique, to analyse metabolic differences in cells from a metastatic breast cancer cell experimental model. Localization and quantification of total (TFA) and total unsaturated (TUSFA) fatty acids were compared between parental cells and its metastatic variants to analyse their differences. Moreover, we performed untargeted lipidomic analysis (ULA) and UPLC-TOF analysis of lipid extracts and the resulting data were further processed following an untargeted analysis strategy (UAS) using the MSROI-MCR-ALS procedure.

Results: RS confirmed the increased TFA and TUSFA in brain metastatic cells compared to the parental cells. Further studies following the UAS and the MSROI-MCR-ALS procedures, revealed that GM2 and GM3 gangliosides are lipid species that changed in brain metastatic *v.s.* non metastatic cells. GM2 increased (4.1 and 5.3 fold) and GM3 decreased (0.8 and 0.5 fold), as a consequence an important decrease of the GM3/GM2 ratio was found in all ganglioside species with different acyl chain length.

Conclusions: Gangliosides are glycosphingolipids mainly localized in the outer leaflet of plasma membrane in lipid-enriched microdomains called lipid rafts, where they are involved in cell-cell recognition, adhesion and signal transduction. In breast cancer an increased HEXA activity and up-regulation of GM2-AP cofactor induce unbalancing GM3/GM2 ratio in lipid rafts priming brain metastasis ability in breast cancer cells.

** This study was supported by grants from the Spanish Ministry of Health and Consumer Affairs FIS-PI14/00336 from the I+D+I National Plan with the financial support from ISCIII-Subdirección General de Evaluación and the Fondo Europeo de Desarrollo Regional (FEDER), by grant 2014 SGR 530 from the Generalitat de Catalunya and from Fundació Privada Cellex Barcelona*

[1] Cadoo, et al. 2013. Surg Oncol Clin N Am 22, 823 - 40. doi: 10.1016/j.soc.2013.06.006.
[2] Sanz-Pamplona et al. 2011. Am J Pathol 179, 564 - 79. doi: 10.1016/j.ajpath.2011.04.037.
[3] Sanz-Pamplona et al. 2012. Mol Biosyst. 8, 2085-2096.
[4] Martínez-Aranda et al. 2015. Oncotarget 6, 44254 - 73. doi: 10.18632/oncotarget.5471.

A GLOBAL TRANSCRIPTOME PROFILING FOR POTATO (SOLANUM TUBEROSUM L.) DURING DORMANCY BREAK USING RNA-SEQ

Fady Mohareb

Head of Bioinformatics, Cranfield University, UK
f.mohareb@cranfield.ac.uk

Potato (*Solanum tuberosum L.*) is one of the most important non-grain food crop in the world and is central to global food security. In the UK alone, 4.05 million tonnes of potato tubers are stored annually [1]. Dormancy properties of potato tubers are key determinants of postharvest life. Premature sprouting of either organ during storage is accompanied by severe loss of quality.

The aim of this project was to provide the molecular understanding of the process of dormancy break in potato. In order to achieve this, we developed a bioinformatics pipeline for RNA-Seq analysis for two genotypic groups of potato of varying dormancy periods.

We have identified a list of key differentially expressed genes for responsible for dormancy control, as well as identifying the main regulatory and metabolic pathways controlling this process.

MACHINE LEARNING GENETIC SIGNATURES (MLGENSIG) BASED ON EXPRESSION AND METHYLATION DATA WITH APPLICATIONS TO THE CANCER GENOME ATLAS PROJECT

Przemysław Biecek

Warsaw University of Technology, Warszawa

How to integrate signals from gene expression and DNA methylation?
In this talk I will present three R packages that we have developed for joint modelling of expression and methylation data.

The statistical methodology is implemented in MLexpResso, an R package with (1) statistical tests for group comparisons based on RNAseq data, (2) statistical tests for methylation data, (3) functions for fusion of both signals and (4) functions for visualisation of differences between groups.

All examples, that will be presented, are based on The Cancer Genome Atlas data. An R package RTCGA is used for accessing, preprocessing and filtering clinical and molecular data from this project.

An R package archivist is used for making results fully reproducible. Due to the frequent updates in TCGS snapshots the reproducibility is a crucial part of modelling.

Dąbrowska et al. 2017.URL: https://github.com/geneticsMiNIng/MLGenSig
Kosiński et al. 2016. https://github.com/RTCGA/RTCGA
Biecek et al. 2017. J StatisticSoftware **Conditionally accepted** URL: https://github.com/pbiecek/archivist

METABOLOMICS AS A USEFUL TOOL IN CANCER RESEARCH

Ernst Plefka

Biocrates Life Sciences AG, Innsbruck

Metabolomics as the comprehensive study of the metabolome is of increasing importance in cancer research and drug development. The metabolic state is reflecting both the downstream information coming from the genome and the upstream information coming from the exposome (like diet, drugs, lifestyle, age, etc.). It is reflected by different metabolic signatures of small molecules.

In the future such metabolic signatures will not only inform about underlying molecular mechanisms of diseases, such as cancer, but also allow to identify metabolic pathways and to reveal biomarkers and metabolic signatures for disease detection or drug response in cancer research and cancer therapie.

Some examples will be given how to these metabolic signatures in different kinds of cancer like breast cancer or melanoma may be used in translational medicine in the future and what might be the contribution of Biocrates Life Sciences AG to this development.

In conclusion Pharmacometabolomics as a new discipline can inform about treatment outcomes and drug response in the future and will be an important tool in future development of precision medicine.

NOVEL BIOPHYSICAL METHODS IN PROTEIN-PROTEIN INTERACTION STUDIES

Piotr Wardega

NanoTemper Technologies

Interactions Analysis and beyond: MicroScale Thermophoresis (MST) is a powerful technique to quantify biomolecular interactions. It is based on thermophoresis, the directed movement of molecules in a temperature gradient. In context of parameters regulating the molecules movement in solution, our technique is highly sensitive to virtually any change in molecular properties, allowing for a precise quantification of events independent of the nature of the investigated specimen.

Here, we present recent progress and developments in MST technology and focus on MST applications beyond standard biomolecular interaction studies. By using different model systems, we introduce alternative MST applications - such as determination of binding stoichiometries and binding modes, thermodynamics and demonstrate the capability of MST to quantify high-affinity interactions with dissociation constants (Kds) in the low picomolar (pM) range as well as protein-protein interactions in pure mammalian cell lysates.

Easy and Rapid Analysis of Protein Stability by nanoDSF: nanoDSF is an advanced Differential Scanning Fluorimetry technology. It detects smallest changes in the fluorescence of tryptophan present in virtually all proteins.

The fluorescence of tryptophans in a protein is strongly dependent on its close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. Furthermore, since no secondary reporter fluorophores are required as in conventional DSF, protein solutions can be analyzed independent of buffer compositions. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control.

Here we present biophysical background behind the Prometheus NT.48 instrument and examples of different applications in both academic and industrial context.

We invite you to visit our booth and discuss your specific research topics where our tools may provide further insight.

Session IV:
Stress Response and Cancer
(Chairperson – Joanna Rzeszowska)

THE LINK BETWEEN RNA STABILITY AND CANCER DEVELOPMENT

Jolanta Jura

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

In response to external and internal stimuli the cells react in changes of the transcriptome profile. The transcriptome is dynamic and can be regulated at many levels. One of them is post-transcriptional mechanisms that rely on specific RNA-protein or RNA-RNA interactions that either result in the targeted degradation of the mRNA or prevent access of the ribosome to the translation start codon. Most mRNAs bear a cis-regulatory elements, important in regulation of their stability and/or availability at the 5' and 3' untranslated regions or within the decoded portion of the molecule.

The members of the Monocyte Chemoattractant Protein-1-Induced Protein family (MCPIP1/2/3/4) are dramatically induced by many stressful agents, such as lipopolysaccharide (LPS) or proinflammatory cytokines. These proteins posses PIN domain (the N-terminus of the PilT protein/**Pi**lT **N** terminus) that possesses ribonucleolytic activity. The active site of PIN domain consists of four aspartate residues that are engaged in coordination of a single magnesium ion localized in a catalytic cleft of this enzyme. Additionally, a special feature shared by this family is a single CCCH zinc finger domain responsible for a direct interaction with RNA.

Previously, MCPIP1 has been described as a protein regulating stability of transcripts coding for cytokines such as: IL-1β, IL-2, IL-6, IL-8, IL-12b, IL-17. However, further studies based on determination of transcriptome and proteome profiles followed by immunoprecipitation assays showed that MCPIP1 degrades transcripts involved in many cellular processes. Its level is significantly modulated not only during inflammatory processes but also in other types of cellular stress, as for example hypoxic conditions. Our results show that MCPIP1 has an impact on cancer development controlling angiogenesis, metabolism, protein quality control and proliferation.

Research presented in this study was supported by Polish National Science Centre (NCN) (grants: 2011/03/B/NZ1/00023 and *2015/17/B/NZ3/01051 assigned to J.Jura). Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University is a partner of the Leading National Research Center (KNOW) supported by the Ministry of Science and Higher Education.*

DYNAMICS AND HETEROGENEITY OF INFLAMMATORY SIGNALLING IN SINGLE CELLS

Paweł Paszek

University of Manchester, Manchester

Cells must accurately decode changing environmental signals to make fate decisions and coordinate tissue-level responses, but often exhibit substantial heterogeneity. Here we use a suite of single cell biology approaches including time-lapse microscopy and transcriptomics in combination with mathematical modelling to understand signalling via the Nuclear Factor kappa B (NF-kappaB) system, a master regulator of inflammatory and immune responses. Our analyses demonstrate that via a number of mechanisms the NF-κB system encodes complex temporal and spatial patterns associated with pathogen infections. Using transcriptomics we show that the NF-καππαB-dependent gene responses are controlled by gene-specific architecture at the single cell level. Altogether, these data demonstrate that seemingly heterogeneous single cell responses are defined by functional constrains, and thus might be predictable.

HARNESSING RADIATION INDUCED BYSTANDER SIGNALLING MECHANISMS FOR RADIOTHERAPY

Carmel Mothersill, Michelle Le, Colin Seymour, Andrew Rainbow, Fiona McNeill

McMaster University, Hamilton, Canada, mothers@mcmaster.ca

Recent efforts to discover the nature of the initial signal triggering the ionising radiation-induced bystander effect have led in two separate directions which appear to be mutually exclusive. Many groups have evidence that exosomes in transferred medium carry information in the form of miRNA which trigger responses in unirradiated cells. However our group have published several papers showing that no medium transfer is necessary and that a UVA signal generated as a result of secondary excitation decay following irradiation is able to trigger the bystander effect in unirradiated cells which are in separate sealed flasks. There are two possible explanations; either these are two alternative mechanisms or the exosomes are generated by the UV signal in both bystander and directly irradiated flasks. In order to test the latter hypothesis, we cultured HCT+/+ cells in medium containing exosome free serum and used our established protocols to generate bystander effects in unirradiated cells in separate flasks. The exosome fractions were harvested from both sets of flasks and from appropriate controls. These fractions were then added to new flasks of never exposed cells to see whether the bystander exosomes could in fact induce a bystander effect. The results suggest that bystander exosomes could indeed induce a bystander effect. Our conclusion is that secondary UV from excitation decay in irradiated cells may have an important role in triggering exosome production in bystander cells without the need for actual medium transfer. This new mechanism opens up the possibility of novel targets for use in radiotherapy.

REGULATION OF INNATE IMMUNITY GENES BY P53 TUMOR SUPPRESSOR PROTEIN

Marek Rusin, Artur Zajkowicz, Małgorzata Krześniak, Agnieszka Gdowicz-Kłosok

Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch,44-101 Gliwice, ul. Wybrzeże Armii Krajowej 15, Poland

The p53 protein coded by the major tumor suppressor gene *TP53* is best studied in the context of cancer formation. It is not surprising because *TP53* is the most frequently mutated gene in human cancers. P53 is a pleiotropic protein, which acting as a transcription factor, can up- and down-regulate the expression of hundreds of genes involved in control of cell cycle, apoptosis, metabolism, DNA repair and other cellular functions. Interestingly, p53 was discovered because it was able to form a tight complex with a large T antigen - a protein coded by an oncogenic virus SV40. Other tumor viruses also code for proteins, which bind and inactivate p53, e.g. the notorious oncogenic papillomaviruses (HPVs) induce p53 degradation by E6 protein. Surprisingly, p53 is also blocked by viruses, which are not carcinogenic, e.g. SARS coronavirus. Moreover, p53 is activated when a cell is infected by some viruses. Hence, there is apparently a strong antagonism between p53 and viruses. The cells have many antiviral proteins at their disposal. The antiviral effector proteins interfere with life cycles of viruses at every stage, from virus entry into cells to the formation of progeny viral particles. These proteins are a part of innate immunity, a system that defends cells against infectious agents, including viruses. This part if immune system was formed during millions of years of evolutionary arms race between cells and pathogens. Many antiviral effectors are up-regulated by interferons - signaling proteins secreted by some infected cells. Interestingly, it was found that many genes stimulated by interferons were also up-regulated by p53. The mechanism of this phenomenon is poorly studied. It is known that p53 directly up-regulates the transcription of some interferon-stimulated genes, e.g. *IFI16*, *ISG15*. Moreover, p53 also positively regulates the expression of transcription factors, e.g. IRF5, IRF7, which directly stimulate the transcription of interferon genes. We found that treatment of cells simultaneously with actinomycin D and nutlin-3a (A+N) strongly stimulated p53. Actinomycin D activates p53 by a mechanism, which is not well understood but it apparently involves activation of some kinases phosphorylating p53. Nutlin-3a activates p53 by inhibiting its negative regulator - MDM2. There is strong synergy between these two substances in inducing p53 phosphorylation on key amino acid residues like serine 46 and serine 392. Consequently, many known p53-target genes were synergistically induced. Our analysis of RNA-seq results revealed that approximately 500 transcripts were up-regulated at least 10-fold by A+N in A549 cells. The bioinformatic analysis of gene expression data showed that in addition to regulators of apoptosis, the A+N treatment preferentially activated antiviral genes. We have selected few of them, which were not previously identified as p53 targets, and we performed experiments strongly indicating that these genes were directly regulated by p53. One of these genes is a master regulator of interferons, coding for a protein triggered by viral cytosolic DNA molecules. Moreover, we found that p53 phosphorylation following A+N treatment was inhibited by C16, a compound believed to be a specific inhibitor of PKR kinase - an antiviral protein coded by interferon-stimulated gene. However, the cells with knocked-down expression of PKR were still sensitive to C16, what indicated that other enzyme was involved in p53 activation leading to the induction of innate immunity genes.

This work was supported by the Polish National Science Center NCN (Grants no.2013/11/B/NZ5/03190 to MR, 2014/15/D/NZ5/03410 to AG-K)

T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA FROM MIRNA TRANSCRIPTOME PERSPECTIVE

Małgorzata Dawidowska[1], Bronisława Szarzyńska-Zawadzka[1], Roman Jaksik[2], Monika Drobna[1], Maria Kosmalska[1], Łukasz Sędek[3], Tomasz Szczepański[3], Michał Witt[1]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland; [2]Silesian University of Technology, Gliwice, Poland; [3]Medical University of Silesia, Zabrze, Poland

T-cell acute lymphoblastic leukemia (T-ALL) is a rare, aggressive and heterogenous subtype of acute lymphoblastic leukemia (ALL), the most common malignancy in children. Clinical, genetic and immunophenotypic heterogeneity of T-ALL and insufficient understanding of its pathogenesis hampers further improvement in stratification and treatment strategies. miRNAs, as important regulators of gene expression, shape cells' phenotype. When aberrantly expressed, miRNAs serve as oncogenes or tumor suppressors (by silencing tumor suppressor genes and oncogenes, respectively). Exploring miRNA transcriptome with the use of next-generation sequencing (NGS) might aid the understanding of both the heterogeneity and pathogenesis of this leukemia.

In this project we aimed to identify miRNA transcriptome of pediatric T-ALL, with the focus on miRNA expression profiles reflecting T-ALL heterogeneity. We aimed to assess the potential of miRNA-seq as a classification and prognostic tool in pediatric T-ALL. We finally aimed to identify novel candidate oncogenic/tumor suppressor miRNAs, their target genes and related pathways, to get insights into T-ALL pathogenesis.

Study group consisted of 34 pediatric T-ALL patients and 5 age-related healthy bone marrow donors. Total RNA, including small RNA fraction, was isolated from CD3+ cells selected immunomagnetically (by negative selection) from bone marrow samples. miRNA sequencing was performed using NextSeq500 Illumina platform (10mln reads/sample, 51bp single-end; NGS Service Exiqon). Raw sequencing reads were adapter trimmed using cutadapt and aligned with bowtie to a modified version of miRBase v21 (mature sequences) created according to the miRge specifications. Unaligned reads were sequentially matched against hairpin miRNA (miRBase v21) noncoding RNAs, Ensembl cDNA database and again to mature miRNA sequences using less stringent criteria. Differentially expressed miRNAs were selected using edgeR. Reference miRNAs for RT-qPCR validation were selected using NormFinder. Target genes for all differentially expressed miRNAs were identified using DIANA-microT, ElMMo, MicroCosm, miRanda, miRDB, PicTar, PITA and TargetScan databases gathered trough the multiMiR Bioconductor library. Genes were assumed to be regulated by a particular miRNA if the association was predicted by more than 5 out of 8 methods used. Target genes for all miRNAs differentially expressed between normal and T-ALL samples were tested for overrepresentation among KEGG, Reactome and Panther pathways as well as Gene Ontology biological processes using Fisher's exact test with Benjamini and Hochberg correction for multiple testing.

We showed that miRNA expression profile is discriminative between pediatric T-ALL and healthy bone marrow controls. We also identified miRNA expression profiles associated with immunophenotypic subtypes of T-ALL (EGIL stages: II, III, IV), reflecting maturation stages of T-cells.

We identified 23 miRNAs overexpressed in T-ALL vs. healthy controls (including known and candidate novel oncogenic miRNAs in T-ALL). Among these are miRNAs with mRNA target genes known to be implicated in T-ALL pathogenesis, including: miR-20b-5p, miR-363-3p, miR-128-3p, miR-181b-5p, miR-181a-5p. We identified 38 underexpressed miRNAs (potential tumor suppressors). Among these are miRNAs with known T-ALL-implicated targets: miR-145-5p, miR-143-3p, miR-27a-5p, miR-24-3p, miR-10b-5p.

By testing overrepresentation of mRNA targets for miRNAs differentially expressed between T-ALL and controls, we showed significant target enrichment in KEGG pathways and GeneOntology terms, including among others: positive regulation of apoptosis, regulation
of kinase activity, interleukin-6-mediated signaling pathway, and lymphocyte differentiation.

With use of NGS, we provide insights into the heterogeneity of pediatric T-ALL at the miRNA transcriptome level. Expression profiles of miRNAs differentially expressed in patients vs. controls and in T-ALL immunophenotypic subtypes (EGIL: II, III, IV) hold potential for improved classification of T-ALL. Individual differentially expressed miRNAs are candidates for functional test to assess their oncogenic/tumor suppressor role in T-ALL and for association analysis (survival & treatment response) to assess their prognostic potential in T-ALL.

Research funded by National Science Centre, Poland grant: 2014/15/B/NZ2/03394 and National Centre of Research and Development (NCRD) grant STARTEGMED3/304586/5/NCBR/2017.

Session V

Radiation Biology and Medicine

(Chairperson – Piotr Widłak)

IMMUNE MODULATORY PROPERTIES OF RADIOTHERAPY – RATIONALES FOR COMBINATION WITH IMMUNOTHERAPY

Udo S. Gaipl

Department of Radiation Oncology, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
udo.gaipl@uk-erlangen.de

Radiotherapy (RT) is a common treatment for cancer and about 60% of all cancer patients will receive it during their course of illness. RT primarily aims to achieve local tumor control. The induction of DNA damage, tumor cell death and the modulation of the tumor microenvironment are the main effects of ionizing irradiation to reduce tumor masses, but also to modulate the immune system. RT might act as an *in situ* cancer vaccine under certain microenvironmental conditions. However, RT also fosters the upregulation of immune suppressive molecules such as the programmed cell death receptor ligand 1 (PD-L1, CD274). Ionizing radiation in general has been demonstrated to impact on the immune system and in dependence on the radiation dose particular immune modulations take place. The presentation will focus on how local irradiation changes the tumor cell phenotype and the tumor microenvironment and consecutively does impact on local and systemic changes in immune cell compositions. The dynamics of immune changes, the radiosensitivity of distinct immune cells as well as biological basis for reasonable combination of RT with immune stimulation will be discussed in detail, as well as how radiation-induced immune suppression can be overcome. Regarding the latter, the impact of radiotherapy and chemotherapy on immune checkpoint molecule expression will be outlined and possible mechanisms for that will be discussed. Based on the pre-clinical knowledge, innovative clinical study concepts of radio-immune treatments will be presented. We conclude that knowledge on immune modulations induced by ionizing radiation is important to optimize multimodal cancer therapies aiming to achieve local and systemic tumor control and to define immune-related biomarkers of radiation exposure for prognosis and prediction.

CAN TUMOUR CONTROL PROBABILITY (TCP) BE PRESERVED WHEN STANDARD FRACTIONATION RADIATION THERAPY IS INTERRUPTED?

Krzysztof Składowski

Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch, Poland

Nowadays high-precision, standard-fractionation radiation therapy (5 fractions over 5 weekdays by 6-7 weeks) is compliant in vast majority of cancer patients. However, some of them has disturbed treatment completion, mainly due to the gaps (accidental or health-related) over fractionation schedule. So called "*Gap-Position Phenomenon*" discovered in 1994 at the Gliwice Institute suggests decrease of TCP to a various degree depending on treatment breaks occurrence at least in head and neck cancer patients receiving definitive radiotherapy. The presentation shows biological modelling of dose-time-volume parameters in order to prevent the risk of TCP gap-dependent loss over the interrupted radiation treatment.

NANORADIOTHERAPY: WINDOW TO THE WORLD OR DEAD END?

Marcin Kruszewski[1,2], Katarzyna Sikorska[1], Teresa Bartłomiejczyk[1], Hanna Lewandowska-Siwkiewicz[1], Barbara Sochanowicz[1], Sylwia Meczynska[1], Anna Lankoff[1], Maria Wojewodzka[1]

[1]Institute of Nuclear Chemistry and Technology, Dorodna 16, 03-195 Warszawa; [2]Institute of Rural Health, Jaczewskiego 2, 20-090 Lublin

Nanomaterials (NMs) are defined as engineered or natural objects having at least one dimension less than 100 nm, whereas nanoparticles (NPs) are the objects having all dimensions less than 100 nm. NPs are a product of natural processes or are man-made. Anthropogenic NPs can be divided into two groups: (1) unintentionally created NPs that include diesel engines exhaust, ashes and other products of combustion, such as PM2.5 and PM10, and (2) NPs intentionally designed by man for different applications in medicine, industry and everyday life, i.e., the so-called "engineered NPs". A small size of NPs, comparable with the size of biomolecules, favours their interaction with biological systems. In addition, a large surface to volume ratio implies different physical, chemical and biological properties, as compared to a bulk material.

Nanotechnology is one of the most rapidly developing fields of science. Here, we are going to describe the use of nanomaterials in medicine, especially in radiotherapy and imaging. Different aspects of the use of NPs in radiotherapy and imaging will be described, including direct use of NPs made of radioisotopes, the use of NPs as a radioisotope carriers and the use of non-radioactive NPs as a radiosensitizers.

This work was supported by Polish National Science Centre projects: 2016/23/D/NZ7/03842 and 2015/19/B/NZ7/02166, and statutory financing for IRH.

MICRORNAS AS BIODOSIMETERS AND BIOMARKERS IN RADIATION ONCOLOGY

Wojciech Fendler[1], Beata Malachowska[1], Khyati Meghani[2], Panagiotis A. Konstantinopoulos[3], Chandan Guha[4,5], Vijay K. Singh[6, 7], Dipanjan Chowdhury[2]

[1]Department of Biostatistics and Translational Medicine, Medical University of Lodz, Poland; [2]Department of Radiation Oncology, Harvard Medical School, Dana-Farber Cancer Institute, Boston, Massachusetts, USA; [3]Medical Gynecologic Oncology Program, Dana-Farber Cancer Institute, Harvard Medical School; [4]Department of Radiation Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York, USA; [5]Department of Pathology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York, USA; [6]Department of Pharmacology and Experimental Therapeutics, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA; [7]Armed Forces Radiobiology Research Institute, Bethesda, Maryland, USA

Effective planning for medical response to a radiologic or nuclear accident is complex. Due to limited resources for medical countermeasures, the key would be to accurately triage and identify victims most likely to benefit from treatment. Similarly, predicting the occurrence of radiation toxicity during radiotherapy is a field of intensive studies in oncology. Our recent works performed in animal models and during clinical studies have advanced the field by formulating an efficient algorithm for the detection of high-dose, potentially lethal irradiation.

Our tool relies on microRNAs – an emergent class of serum biomarkers that show remarkable stability, ease of quantification and good replicability. The diagnostic model was devised by a series of irradiation experiments on mice and non-human primates (*Macaca mulatta*). The diagnostic model itself is a two-tiered classifier that uses miR-133b, miR-215, miR-375 expression to identify exposed individuals (Area under the ROC curve 0.99 (95%CI 0.98-1.00)) and, by using sex-adjusted expression levels of miR-30a and miR-126 provides a for irradiation and an AUC of 0.79 (95%CI 0.56-1.00) to identify NHPs that would die after the exposure to doses within the LD_{30}-LD_{70} range. Furthermore, by overlapping these results we identified a subset of miRNAs that after bioinformatic analyses revealed evolutionary conservation of miRNAs themselves and their promoter regions, evidencing mechanistic involvement in the mitigation of radiation exposure's effects.

In conclusion, measurement of serum miRNA expression may be used as an efficient diagnostic tool in scenarios of mass exposure to unknown doses of radiation, in order to facilitate screening for patients that will require bone marrow transplantation to survive.

Session VI

Molecular Biomarkers

(Chairperson – Katarzyna Lisowska)

FINE-TUNING CLINICAL PROTEOMICS FOR BIOMARKER IDENTIFICATION AND QUALIFICATION

Marc Baumann

Medicum, Department of Biochemistry and Developmental Biology, Meilahti Clinical Proteomics Core Facility, University of Helsinki, Helsinki, Finland

Clinical proteomics has yielded some early positive results for the identification of potential disease biomarkers indicating the promise for this analytical approach to improve the current state of the art in clinical practice. However, the inability to verify some candidate molecules in subsequent studies has led to skepticism among many clinicians and regulatory bodies, and it has become evident that commonly encountered shortcomings in fundamental aspects of experimental design and the limitations of the current technology mainly during biomarker discovery must be addressed in order to provide robust data. In this perspective, it is asserted that successful studies generally use suitable statistical approaches for biomarker definition and that the results can be confirmed in independent test sets by various means. In this presentation I will show that the understanding of the definition of a biomarker is actually surprisingly broad and by no means reflecting only the limitations and shortcomings in clinical proteomics. I will then highlight the new trends in overcoming current technical limitations in clinical proteomics and finally end with a vision for the future.

OSTEOPONTINS AS BIOMARKERS IN BREAST CANCER

Piotr Ziółkowski[1], Georg F. Weber[2]

[1]Department of Pathology, Wrocław Medical University, Poland; [2]College of Pharmacy, University of Cincinnati Academic Health Center, Cincinnati, Ohio, USA

Introduction: Progress in imaging and biopsy of breast tissue has enabled the detection of early lesions with variable degrees of risk for transformation, raising the question who should receive preemptive treatment to counteract the potential for a progression to breast cancer. Because the secreted metastasis mediator Osteopontin (OPN) is a marker for breast cancer progression, its presence in premalignant breast lesions may reflect progression risk. The splice variant Osteopontin-c is absent from healthy tissue but it is associated with about 75% of breast cancer cases.

Methods: By immunohistochemistry, (1) we analyzed the association of Osteopontin variant expression with pre-cancerous breast disease. We covered healthy breasts, hyperplasias, papillomas, and carcinomas in situ from 415 women to assess a) staining for OPN exon 4 or OPN-c in low-risk to high-risk lesions, and b) correlations between staining and relapse or survival, and (2) we have also analyzed 671 patients, comprising a cohort of 291 paraffin blocks plus a population-based case-control study of 380 arrayed breast tumor tissues.

Results: (1) The markers correlated with risk. They were prognostic for relapse and survival. More than 95% of women, who experienced a relapse had pathology scores of 2-3 for OPN-c intensity at the time of initial diagnosis. 0% of women free of OPN-c (pathology score 0), and about 10% of OPN-c pathology score 1 relapsed over 5 years. When combining OPN-c and OPN exon 4 staining, all of the low intensity patients were alive after 5 years, whereas women in the high category have a 50% chance to die within 5 years. Of patients who succumbed, close to 80% had a high score at the time of initial diagnosis.

(2) We found that high staining intensity of nuclear Osteopontin-c was strongly associated with mortality in patients with invasive breast cancer. Cytosolic staining for exon 4, reflective of Osteopontin-a and -b also predicted poor outcome. Consistent with its role in tumor progression, not tumor initiation, Osteopontin-c was not correlated with proliferation markers (Ki-67, cyclins A, B, E and D), neither was it correlated with ER, PR or HER2.

Summary: The addition of OPN splice variant immunohistochemistry to standard pathology work-ups has the potential to aid decision making in breast cancer prevention, may have prognostic benefit in breast cancer diagnosis, and taking a decision about the treatment based on analysis of 119 breast cancer cases treated with radio- or/and chemotherapy.

THE PHENOMENON OF MEMBRANOUS/CYTOPLASMIC MIB-1 IMMUNOPOSITIVITY IN BREAST CANCER

Joanna Niemiec[1], Agnieszka Adamczyk[2], Aleksandra Ambicka[1], Anna Mucha-Małecka[3], Wojciech M. Wysocki[4], Kaja Majchrzyk[2], Janusz Ryś[1]

[1]Department of Tumor Pathology; [2]Department of Applied Radiobiology; [3]The Oncology Clinic; [4]Department of Surgical Oncology, Centre of Oncology, Kraków, Poland

Objectives: Applying the MIB-1 clone of anti-Ki-67 antibody (which is currently endorsed for assessment of the Ki-67 labeling index), in some neoplasms a membranous/cytoplasmic staining pattern is observed. According to current recommendations, the above-mentioned staining, if observed in tumor cells, should be ignored while creating a Ki-67 index. For pathologists, a recommendation on how to proceed in such cases should be valuable.
Therefore the pattern of immunonopositivity of MIB-1 and BGX-Ki-67 clones; as well as their labelling inexes were compared.
Methods: In a group of 156 patients with invasive ductal breast cancer based on immunohistochemistry with MIB-1 and BGX clones; both labeling index (MIB-1LI; BGXLI) and pattern of immunopositivity were evaluated both in primary tumors (T) and synchronous lymph node metastasis (LNM).
Results: In addition to nuclear MIB-1 staining, membranous/cytoplasmic labeling was found in 23 of 145 primary tumors and 19 of 144 T LNM. In these cases, BGX-Ki-67 showed exclusively nuclear labeling and presented significantly higher labeling index. Survival analysis revealed that, high BGXLI(T) was a significant independent negative prognostic factor for disease-free survival. Moreover, based on BGXLI(T)/BGXLI(LNM), patients with high MIB-1LI(T) were stratified into low- and high-risk carriers.
Conclusions: In carcinomas with membranous/cytoplasmic MIB-1 staining, additional assessment of BGXLI may be recommended. It could help in defining breast cancer subtype and in selection of individuals at risk who, despite appropriate therapy, would benefit from more frequent controls aimed at earlier implementation of second-line treatment.

The study was supported by grant number NN 401 096 137 from the Polish Ministry of Science and Higher Education.

LUNG CANCER SREENING – MOVING FORWARD

Witold Rzyman

Department of Thoracic Surgery, Medical University of Gdańsk, Gdańsk, Poland

Since February 2015 lung cancer screening by low-dose computed tomography (LDCT) in high-risk individuals is covered by the US health insurance. This considerable step ahead towards a reduction of lung cancer mortality in the future, has its roots in the National Lung Screening Trial that showed mortality reduction of more than 20%. It appears likely that LDCT will be the mainstay of early lung cancer detections for years to come. On the other hand, it would be good to improve the diagnostic work-up efficiency for lung cancer screening as it could improve the cost efficiency of the screening process while also reducing the potential for "harm". Further research could also suggest more tailored approaches to the surgical management of screen-detected lung cancer that could reduce the potential for overtreatment.

In Europe, cost-effectiveness and low detection rate seem to be stumbling blocks to lung cancer screening implementation. The potential step forward is better selection of screening population. There is intensive search underway for molecular signature of early lung cancer that would enhance preselection of LDCT screening candidates. The consortium consisting of Gdańsk Medical University, Gliwice Oncology Institute and Silesian Technology Institute has participated since 2010 in lung cancer biomolecular signature research program based on blood samples collected from Gdańsk Lung Cancer Screening participants. Three independent early lung cancer signatures were identified and submitted for patent verification. In this presentation the concept of research in the field will be presented along with difficulty-laden scenario of research support by biotechnological and pharma industries as well as actual implementation to everyday practice in Polish reality.

PERSONALIZED TREATMENT FOR OVARIAN CANCER

Katarzyna M. Lisowska[1], Katarzyna A. Kujawa[1], Aleksander J. Cortez[1], Patrycja Tudrej[1], Aleksandra Stańczak[2], Maciej Wieczorek[2], Magdalena Olbryt[1]

[1]Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Institute – Oncology Center, Gliwice Branch, 44-101 Gliwice, Poland; [2]Innovative Drugs R&D Department, Celon Pharma Inc., Mokra 41a, 05-092 Lomianki/Kielpin, Poland

Ovarian cancer is the most lethal gynecological malignancy in the Western world. So far, there is lack of methods recommended for screening and early detection. As the disease develops without any specific warning symptoms for a long time, it is usually diagnosed at an advanced stage. State of the art treatment for advanced ovarian cancer is maximal possible debulking surgery and adjuvant chemotherapy based on platin derivatives and taxanes. Although majority of patients respond well to this treatment, they frequently develop recurrence and chemoresistance.

Currently, there are many biological drugs under development and tested in the ongoing clinical trials; some have recently entered into clinics. These drugs are mostly directed against molecular targets and pathways that are indispensable for cancer cells proliferation, tumor growth and escape from immune surveillance and death signals. These are e.g. anti-angiogenic factors, inhibitors of growth factor signaling, polyADP-ribose polymerase (PARP) inhibitors, or folate receptor inhibitors. In addition, there are many immunotherapeutic approaches tested in early phase trials. So far, these new agents and therapeutic approaches were not shown to cure advanced ovarian cancer, but they may lead to the delay of recurrence or stabilization of the disease. Thus, there is a real perspective that ovarian cancer will become a kind of manageable chronic disease. However, at present the cost-effectiveness of the new drugs is challenging from the perspective of national health care systems.

Our recent studies have been focused on the search for new prognostic markers for ovarian cancer. We have shown that several genes/proteins related with tumor stroma structure and function (e.g. fibronectin, periostin, integrin β-like protein 1) are related with poor prognosis when upregulated in ovarian cancer [1, 2]. In addition, we are engaged in in vitro testing of a new inhibitor of fibroblast growth factor (FGFR) developed by Polish pharmaceutical company Celonpharma. Interestingly, this inhibitor, CPL-304-110, shows higher efficacy against ovarian cancer cells than control inhibitor.

This study was partially supported within "CELONKO" project (STRATEGMED2/ 266776/17/NCBR/2015), co-financed by the Polish National Center of Research and Development and pharmaceutical company CelonPharma S.A.

[1] Lisowska et al. 2014. Front Oncol DOI:10.3389/fonc.2014.00006.
[2] Lisowska et al. 2016. J Cancer Res Clin Oncol DOI:10.1007/s00432-016-2147-y.

Poster abstracts

Number next to the abstract title correlates with poster number in three groups:
A – Bioinformatics and modeling (A1-A28)
B – Regulation of cellular processes (B1-B56)
C – New molecules, new methods and therapies (C1-C40)

[C-6] RELATION BETWEEN HER2 GENE STATUS AND SELECTED POTENTIAL BIOLOGICAL FEATURES RELATED TO TRASTUZUMAB RESISTANCE AND ITS INFLUENCE ON SURVIVAL OF BREAST CANCER PATIENTS` UNDERGOING TRASTUZUMAB ADJUVANT TREATMENT

Agnieszka Adamczyk[1], Anna Kruczak[2] , Agnieszka Harazin-Lechowska[2], Aleksandra Ambicka[2], Aleksandra Grela-Wojewoda[3], Małgorzata Domagała-Haduch[3], Anna Janecka-Widła[1], Kaja Majchrzyk[1], Janusz Ryś[2], Joanna Niemiec[2]

[1]Department of Applied Radiobiology, [2]Department of Tumour Pathology, [3]Department of Systemic and Generalized Malignancies, Maria Sklodowska-Curie Institute-Oncology Centre, Cracow Branch,31-115 Cracow, ul. Garncarska 11, Poland

Human epidermal growth factor receptor type 2 (HER2) is one of the most important oncogenes in breast cancer and plays important role in cellular processes such as: proliferation, invasion, metastasis and enhanced survival of cancer cells. Overexpression of HER2 is observed in about 20-25% of invasive breast cancer cases. To block activity of HER2, trastuzumab, which is an antibody against the extracellular fragment of HER2, is currently implemented in treatment schedules applied for breast cancer.

The aim of the study was to investigate if parameters associated with HER2 status (HER2 gene copy number, HER2/CEP17 ratio, polysomy) are linked to various potential biological features related to trastuzumab resistance (PTEN, IGF-1R, MUC4, EGFR, HER3, HER4 expression and mutation status of PIK3CA) as well as their relation to survival of HER2 positive breast cancer patients treated with adjuvant chemotherapy and trastuzumab.

The investigated group consisted of 117 patients with invasive ductal breast cancer ($T\geq1$, $N\geq0$, M0) with overexpression of HER2, who underwent radical surgery between 2007 and 2014. Status of ER, PR, HER2 expression was retrieved from patients files. HER2 gene copy number has been investigated by FISH using PathVision HER-2 DNA Probe Kit II. Expression of PTEN, IGF-1R, MUC4, EGFR, HER3, HER4 was assessed immunohistochemically on formalin-fixed paraffin-embedded tissue sections. PIK3CA mutations (H1047R and E545K) status was determined by qPCR analysis.

Higher level of HER2 protein (IHC 3+) and ER negativity corresponded to higher HER2 copy number and HER2/CEP17 ratio ($p<0.001$). Tumours with polysomy were characterized by higher HER2 gene copy number but lower HER2/CEP17 ratio ($p<0.026$, $p<0.001$ respectively). Patients with tumours featuring HER3 immunonegativity or low HER2/CEP17 ratio (≤4) were characterized by 100% metastasis-free survival ($p=0.018$, $p=0.062$, respectively). Presence of two negative factors simultaneously: HER3 expression with high HER2/CEP17 ratio also allowed to distinguish group of patients with unfavorable prognosis with ($p=0.001$)

HER2/CEP17 ratio seems to have little effect on metastases-free survival. However, high HER2/CEP17 ratio combined with HER3 expression clearly distinguished patients with poorer prognosis.

The study was financed by the National Science Centre, based on the decision numbered DEC-2013/09/B/NZ5/00764.

[C-17] COORDINATION COMPOUNDS OF PLATINUM(II) WITH 4'-SUBSTITUTE 2,2':6',2"-TERPIRYDINE - STRUCTURE, PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES

Sandra Altmann[1,2], Magdalena Skonieczna[3,4], Anna Maroń[5], Katarzyna Czerwińska[5], Dorota Zygadło[1,2], Agata Szlapa-Kula[6], Stanisław Krompiec[6], Alicja Ratuszna[1,2], Barbara Machura[5], Agnieszka Szurko[1,2]

[1]August Chełkowski Institute of Physics, University of Silesia, Uniwersytecka 4, 40-007 Katowice, Poland [2]Silesia Center for Education and Interdisciplinary Research, 75 Pułku Piechoty 1A, 41-500 Chorzów, Poland [3]Silesian University of Technology, Center Biotechnology Bioengineering and Bioinformatics, Gliwice, Poland [4]Silesian University of Technology, Institute of Automatic Control, Gliwice, Poland [5]Department of Crystallography, Institute of Chemistry, University of Silesia, 9th Szkolna St, 40-006 Katowice, Poland [6]Department of Inorganic, Organometallic Chemistry and Catalysis, Institute of Chemistry, University of Silesia, 9th Szkolna St., 40-006 Katowice, Poland

The incidence of cancer is constantly increasing, therefore we are looking for new medicinal substances, that can be used in chemotherapy. Platinum(II) based drugs are leading chemotherapeutics used in most cancer therapeutic schemes since 1969, when anticancer activity of cisplatin was discovered[1]. The limitation of the use of cisplatin and its commercially available derivatives is conditioned by numerous side effects (neurotoxicity, myelotoxicity, nephrotoxicity, etc.), as well as the tumor cell resistance to these drugs. To overcome the drawbacks of current platinum(II)-based drugs there has been a search towards new platinum drugs that are structurally different to cisplatin or demonstrate different modes of binding[2]. In this regard, platinum(II) compounds with 4'-substituted derivatives of 2,2':6',2"-terpyridine as cytostatic potential are interesting candidates for further research[3].

For four coordination compounds of platinum(II) with 4'-substituted derivatives of 2,2':6',2"-terpyridine with the general formula [PtCl(4'-R-terpy-κ3N)]X (where R = 2,2'-bitiofen-5-yl, 2,4-difluorophenyl, X = BPh4-, 2-pyridyl, 3-pyridyl, X = CF3SO3-) there have been described physicochemical properties and biological activity in relation to their anticancer potential. These compounds were obtained by known synthetic methods using two different platinum(II) precursors - K2[PtCl4][4,5] and [PtCl2(PhCN)2][6], and their molecular structure was confirmed by X-ray structural analysis, elementary analysis, NMR and FT-IR spectroscopy. Their luminescence properties were also determined.

As part of preliminary biological in vitro studies we investigated cytotoxicity of the platinum complexes in several cancer and normal cell lines using the standard MTS assay. Our research has shown special properties of one of the four represented compounds [PtCl(4'-(2,4-F2C6H3)-terpy-κ3N)]BPh4. Strong cytotoxicity to glioblastoma cells A-172 (IC_{50} = 0,43μM) qualifies this coordination compound for further, more detailed studies such as cell cycle analysis by flow cytometry and apoptosis/necrosis analysis also by flow cytometry (Annexin-V Assay). The cell cycle distribution, ROS production, together with mitochondrial mass and potential analysis have confirmed the apoptosis activity of the tested compounds.

The effect of action of the compound with platinum is visible no earlier than after 72 hours, when apoptosis (marked with Annexin-V) is clearly obvious. Also sub-G1 appears in the cell cycle. After 6 hours a virtual explosion of ROS is observed, which causes cell cycle arrest – the cells are in the G1 block and do not divide. It is also confirmed by the mitochondrial mass (no growth, continuous fall). Furthermore, mitochondrial potential decreases after 48 hours, which correlates with apoptosis and denotes the spread of mitochondria leading to cell death.

Reference:
[1] B. Rosenberg et al. Nature 222 (1969) 385
[2] S. J. Lippard. Chem. Rev. 116 (2016) 3436
[3] M. Fanelli et al. Coord. Chem. Rev. 310 (2016) 41
[4] R.N. Keller, T. Moeller, Inorg. Synth. 7 (1963) 247
[5] G. Annibale et al. Polyhedron 14 (1995) 45
[6] S. C. Dhara, Indian J. Chem. 8 (1970) 193

[C-1] APPLICATION OF FLUORESCENT IN SITU HYBRIDIZATION FOR INVESTIGATION OF MICROBIAL COMMUNITY COMPOSITION IN ANAMMOX BIOMASS

Anna Banach[1], Sebastian Student[2], Aleksandra Ziembińska – Buczyńska[1]

[1]Environmental Biotechnology Department, Silesian University of Technology, Gliwice, Poland; [2]Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland

Fluorescent in Situ Hybridization (FISH) plays an important role in a variety of research areas, including gene mapping, studies of biological processes, cytogenetics and tumor biology. This technique is also widely used in microbial ecology for investigation of microbial community composition. Hybridization with rRNA-targeted fluorescent oligonucleotide probes, allows the identification and quantification of individual cells in samples from natural ecosystems or technological systems. FISH probes are also frequently used to detect and quantify the number of microorganisms in wastewater treatment plants, in nitrifying and anammox-based reactors.

In the present study FISH protocol was optimized for determination the bacterial community structure in activated sludge performing anammox process in Sequencing Batch Reactor. In addition, method of sample preparation for FISH was optimized for detection microorganisms immobilized in the carriers. FISH technique were used both for suspended biomass in the reactor and for immobilized anammox biomass. Specific probes targeting bacterial RNA have been used for different bacterial groups involved in the nitrogen cycle: anammox bacteria (Amx368, Amx820, Amx1015 and general Planctomycetes-specific probe: Pla46), ammonia oxidizing bacteria (probes for Betaproteobacterial ammonia-oxidizing bacteria: Nso190, Nso1225) and nitrite oxidizing bacteria (probe for phylum Nitrospirae: Ntspa712).

Optimizing samples preparation procedure and hybridization parameters allowed to observe 3D anammox aggregates under confocal microscope, and characterization of the investigated biomass was possible. The spatial organization of anammox bacteria and coexistence with other microorganisms in flocks from suspended biomass were revealed. In addition, optimization of sample preparation for immobilized anammox biomass was helpful to determine presence of specific groups of microorganisms entrapped in the carriers and immobilized on bacterial cellulose.

The research was supported by National Science Centre grants: UMO-2013/09/D/NZ9/02438 and 2016/23/N/NZ9/02147

[A-24] NEONATAL HEAD SELECTIVE BRAIN COOLING – SENSITIVITY OF OVERALL HEAT TRANSFER ON SELECTED MATERIAL DATA

Dominika Bandoła[1], Wojciech Walas[2], Andrzej J. Nowak[1*], Ziemowit Ostrowski[1]

[1]Biomedical Engineering Laboratory, Institute of Thermal Technology, Silesian University of Technology, Gliwice, Poland; [2]Department of Children Intensive Care, University Hospital Clinic, Opole, Poland
**Correspondence: Konarskiego 22, 44-100 Gliwice, Poland, andrzej.j.nowak@polsl.pl*

The purpose of this work is to present the results of initial calculations concerning the heat transfer phenomena between the neonate's brain and the cooling device (Olympic Cool-Cap® System, Natus Medical Inc., USA) during selective hypothermia therapy.

In about two to four cases per thousand births, newborns suffer from hypoxic-ischaemic encephalopathy (HIE) which is the brain damage caused by a shortage of the oxygen and reduced cerebral blood flow.

A few years ago the effective, hypothermal methods of treatment were proposed. They can be classified into two types: body cooling (by use of the cooling wrap) and selective brain cooling (carried out by Cool-Cap). Both of them can improve the neurological outcome, reduce long-term neurodevelopmental sequelae and even prevent deaths [1].

The effectiveness of the hypothermal therapy is related to the time and intensity of cooling and rewarming process. Therefore, the cooling should be conducted as fast as possible but rewarming - as slowly as possible. This may also prevent adverse events. Especially the last stage of therapy – rewarming – is considered to be essential and the most difficult. Neonatologists agree that this should be improved and standardized. So far, they manually manage the process based on three temperature measurements: rectal, abdominal skin and forehead skin. Nonetheless, it is impossible to prepare the guidelines without data of the temperature field within the newborn's body and appropriate heat flux transported from and into the newborn's brain [1].

This paper concerns the part of studies focused on the heat transfer between body and the Cool-Cap device.

Sensitivity analysis of the heat transfer on various materials of the cap and its insulation was performed. Moreover, the impact of the ambient conditions (temperature and the heat transfer coefficient) was analyzed. Those calculations were completed using Computational Fluid Dynamics model of the simplified Cool-Cap device. The boundary conditions were chosen based on real measurement data to mimic real therapy conditions.

[1] J.E.Łaszczyk, A.J.Nowak, The Analysis of the Newborn's Brain Cooling Process. Measurements and CFD modelling, Lambert Academic Publishing, 2014

The research was supported by Faculty of Energy and Environmental Engineering within Ministry of Science and Higher Education (Poland) statutory research funding scheme. This help is gratefully acknowledged herewith.

[A-14] HOW DOES SAMPLE PREPARATION IN MALDI-MSI IMAGING INFLUENCE THE MOLECULAR SIGNATURE

Katarzyna Bednarczyk[1], Denis A.Sammour[2], Carsten Hopf[2], Joanna Polańska[1]

[1]Data Mining Group, Faculty of Automatic Control, Electronics, and Computer Science, Silesian University of Technology, Gliwice, Poland; [2]Center for Applied Research in Biomedical Mass Spectrometry (ABIMAS), Mannheim University of Applied Sciences, Mannheim, Germany

Introduction: Mass spectrometry imaging is a standard analytical technique which allows measuring the space-oriented distribution of different molecules in tissue-based studies. Nowadays this technique is used for various types of research in the biomedical field, especially in cancer study. Developments in MSI technology have led to the emergence of various workflows of sample preparation methods. Recent works focus on finding proper scores that could be used for quantitative comparison of methods.
Aim: This work is an attempt to evaluate the homogeneity of tissue molecular fingerprint obtained with the use of 5 different methods of sample preparation.
Material and methods: Five sample preparation methods, have been tested on liver and gist tissue samples. These five methods were different in terms of dewaxing, heat-induced antigen retrieval, enzyme application, tissue digestion and MALDI matrix application. MALDI ToF spatially distributed spectra were collected by MALDI-TOF/TOF Bruker Daltonik spectrometer with positive mode in a mass range of m/z 500-5000. Spectra preprocessing consisted of several steps, including resampling to common m/z channels, noise reduction based on Savitzky–Golay filter, adaptive baseline correction, PAFFT alignment, and normalization to mean TIC. The average spectrum per each sample preparation technique was subjected to Gaussian mixture modelling (GMM), and then GMM component filtering, spectra and GMM components convolution, and finally z-score normalization to determine the features' intensities (peptide abundance) for comparative analysis. Comparative analysis of GMM model complexity and the coefficient of abundance variation applied to all peptides were used and gave the information of repeatability and reproducibility of the different methods on gist and liver tissue samples. Kruskal–Wallis test, Benjamini–Hochberg multiple comparison correction and Dunn's multiple comparison test were used to find peptides significantly different between methods.
Results: The common GM model constructed for all sample preparation methods together consisted of 4605 peptides (features) in case of liver tissue, and 4723 while gist samples were considered. The coefficient of peptide abundance variation (CV) for each method served as performance index in homogeneity study. The most stable method, independently of tissue type, was the method numbered as 3, adapted from Heijs et al. (2015) for use with FFPE tissue, with median(CV)liver = 42% and median(CV)GIST = 38%. The median(CV)liver for the remaining methods were equal to 54% (method 1), 55% (method 2), 49% (method 4), 47% (method 5), respectively, while median(CV)GIST were equal to 52% (method 1), 51% (method 2), 52% (method 4), 48% (method 5). The comparative analysis revealed 204 peptides significantly differentiating across sample preparation methods for liver tissue and 808 for gist tissue. While the GM models were constructed for each method independently, the most complicated model was obtained for method 2 with 27146 components for GIST samples, while the simplest model contained only 22030 components (method 3).
Conclusions: Our study allowed to select the most stable and repeatable sample preparation method. Comparative statistical analysis identified a set of the most variable peptides across methods. We observed greater molecular complexity in the cancer tissue.

The work was financially supported by National Science Centre, Poland NVN BiTIMS grant no 2015/19/B/ST6/01736 (JP), and BKM grant no BKM/508/RAU1/2017/t.28 (KB)

[B-28] CHANGES IN EXPRESSION PROFLES OF GENE RELATED TO THE AUTOPHAGY

Martyna Bednarczyk[1], Małgorzata Muc-Wierzgoń[1], Klaudia Simka[1], Urszula Mazurek[2]

[1]Department of Internal Medicine, School of Public Health in Bytom, Medical University of Silesia in Katowice, Poland; [2]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, Poland

Autophagy is a self-digesting mechanism responsible for removal of long-lived proteins, damaged organelles, and malformed proteins during biosynthesis by lysosome. It plays two basic roles in cell: on the one hand, it removes the damaged cell components or organelles resulting from autodigestion, on the other, being a catabolic process, it enables generation of substrates necessary to maintain energetic homeostasis during cell's restricted access to nutrients. Also, it is considered to promote programmed cell death type II.

There are three forms of autophagy depending on the way the substrate is delivered to lysosome: macrophagy, chaperone-mediated autophagy and specific autophagy, the latter causing removal of specific structures (e.g. mitophagy in mitochondria).

The aim of our research was to evaluate changes in transcriptive activity of genes involved in the process of autophagy in four clinical stages of colon cancer compared to the controls, and to find out which of the transcripts could differentiate normal colon from that affected by cancer, thus having a diagnostic or prognostic or predictive value.

The study material consisted of biopsy specimens from colon cancer in four clinical stages, confirmed by histopathologist as adenocarcinoma, and biopsy specimens from normal colon operative margin (controls).

435 mRNAs involved in regulation of autophagy, detected on the microarray HG-U133A (Affymetrix, Santa Clara, CA) were analysed. Evaluation of differentiating genes was carried out using the PL-Grid Infrastructure (http://www.plgrid.pl/).

Stastistical analysis one-way ANOVA ($p < 0.05$) revealed that among the group of 435 mRNA of the genes connected with autophagy, 50 mRNA could differentiate cancerous specimens from the control C. Among these transcripts, 4 mRNAs were differentially expressed in CSI, 9 mRNAs were differentially expressed in CSII, 12 mRNA were differentially expressed in CSIII and 29 mRNA were differentially expressed in CSIV in comparison to control specimens (Tukey post hoc test, $p < 0.05$).

The differences in mRNA concentration profiles of the genes linked with autophagy as shown in adenocarcinoma specimens may suggest that autophagy plays a role in pathogenesis of adenocarcinoma. mRNA concentration profile of the genes connected with autophagy may be considered a supplementary marker in differentiating adenocarcinoma from controls depending on clinical stage of cancer.

[B-44] THE REGULATION OF EXPRESSION OF LGALS3 (GALECTIN-3) GENE BETWEEN OVARIAN CANCER CELL LINES BY EXOSOMES CONTAINING MIR-424-3P

Dominik Bieg, Ewa Nowak, Sabina Gałka, Paulina Borzdziłowska, Ilona Bednarek

Department of Biotechnology and Genetic Engineering School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec; Medical University of Silesia, Katowice, 41-200 Sosnowiec, ul. Jedności 8, Poland

Despite the fact that ovarian cancer accounts for only 3% of all female cancers, it still remains one of the most lethal gynecological cancer. One of the reason of high mortality is resistance for chemotherapy. LGALS3 is an oncogene, coding galectin-3, whose expression is commonly altered in many cancers (including ovarian cancer). Due to its anti-apoptotic properties galectin 3 is linked with ovarian cancer cells' chemoresistance. Increasing evidence suggests, that the expression of galectin-3 is regulated by miR-424-3p. On the other hand, miRNAs can be transmitted by exosomes, which are particles 30-100nm in diameter, secreted by cells under physiological and pathological conditions. These phospholipid bilayer-enclosed particles are carriers of proteins, lipids, mRNAs, miRNAs, and non-coding RNAs and by this they play a role in intercellular communication.

The present study attempted to investigate whether it is possible to regulate the expression of LGALS3 gene in OVP-10 ovarian cancer cells by exosomes, containing miR-424-3p, derived from A2780 ovarian cancer cells.

The OVP-10 and A2780 ovarian cancer cells were cultured under standard conditions (37˚C; 5% CO2; RPMI medium supplemented with 10% Exosome-Depleted FBS and 50µg/ml gentamycin). The OVP-10 cells were treated with exosomes isolated from the A2780 cell culture medium by Total Exosome Isolation Reagent (ThermoFisher). Afterwards RNA was extracted from the OVP-10 cells using TRI Reagent® Solution (Ambion®). Then the expression profile of LGALS3 and miR-424-3p was examined by Real Time™ RT-PCR (reverse transcription PCR) and Real Time™ stem-loop RT-PCR.

Conclusion: A2780 cancer cells secrete exosomes containing the particles of miR-424-3p, which can regulate the expression of LGALS3 gene in OVP-10 cells.

The research was supported by Medical University of Silesia funding: KNW-1-043/N/6/B, KNW-1-090/N/7/B and KNW-2-B20/N/7/N. Part of presented results is included in Ph.D. thesis: Role of modulation of galectin-3 expression by miR424-3p mimic and morin in cisplatin-induced apoptosis of ovarian cancer.

[C-22] GRAFTED COPOLYMERS OF CHOLINE DERIVATIVE AS NOVEL DRUG DELIVERY SYSTEMS

Rafał Bielas, Dorota Neugebauer

Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, 44-101 Gliwice, ul. Strzody 9, Poland

Polymeric drug carriers are often used in many different therapies. Generally they are used for encapsulation of biologically active substances or covalent binding of pharmaceutics with bonds prone to degradation.[1] We propose a novel solution focused on a drug binding system consisting of a linear polymers with ionic bonds.[2,3] The use of graft copolymers for this purpose should considerably slow down the release rate of the biologically active substance and thus improve its properties as a prolonged delivery carrier.

A series of graft copolymers based on choline methacrylate and methyl methacrylate was obtained using atom transfer radical copolymerization (ATRP). Reaction products were obtained with the assumed conversions, which resulted in copolymers with side chains containing 20, 58, and 78 of repeating units, respectively.

Size exclusion chromatography (SEC) analysis of polymers was performed, confirming the low molecular weight distributions (1.26-1.48). Hydrodynamic diameters reaching less than 100 nm were also measured using dynamic light scattering.

Polymers have been tested for the use as carriers of biologically active substances that are ionically bound to the polymer matrix. The model drug chosen in this study was salicylic acid with anti-inflammatory activity. Characteristics of the obtained polymeric carriers was completed by drug release profiles in PBS at pH 7.4.

[1] X. Guo et al. J.Polym.Sci. Part A Polym. Chem., 2016, 54, 3525-3550
[2] R. Bielas et al. ACS Sustain.Chem.Eng., 2016, 4, 4181-4191
[3] R. Bielas et al. New J. Chem., 2017 (doi: 10.1039/C7NJ02667F)

[B-13] CHANGES OF SUPEROXIDE RADICAL LEVELS IN DIVIDING AND NON-DIVIDING ME45 CELLS

Patryk Bil, Sebastian Wołkowicz, Sebastian Student, Joanna Rzeszowska-Wolny

Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 16, Poland; Biotechnology Centre, Silesian University of Technology, 44-100 Gliwice, ul. Krzywoustego 8, Poland

Reactive oxygen species (ROS) are known for their role in the cell's oxidative stress response. ROS are also a vital part of many regulation processes in cells and our goal was to study how one free radical (superoxide radical) changes its levels in cancer cells. In this study we have used a melanoma cell line (Me45) and stained it with MitoSOX dye (red mitochondrial superoxide indicator) to visualize where superoxide is located in cells and how its levels are changing through a cell's life. We also stained those cells with Hoechst 33342 dye to visualize the location of the cell's nucleus, and then we used time-lapse fluorescence microscopy to observe single cells. The cells were observed for 72 hours and the read-out time interval was 30 minutes. We observed that cells from one of the populations had slower progression through the cell cycle (or even cell cycle arrest) and the other population of cells had normally proliferating cells. We selected non-proliferating cells and a few dividing cells that could be fully segmented, and then acquired their total superoxide levels and superoxide concentration levels with the use of ImageJ software (plugin: LineageTracker). As we expected, in most cases cell nuclei were free from superoxide radical, but we found a few cells with high levels of superoxide in their nucleus which seemed to result in some kind of cytoskeleton block or the cell's death. In the dividing cells we observed that the total level of superoxide radical grew till late G2 phase, and it dropped notably just before cell division. In our observation of the non-dividing cells the total superoxide level increased through time, but these cells appeared to regulate superoxide concentration differently in each case – in some cases the concentration was dropping, in others rising, and in some it was fluctuating. These results show that changes in superoxide radical levels are not strictly connected to the cell cycle and that superoxide radical can be utilized differently by cells from the same population.

This work was supported by the Polish National Science Centre (Grant 2015/19/B/ST7/02984)

[A-15] AUTOMATED DETECTION OF LYMPHOCYTES IN WHOLE SLIDE HISTOPATHOLOGICAL IMAGES USING MIMSEG2 APPROACH

Franciszek Binczyk, Michal Marczyk, Joanna Polanska

Data Mining Group, Institute of Automatic Control, Silesian University of Technology, ul. Akademicka 16, 44-100 Gliwice, Poland

Recently, the concentration of lymphocytes infiltrating tumour tissue estimated using histopathological images became a promising prognostic marker of tumour malignancy. The crucial step in estimation of the concentration is a detection of single lymphocytes in H&E stained images. In literature there are several algorithms that are mostly dedicated to analysis of small and medium size images. The problem grows when the whole slide image with tens of millions pixels is analysed. We propose the efficient signal-based algorithm to automated detection of lymphocytes in high resolution H&E images by application of unsupervised MiMSeg2 algorithm.

The proposed algorithm consists of two major steps. In the first step, the whole image is clustered into number of different segments and then the segment containing mainly lymphocytes and other cells is automatically identified. The image segmentation is preceded by decomposition of R,G and B colour channels with use of Gaussian mixture model and construction of conditional probability functions. Next, these functions are mapped to image pixels to estimate the probability that pixel belongs to the one of the identified segments. Finally, clustering in the domain defined by pixel conditional probabilities is done using k-means algorithm. Dunn index is used to find the number of segments. In the second step, the identification of lymphocytes that are characterized by specific shapes and sizes is performed. The shape is evaluated by fitting an ellipse or set of ellipses to image fragments with identified cells and/or lymphocytes. Size, sphericity and texture homogeneity were used to distinguish individual lymphocytes. All algorithms were implemented and optimized to handle a very large dataset.

Due to the lack of annotated whole slide images, the proposed methodology was evaluated on two publicly available datasets with smaller fragments of H&E images. The first set contains 100 images of breast carcinoma tissue with 3,064 manually delineated lymphocytes. The second set contains 100 images collected for a samples of colorectal adenocarcinoma. A total number of 29,756 nuclei were marked, out of which there were 22,444 that were associated as epithelial, inflammatory, fibroblast or miscellaneous. In this work we were looking for inflammatory nuclei.

The preliminary results of lymphocytes detection are very promising. In the first set, the proposed solution was able to achieve 81.65% PPV (95% CI: 79.26% - 84.02%) 86.70% (95% CI: 84.29% - 89.10%) sensitivity. For the second dataset 85.14% PPV (95% CI: 83.05% - 87.23%) and 77.42% (95% CI: 75.57% - 79.27%) sensitivity was obtained, however all inflammatory cells were detected. The results are almost as good as application of supervised deep learning approach that needs many manually annotated lymphocytes in training phase. These results prove that proposed unsupervised approach allows for identification of lymphocyte cells at sufficient level. Current work is focused on tuning and expansion of the second part of the algorithm to increase the detection performance.

FB was financed by project granted by Narodowe Centrum Nauki: UMO-2015/19/B/ST6/01736, MM and JP were financed by grant no. 02/010/BK17/0060/9; BK-204/RAU1/2017/9.

[C-14] PROAPOPTOTIC PROPERTIES OF DERIVATIVES OF 2(5H)-FURANONE

Anna Byczek-Wyrostek[1,2], Radosław Kitel[1,2,3], Joanna Wawrzeczko[1], Anna Kasprzycka[1,2], Krzysztof Walczak[1,2]

[1]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, 44-100 Gliwice, Krzywoustego 4, Poland; [2]Centre of Biotechnology, Silesian University of Technology, Krzywoustego 8, 44-100 Gliwice, Poland; [3]Department of Organic Chemistry, Jagiellonian University, Ingardena Street 3, 30-060 Krakow, Poland

Background: Several reports have confirmed the anticancer activity of compounds bearing a 2(5H)-furanone ring in their structure. 5-Butoxy-3,4-dichloro-2(5H)-furanone is cytotoxic at high millimolar concentration in murine colon cancer cell lines. Its modifications led to 3,4-dichloro-5-(oxirane-2-ylmethoxy)-2(5H)-furanone derivative with cytotoxicity in nanomolar range in the same cancer cell lines. (E)-3-(4-fluorophenyl)-N-(4-((2-methylene-5-oxo-2,5-dihydrofuran-3-yl)methyl)phenyl) inhibits the formation of complex DNA-topoisomerase I. We also know a natural compound – rubrolides – which displays anti-cancer activity in HT-29, MEL-28, P-388 and A549 cell lines.
Materials and methods: The cytotoxicity of 5-substituted derivatives of 2(5H)-furanone was marked through the MTT assay, and the impact of the derivatives on the cell cycle has been determined by flow cytometry. Caspase-3 and cyclin B1 protein were analyzed in western blots.
Results: The compound with isopropyl group in C-5 position of the 2(5H)-furanone ring exhibits higher activity than 3,4-dichloro-5-hydroxy-2(5H)-furanone. This compound arrests the cell cycle in G2 phase and is also able to trigger apoptosis.

This work was financially supported by the National Science Centre (Poland) under the project number 2016/21/B/NZ7/01766.

[A-6] STRUCTURAL ASPECTS OF SELECTIVE INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE

Maria Bzówka[1,2], Artur Góra[1], Jan Víteček[3,4], Lukáš Kubala[3,4], Jiří Damborský[3,5]

[1]Tunneling Group, Biotechnology Centre, Silesian University of Technology, ul. Krzywoustego 8, 44-100 Gliwice, Poland; [2]Department of Chemistry, Silesian University of Technology, ks. Marcina Strzody 9, 44-100 Gliwice, Poland, [3]International Clinical Research Center, Center of Biomolecular and Cell Engineering, St. Anne's University Hospital Brno, Pekařská 53, 656 91 Brno, Czech Republic, [4]Institute of Biophysics, AS CR, Královopolská 135, 612 65 Brno, Czech Republic, [5]Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment RECETOX, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Nitric oxide synthases (NOSes) are enzymes catalyzing the production of nitric oxide (NO). It is a crucial signaling molecule that regulates various physiological functions. NO acts in the cardiovascular system as a key regulator of vascular tone, it can prevent platelet activation, regulate myocardial contractility. NO is also a neurotransmitter and it is involved in immune response. Besides that, NO can be involved in numerous pathological processes like septic shock, hypertension, and atherosclerosis. The overproduction of NO contributes pathogenesis of acute and chronic inflammatory processes and NO has been recognized as one of the main signaling molecule involved in these processes. In mammals, NO is produced by one of the three NOS isoforms: neuronal (nNOS), endothelial (eNOS) or inducible (iNOS) [1,2]. Due to the importance of NO in various pathological processes, NOSes are significant pharmacological targets. A great effort has been made to design inhibitors of NOSes. The most widely used classification of inhibitors relies on the identification of the site of inhibitor binding to the NOS enzyme. Computer-aided study based on crystallographic data mapped the active site of mammalian NOS isoforms [3].

In the presented study, 40 crystal structures of iNOS deposited in PDB have been revisited in order to summarize which parts of the iNOS were targeted up to recent. The structures of iNOS-ligand complexes were analyzed using Ligplot [4], aiming to identify the amino acids targeted by inhibitors. Then, hierarchical clustering method [5] was used to group amino acids based on the interaction similarity. Presented analysis provides information about the most important amino acids which contributes to the binding. In total, 28 amino acids and the heme were identified as interacting with ligands, including 4 of them which have not been identified by any previous research.

[1].Jaffrey SR et al. Annu Rev Cell Dev Biol. 1995;11:417–40.
[2].Bogdan C. Nature immunology. Nature Publishing Group; 2001;2: 907–16.
[3].Ji H et al. J Med Chem. 2003;46: 5700–11.
[4].Wallace A C et al. 1995; Prot. Eng., 8, 127-34.
[5].Ward Jr, JH. J. Am. Stat. Assoc. 1963; 58: 236–44.

This work was supported by the National Science Centre, Poland (DEC-2015/18/M/NZ1/00427) and project no. LQ1605 from the National Program of Sustainability II (MEYS Czech Republic).

[B-22] DIFFERENT METHYLATION PROFILES AND THEIR ALTERATIONS AMONG GENE ASSOCIATED AND INTERGENIC GENOME REGIONS IN AML

Agnieszka Cecotka, Joanna Polanska

Data Mining Group, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100 Gliwice, Akademicka 16 Poland
Corresponding author: agnieszka.cecotka@polsl.pl

Introduction: DNA methylation is an epigenetic process controlling transcription mechanism. Its role is different according to genome region. DNA methylation was most thoroughly examined in gene promoter regions. Hypermethylation of this region can block initiation of transcription. Hypomethylation – on the contrary – can enhance transcription process. Low DNA methylation level in gene body region can hypothetically slow down an elongation process. Changes in intergenic regions might induce whole genome instability. The aim of the study was to examine differences of methylation profiles and their alterations among gene promoter, body and intergenic regions.
Materials and methods: Analyzed data was downloaded from GEO database (GSE63409) and contained 5 samples of hematopoietic stem cells (HSC) from healthy donors (control) and 14 samples of CD34+38- cells from Acute Myeloid Leukemia (AML) patients. Experiment was conducted on Illumina Infinium HumanMethylation450k array and provided methylation level for 485 512 sites of genome for each patient. Raw data were normalized with minfi package in Bioconductor. After normalization methylation level is defined as β value ranging from 0 to 1, while 0 means no methylation and 1 means full methylation. Methylation level in each genome region was tested for $\beta>0.5$ (high methylation) and $\beta<0.5$ (low methylation) for both control and AML sample. To check demethylation level, AML and control samples were compared using statistical tests. In every case nonparametric test was applied, because of nonnormality of β value distribution.
Results: In both groups – AML and control there are more high methylated than low methylated sites within whole genome. The situation changes in different genome regions. In gene promoter regions most of the sites are low methylated, contrarily to gene body regions and intergenic regions, where most of the sites are high methylated (intergenic region is highest methylated). For comparison between AML and control, up methylation phenomenon is stronger than down methylation. However, demethylation (up or down methylation) is strongest for intergenic regions, then for gene body regions and at the end for gene promoter regions.
Conclusion: DNA methylation differentiates genome regions. Its level is lowest for gene promoter regions and highest for intergenic regions. Also alterations of DNA methylation vary within genome regions. They are strongest in intergenic regions and weakest for gene promoter regions. Independently of genome region, up methylation is stronger than down methylation in case of AML. Role of DNA methylation is known for promoter regions, but it still stays unrevealed for intergenic regions.

The work was financially supported by SUT grant BKM-508/RAU1/2017/t.29 (AC) and SUT grant BK-204/RAU1/2017/9 (JP).Calculations were carried out using infrastructure of GeCONiI (POIG.02.03.01-24-099/13).

[B-7] ISOFORMS OF THE MOUSE PMAIP1/NOXA TRANSLATED FROM INTERNAL ATG CODONS RETAIN THE PROAPOPTOTIC ACTIVITY

Marek Chadalski, Agnieszka Toma-Jonik, Joanna Korfanty, Patryk Janus, Natalia Vydra, Wieslawa Widlak

Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, 44-101 Gliwice, Wybrzeze Armii Krajowej 15, Poland

PMAIP1 (phorbol-12-myristate-13-acetate-induced protein 1), also known as NOXA (Latin for damage), is a proapoptotic member of the BCL-2 protein family. It belongs to a BH3-only proteins, which contain only a single BH-domain. PMAIP1/NOXA is the primary p53-responsive gene. We found that it is also activated by HSF1 in response to heat shock. Although murine and human Pmaip1/PMAIP1 genes are activated by similar transcriptional mechanisms, there are significant structural and regulatory differences between them. The mouse gene codes for ~11.5 kDa protein (103 residues) with two BH3 domains, while the human gene lost the part of the sequence and codes for ~6 kDa protein (54 residues) containing one BH3 domain.

Looking for PMAIP1 protein by Western blot, we observed much larger PMAIP1 form of ~18-20 kDa in some mouse tissues than the expected one of 11,5 kDa. The form of the expected size appeared following heat shock and was considerably less abundant. Interestingly, we found also ~6 kDa form of the mouse PMAIP1 matching to the size of the human protein. All forms were specifically blocked in peptide competition assay. To elucidate if these two forms could originate from different transcripts we performed 5'RACE. The test revealed that there is only one Pmaip1 transcript, although it starts several bases upstream the sequence shown in databases. Then, we tested whether translation could start from internal ATG codon. We cloned the mouse Pmaip1 gene (fused with Egfp) and separately mutated all in-frame ATG codons. Mutation of the first ATG codon led to the production of the shorter protein, from one of two internal ATG codons. Using live imaging microscopy we confirmed that all mutants produced active PMAIP1 which was able to induce apoptosis.

This work was supported by grant no 2014/13/B/NZ3/04650 from the Polish National Science Centre.

[B-45] ITGBL1 OVEREXPRESSION STIMULATES OVARIAN CANCER CELL CHEMORESISTANCE AND HAS NO EFFECT ON PROLIFERATION RATE

Alexander J. Cortez, Katarzyna A. Kujawa, Patrycja A. Tudrej, Ewa I. Kempa, Katarzyna M. Lisowska

Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Institute Oncology Center Gliwice Branch, Poland

Background: In our previous microarray study we analyzed gene expression profile of 72 high grade serous ovarian cancers(HG-SOC) [1]. We identified two molecular subgroups of HG-SOC with distinct gene expression profiles and survival [2]. Among differentially expressed genes was an Integrin beta-like1 gene(ITGBL1). ITGBL1 is a poorly characterized protein, structurally cognate with integrin β. Our aim was to study whether and how ITGBL1 can influence the phenotype of ovarian cancer cells.
Methods: ITGBL1 coding sequence was PCR-amplified from cDNA and cloned into pLNCX2 vector. Retroviral system was used to obtain two ovarian cancer cell lines: OAW42/ITGBL1(+) and SKOV3/ITGBL1(+) with overexpression of ITGBL1. Control cell lines were obtained by transduction with empty vector. Proliferation and cytotoxicity were analyzed using MTS and crystal violet assay.
Results: We compared proliferation of control OAW42 and SKOV3 cells with isogenic cell lines containing ITGBL1 construct. The results indicate that ITGBL1 overexpression has no effect on proliferation of ovarian cancer cells. We also evaluated an influence of ITGBL1 protein on ovarian cancer cells sensitivity to cisplatin and paclitaxel, and both cell lines overexpressing ITGBL1 were significantly more resistant to cisplatin and to paclitaxel, as compared to the control lines.
Conclusions: Our results indicate that although ITGBL1 has no effect on ovarian cancer cells proliferation rate, it may however increase their resistance to cisplatin and paclitaxel. These results are in line with our previous observation that patients with HG-SOC showing higher ITGBL1 mRNA expression have significantly shorter OS [2].

[1]K.M.Lisowska,et al.(2014),Front.Oncol. DOI:10.3389/fonc.2014.00006.
[2]K.M.Lisowska,et al.(2016),J.Cancer Res.Clin.Oncol.DOI:10.1007/s00432-016-2147-y.

[B-46] MODULATION OF THE NRF2 SIGNALING PATHWAYS BY A COMBINATION OF PHYTOCHEMICALS IN HUMAN HEPATOCELLULAR CARCINOMA CELLS

Marta Cykowiak, Jarosław Paluszczak, Hanna Szaefer, Violetta Krajka-Kuźniak

Department of Pharmaceutical Biochemistry, Poznan University of Medical Sciences, 60-781 Poznań, ul. Święcickiego, Poland

Hepatocellular carcinoma (HCC) is the 5th most frequent malignancy in the world. The survival rates in HCC patients remain poor especially in more advanced disease stages when the risk of recurrence is higher. Research of the last decades has demonstrated that the understanding of the interaction of nutrition and health plays a crucial role in disease prevention and therapy. Naturally occurring phytochemicals such as phenethyl isothiocyanate, indole-3-carbinol and xanthohumol are known to act as chemopreventive agents in experimental models. Recently, it has been suggested that the results of chemoprevention can be improved by the use of combinations of compounds instead of individual phytochemicals.

The aim of the study was to investigate the influence of the aforementioned phytochemicals and their combinations on the Nrf2 pathway activation in HCC cells. HepG2 cells were cultured in standard conditions and treated with phenethyl isothiocyanate, indole-3-carbinol and xanthohumol or their combination at the doses of 1 μM, 5 μM and 10 μM. Cell viability was assessed with the MTT assay. The level of gene expression and protein content were assessed by RT-PCR and Western blot, respectively.

The results of MTT analysis showed that the highest cytotoxicity was induced by the individual and combined use of xanthohumol and phenethyl isothiocyanate. Among the studied phytochemicals, phenethyl isothiocyanate increased the expression of Nrf2 to the most extent. Western blot analysis showed the occurrence of translocation of Nrf2 from cytosol to nucleus as an effect of the treatment with individual phytochemicals and their combination.

The activation of Nrf2 was assessed by measuring its binding to an oligonucleotide containing the ARE consensus sequence. The results show that xanthohumol and phenethyl isothiocyanate as well as their combination increases to the greatest extent the binding of Nrf2 to ARE in HepG2. The activation of Nrf2 induces the expression of cytoprotective enzymes such as glutathione–S-transferase (GST) or superoxide dismutase (SOD). Indeed, phenethyl isothiocyanate, indole-3-carbinol and xanthohumol alone increased both GST and SOD transcript and protein level. Importantly, their combinations significantly enhanced this effect.

Overall, the results of this study indicate that the use of the combination of the tested phytochemicals has greater potential to modulate the Nrf2 pathway and thus augment their chemopreventive and/or adjuvant chemotherapeutic capacity of the phytochemicals.

This work was supported by grant no 2016/21/B/NZ5/01390 from the National Science Centre, Poland.

[B-18] CANCER –DERIVED EXOSOMES INFLUENCE THE LEVEL OF IMMUNE SIGNALLING PROTEINS

Liliana Czernek, Markus Düchler

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 112 Sienkiewicza Street, 90-363 Lodz, Poland

Cancer-derived exosomes support the survival and progression of tumors in many ways and also contribute to the neutralization of the anti-cancer immune response. In general, exosomes can modify the transcriptional profile of the recipient cells by receptor activation, or by directly changing gene expression through delivered nucleic acids. We want to investigate the impact of melanoma-derived exosomes on immune signalling pathways by evaluating their influence on the expression level of immune signalling proteins. Extracellular vesicles were isolated from the culture medium of a human malignant melanoma cell line (A375) by differential centrifugation. The presence and size of the particles was verified by atomic force microscopy and nanoparticle tracking analysis (NTA). Cancer-derived exosomes were also characterised by flow cytometry using fluorescently labelled antibodies for characteristic tetraspanins – CD9 and CD63.

The target protein we investigated was chosen based on a previous MicroArrays analysis of miRNAs found in exosomes. By various bioinformatics tools a gene was identified that was targeted by the largest number of exosomal miRNAs. This gene codes for the T-cell nuclear factor, which causes the activation of target genes in the immune response. NFAT5 is a member of the nuclear factor of activated T cells (NFAT) family of transcription factors. NFAT5 exists as a homodimer and is able to form stable dimers which further can bind to DNA elements. It was originally identified as inducer of cytokine gene expression in T cells. We wanted to verify that the miRNAs present in exosomes when added to target cells induce silencing of protein expression. Experiments were performed on several cell lines, including HeLa – human cervix carcinoma, a human ovarian cancer cell line developed in our institute (OvC16) and a human acute T lymphoblastic leukaemia cell line (MOLT4) and carried out by the Western Blot method.

Our results demonstrate that NFAT5 is expressed not only in T cells (MOLT4) as expected, but also in cancer cell lines (HeLa, OvC16). Furthermore, melanoma-derived exosomes influence NFAT5 protein level. In conclusion, by reducing the level of immune signalling proteins, cancer-derived exosomes are able to alter immune cell functions and potentially contribute to tumor immune escape.

This research was financially supported by The National Science Centre in Poland, Project Number UMO-2016/21/B/NZ7/02747

[C-29] ELECTROCHEMICALLY MODIFIED ITO AS BIOLOGICALLY ACTIVE SUBSTRATE FOR MEDICAL DEVICES

Dominika Czerwińska[1], Katarzyna Krukiewicz[1], Karol Erfurt[2], Anna Chrobok[2], Manus Biggs[3]

[1]Department of Physical Chemistry and Technology of Polymers, [2]Ionic Liquids Group, Silesian University of Technology, Gliwice, Poland, [3]The Biggs Lab, Centre for Research in Medical Devices, National University of Ireland, Galway

Neural cells are capable of processing and transmitting information in the form of electrical signals. When the malfunction of neural tissue occurs, the breakage in the bioelectric circuit may cause numerous neurological disorders, such as paralysis, pain, epilepsy or loss of sensations. Neuroelectrodes are medical devices that are designed to stimulate neurons through electrical impulses, and can be used to treat or prevent the progression of neural diseases [1]. These electrodes are made of various biocompatible materials such as platinum, iridium oxide or conductive polymers. Recently, indium tin oxide (ITO) has been found as the advantageous substrate with the biocompatibility superior to conventional materials [2].

In the present study, the surface of ITO was electrochemically modified through oxidation and reduction processes in the presence of poly(styrene sulfonate) and various ionic liquids. The electrochemical performance, in terms of charge storage capacity and impedance, was evaluated through cyclic voltammetry and electrochemical impedance spectroscopy. SEM with EDS were used to have an insight into the surface chemistry and morphology of modified substrate. The biocompatibility of modified ITO was determined by means of cell studies with embryonic ventral mesencephalon cells.

The results show that each of the applied surface modifications, both oxidation and reduction processes, had a beneficial effect on increasing the conductivity of ITO and the development of neural cells. The use of biocompatible ionic liquids has increased the adhesion of cells to the surface. The best electrochemical performance together with biological properties gave tetrabutylammonium glutamine and (2-D-glucopyranosyloxyethyl)trimethylammonium bistriflamide.

[1]. Fattahi P. et al. Advanced Materials 26 (2014) 1846.
[2]. Vallejo-Giraldo C. et al. Advanced Functional Materials 2017 1605035.

This research is supported in part by a research group grant from Science Foundation Ireland (SFI) and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073. MB is an SFI, Starting Investigator SIRG COFUND fellow, grant no. 11/SIRG/B2135. DC and KK are grateful to the National Science Centre in Poland for financing the research in the framework of SONATA program (2016/23/D/ST5/01306).

[B-24] T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA FROM miRNA TRANSCRIPTOME PERSPECTIVE

Małgorzata Dawidowska[1], Bronisława Szarzyńska-Zawadzka[1], Roman Jaksik[2], Monika Drobna[1], Maria Kosmalska[1], Łukasz Sędek[3], Tomasz Szczepański[3], Michał Witt[1]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland; [2]Silesian University of Technology, Gliwice, Poland; [3]Medical University of Silesia, Zabrze, Poland

T-cell acute lymphoblastic leukemia (T-ALL) is a rare, aggressive and heterogenous subtype of acute lymphoblastic leukemia (ALL), the most common malignancy in children. Clinical, genetic and immunophenotypic heterogeneity of T-ALL and insufficient understanding of its pathogenesis hampers further improvement in stratification and treatment strategies. miRNAs, as important regulators of gene expression, shape cells' phenotype. When aberrantly expressed, miRNAs serve as oncogenes or tumor suppressors (by silencing tumor suppressor genes and oncogenes, respectively). Exploring miRNA transcriptome with the use of next-generation sequencing (NGS) might aid the understanding of both the heterogeneity and pathogenesis of this leukemia.

In this project we aimed to identify miRNA transcriptome of pediatric T-ALL, with the focus on miRNA expression profiles reflecting T-ALL heterogeneity. We aimed to assess the potential of miRNA-seq as a classification and prognostic tool in pediatric T-ALL.

We finally aimed to identify novel candidate oncogenic/tumor suppressor miRNAs, their target genes and related pathways, to get insights into T-ALL pathogenesis.

Study group consisted of 34 pediatric T-ALL patients and 5 age-related healthy bone marrow donors. Total RNA, including small RNA fraction, was isolated from CD3+ cells selected immunomagnetically (by negative selection) from bone marrow samples. miRNA sequencing was performed using NextSeq500 Illumina platform (10mln reads/sample, 51bp single-end; NGS Service Exiqon). Raw sequencing reads were adapter trimmed using cutadapt and aligned with bowtie to a modified version of miRBase v21 (mature sequences) created according to the miRge specifications. Unaligned reads were sequentially matched against hairpin miRNA (miRBase v21) noncoding RNAs, Ensembl cDNA database and again to mature miRNA sequences using less stringent criteria. Differentially expressed miRNAs were selected using edgeR. Reference miRNAs for RT-qPCR validation were selected using NormFinder. Target genes for all differentially expressed miRNAs were identified using DIANA-microT, ElMMo, MicroCosm, miRanda, miRDB, PicTar, PITA and TargetScan databases gathered trough the multiMiR Bioconductor library. Genes were assumed to be regulated by a particular miRNA if the association was predicted by more than 5 out of 8 methods used. Target genes for all miRNAs differentially expressed between normal and T-ALL samples were tested for overrepresentation among KEGG, Reactome and Panther pathways as well as Gene Ontology biological processes using Fisher's exact test with Benjamini and Hochberg correction for multiple testing.

We showed that miRNA expression profile is discriminative between pediatric T-ALL and healthy bone marrow controls. We also identified miRNA expression profiles associated with immunophenotypic subtypes of T-ALL (EGIL stages: II, III, IV), reflecting maturation stages of T-cells.

We identified 23 miRNAs overexpressed in T-ALL vs. healthy controls (including known and candidate novel oncogenic miRNAs in T-ALL). Among these are miRNAs with mRNA target genes known to be implicated in T-ALL pathogenesis, including: miR-20b-5p, miR-363-3p, miR-128-3p, miR-181b-5p, miR-181a-5p. We identified 38 underexpressed miRNAs (potential tumor suppressors). Among these are miRNAs with known T-ALL-implicated targets: miR-145-5p, miR-143-3p, miR-27a-5p, miR-24-3p, miR-10b-5p.

By testing overrepresentation of mRNA targets for miRNAs differentially expressed between T-ALL and controls, we showed significant target enrichment in KEGG pathways and GeneOntology terms, including among others: positive regulation of apoptosis, regulation of kinase activity, interleukin-6-mediated signaling pathway, and lymphocyte differentiation.

With use of NGS, we provide insights into the heterogeneity of pediatric T-ALL at the miRNA transcriptome level. Expression profiles of miRNAs differentially expressed in patients vs. controls and in T-ALL immunophenotypic subtypes (EGIL: II, III, IV) hold potential for improved classification of T-ALL. Individual differentially expressed miRNAs are candidates for functional test to assess their oncogenic/tumor suppressor role in T-ALL and for association analysis (survival & treatment response) to assess their prognostic potential in T-ALL.

Research funded by National Science Centre, Poland grant: 2014/15/B/NZ2/03394 and National Centre of Research and Development (NCRD) grant STARTEGMED3/304586/5/NCBR/2017.

[C-10] ISOLATION OF HUMAN AND MOUSE BONE MARROW MESENCHYMAL STEM CELLS

Wojciech Fidyk, Agnieszka Toma-Jonik, Agnieszka Ciomber, Aleksander Sochanik, Joanna Jazowiecka –Rakus

Maria Skłodowska-Curie Institute – Oncology Center, Gliwice Branch, Wybrzeże Armii Krajowej 15, 44-101 Gliwice, Poland

Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into a broad range of cell lineages such as osteoblasts, chondrocytes and adipocytes. The main source of MSCs described in many laboratory protocols is the bone marrow, although they can be easily found in other tissues, mainly in adipose tissue, cord and peripheral blood. MSCs acquired from the bone marrow are used as vehicles in cell-based therapies, in tissue repair and engineering. Two main stem cells populations that are residual in the bone marrow are hematopoietic stem cells and mesenchymal stem cells. BM-MSCs are usually isolated owing to their physical adherence to plastic cell culture plate surface. Here, we describe an isolation method of human and mouse MSCs from the bone marrow.

Mouse BM-MSCs were isolated from tibias, femurs and humeri of 6-8 week old mice. Muscles, tendons and ligaments were completely removed from the bones by using scissors and scalpel. Bones were transferred to a dish with α-MEM medium on ice. Then two ends of the bone were excised and the bone marrow cavity was flushed with α-MEM until the bone appeared to be white. Cells were filtered through a 70μm strainer and cultured in complete α-MEM containing 10% fetal bovine serum (FBS). The bones were dissected into explants and incubated with shaking (200 rpm) for 2 h in α-MEM with collagenase added. Released cells were cultured as above. Bone explants were carefully located on Petri dish with α-MEM to allow MSCs leave the bone marrow cavity. After cells reached monolayer stage they were characterized for mouse MSCs markers: CD105, CD29 and Sca1.

Human BM-MSCs were isolated from bone marrow of healthy human bone marrow donors as a part of routinely collected BM samples. Bone marrow (2mL) was transferred to a culture dish containing α-MEM medium supplemented with 10% FBS. The cells were left to adhere for 48-72h. Next, the medium change removed the leftover bone marrow cells allowing the adherent MSCs to remain in the culture dish. After reaching monolayer stage the cells were collected and analyzed by means of flow cytometry for MSCs markers. Analysis demonstrated 90+% of typical MSCs markers to be present in the analyzed cell cultures (CD73+CD90+CD105+).

This work was supported by grant no 2016/22/M/NZ6/00418 from the Polish National Science Centre.

[C-30] THE IMPACT OF NATURAL ANTIOXIDANTS TO MINIMIZE INDUCTION OF GENETIC CHANGES IN HUMAN LEUKOCYTES OF NUCLEAR MEDICINE STAFF

Aneta Gajowik, Małgorzata M. Dobrzyńska

National Institute of Public Health – National Institute of Hygiene, Department of Radiation Hygiene and Radiobiology, 00-791 Warsaw, 24 Chocimska Street, Poland

Ionizing radiation induces a series of DNA damage directly or through the formation of highly reactive free radicals. A number of studies indicate that antioxidants of exogenous origin are particularly promising as radiation protectors/ modifiers which have the ability to protect DNA as well as to induce DNA repair. Lycopene and resveratrol are strong antioxidants which may be used to minimize toxicity even applied after radiation and act as mitigators.

Lycopene (LYC) is an acyclic isomer of β-carotene synthesized by autotrophic bacteria or plants like red fruits and vegetables, including tomatoes, watermelons, pink grapefruits, apricots, pink guavas and papaya.

Resveratrol (RSV) is a polyphenol structurally similar to diethylstilbestrol and estradiol. This antioxidant is present in grapes, peanuts, strawberry, blueberry, cranberry, mulberry, lingberry, sparkleberry, bilberry and also in wine, especially in red.

The aim of the study was to evaluate the effect of RSV or LYC on the kinetics of DNA repair in leukocytes of peripheral blood of individuals occupationally exposed to ionizing radiation. Samples of human peripheral blood were aseptically collected in heparinized sterile tubes from nuclear medicine workers. 10 µl of blood was collected into Eppendorf tubes and than incubated for ½ or 1 h in a water bath at 37 °C with LYC at doses 10 and 20 µl/ml or RSV at doses 0.1 and 0.5 mM/ml. After centrifugation and removal of excess supernatant, microscopic preparations were made. Frequency of DNA damage was evaluated by the comet assay (Tail Moment). For the determination of viability assay of leukocytes have been used trypan blue.

The results show that viability of leukocytes was ca. 95 %. With increased incubation time, the degree of DNA damage rose. After 0.5 h incubation, there were no significant differences as compared to controls except for the 0.1 mM RSV/ml dose, which resulted in increased DNA damage. After 1 h of incubation, the value of Tail Moment was decreased only after LYC supplementation in dose of 20 µM/ml. A lower dose of LYC did not change the degree of DNA damage, whereas RSV in both doses increased the frequency of DNA strand breaks.

In conclusion, LYC at a suitable dose may accelerate the repair of DNA damage induced by ionizing radiation in human peripheral blood leukocytes.

The work was financed by the National Institute of Public Health - National Institute of Hygiene within the framework of statutory research (Scientific Project No. N11ZŚ / 2017).

[B-36] SELECTIVE RELEASE AND TROPISM OF PORCINE ENDOGENOUS RETROVIRUSES - POTENTIAL ROLE IN RETROVIRAL GENE TRANSDUCTION

Sabina Gałka, Daria Matczyńska, Ewa Nowak, Michał Szapski, Ilona Bednarek

Department of Biotechnology and Genetic Engineering, School of Pharmacy with the Division of Laboratory Medicinein Sosnowiec, Medical University of Silesia, ul. Jedności 8, 41-200 Sosnowiec, Poland

It is known that endogenous retroviruses are the cause of diseases. They are also involved in carcinogenesis, especially in breast cancer, melanoma and germ cell tumors. In these cases viral particles, as well, as the retroviral envelope protein (Env), can be detected. It was shown, that retroviral Env protein induces epithelial to mesenchymal transition (EMT), very often associated with metastasis and tumor aggressiveness. Insertional mutagenesis, immunosupression and/or direct oncogenic activity of retroviral protein leads to tumor formation. Retroviral integration can silence or activate nearby genes and induce oncogenesis, and may also contribute to the recruitment of endogenous human retroviruses (HERVs). What is important, in case of xenotransplatation of porcine organs or cells, retroviral transmission may also lead to the emergence of PERV/ HERV viral hybrids. Knowledge of the determinants of retrograde tropism is a critical element in understanding the conditions of transmission of retroviruses from the donor to the recipient.

The aim of the study was to characterize the sequence of porcine endogenous retroviruses (PERVs) genes (envA, envB, gag, pol) in pig PK-15 cells and PK-15 cells' supernatant, (donor of PERVs for infection), as well as in in vitro PERVs' infected HEK-293 and HeLa human cell lines.

The results showed the presence of polymorphic variants in PK-15 cells: one polymorphic localization in each envA, envB and gag genes, and 19 ones in pol gene. What is important only one of this variants was present in the virions in the supernatant of PK-15, and then was embedded in the HEK-293 and HeLa cell genome sequences. Atypically, in HeLa infected cells only one polymorphic variants in gag gene was observed: 2331G>A and 2400A>G. Moreover, HeLa cells were infected only by PERVA subtype.

Also, the results demonstrated a large variety of sequences of PERVs in PK-15 cells, especially within the pol gene. The obtained data may suggest selective release of variant PERV virions by PK-15 cells with strictly defined sequences of the gag, envA, envB and pol genes. Despite the same virus source, there are differences in the insertion of PERVs sequences between different human cell lines (between normal, HEK-293, and HeLa cancer cells). It is advisable to conduct further studies to enhance the relationship between the transmission of selected variants of the viral genes and the ability to integrate into the recipient cell genome.

The study was supported by Medical University of Silesia funding (KNW-1-043/N/6/B, KNW-1-090/N/7/B). Part of presented results is included in Ph.D. thesis: Infection of human cells by endogenous porcine retroviruses (PERV, porcine endogenous retroviruses). In vitro studies.

[C-2] HOW ACTIVATED SLUDGE BACTERIAL COMMUNITY RESPONDS TO A SUDDEN pH DECREASE?

Filip Gamoń, Anna Banach, Mariusz Tomaszewski, Aleksandra Ziembińska-Buczyńska

Environmental Biotechnology Department, Silesian University of Technology, Gliwice 44-100, Akademicka 2, Poland

In the Anaerobic Ammonium Oxidation (anammox) process ammonia nitrogen is converted into dinitrogen gas, using nitrite as an electron acceptor. The anammox process is performed by anammox bacteria belonging to the phylum Planctomycetes and it strongly depends on these bacteria activity, which may be affected by pH change. The optimum pH for the growth and activity of anammox bacteria used in wastewater treatment was reported between 6.7 - 8.3.

In the present study the biodiversity change of the anammox, ammonia oxidizing bacteria (AOB), and all bacteria biocenosis during pH decrease (from 7.89 to 6.66) in Sequencing Batch Reactor (SBR) was investigated. To examine the change of microbial community biodiversity and its genotypic structure, PCR – DGGE (Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis) method was used with the specific primers sets: 338f-GC/518r - for all bacteria in the biocenosis; AMOf/AMOr - for ammonia oxidizing bacteria; Pla46f-GC/518r - for Plactomycetes. Obtained PCR products were separated by DGGE. Densitometric analysis has been done on the basis on obtained fingerprints, then Shannon and Simpson biodiversity indexes were calculated.

The analysis revealed that both AOB and all bacteria in the biocenosis have a gentle decreased biodiversity, due to pH decrease. In SBR bacterial biodiversity for Plactomycetes presented as Shannon index decreased from 2.78 (in 303 day of experiment) to 2.38 (in 334 day of experiment), while Simpson index increased from 0.06 to 0.11. This change was followed by pH decrease from 7.89 to 6.66. These results let us suspect, that the sudden pH change was the cause of biodiversity decrease in the system.

The research was supported by National Science Centre grant: UMO-2013/09/D/NZ9/02438

[C-18] IRON CHELATORS IN PHOTODYNAMIC THERAPY

Robert Gawecki[1], Anna Mrozek-Wilczkiewicz[1], Katarzyna Malarz[1,2], Marta Rejmund[2], Jarosław Polański[2], Alicja Ratuszna[1], Robert Musioł[2]

[1]A.Chełkowski Institute of Physics and Silesian Centre for Education and Interdisciplinary Research, University of Silesia in Katowice, 75 Pułku Piechoty 1A, 41-500 Chorzów, Poland [2]Department of Organic Chemistry Division, Institute of Chemistry, University of Silesia in Katowice, Szkolna 9, 40-006 Katowice, Poland
e-mail: robert.gawecki@us.edu.pl

Photodynamic therapy (PDT) is characterised by low invasiveness and high selectivity towards normal cells, making this method an attractive form of treatment compared to chemotherapy and radiotherapy. The essence of this therapy is singlet oxygen and free radicals generation by absorption of appropriate wavelength of light by a 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) - 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 incorporate into PDT, what will inhibit PpIX conversion [3].

The aim of this study was to investigate the effect of the novel derivatives of thiosemicarbazones (TSC) on PpIX production. The cellular model was chosen based on level of enzyme activity involved in the heme biosynthesis pathway. Selected compounds have high ability for complexing iron ions. Due to the cellular environment, inactive compounds were selected (IC50> 25 μM). Preliminary results have confirmed the utility of some derivatives of TSC in ALA-PDT therapy.

[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

[B-8] IMPACT OF ANGIOGENESIS-REGULATING GENE POLYMORPHISMS ON PROGNOSIS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA, HNSCC

Agnieszka Gdowicz-Kłosok[1], Małgorzata Krześniak[1], Tomasz Rutkowski[2], Iwona Matuszczyk[1], Iwona Domińczyk[1], Monika Pietrowska[1], Krzysztof Składowski[2], Dorota Butkiewicz[1]

[1]Center for Translational Research and Molecular Biology of Cancer, [2]I Radiation and Clinical Oncology Department, Maria Skłodowska-Curie Institute-Oncology Center, Gliwice Branch, Gliwice, Poland

In Poland, head and neck cancer (HNC) is the sixth most common malignancy in men and twelfth among women. Annually, 6,000 cases and nearly 4,000 deaths are attributable to this group of cancers. Up to two-third of HNC patients present with advanced stage disease. Radiotherapy alone (RT) or in combination with cisplatin-based chemotherapy (CHRT) are the mainstays of treatment in locally advanced HNC. Head and neck tumor progression depends on angiogenesis and reconstruction of extracellular matrix. As a result of rapid cancer cells proliferation hypoxia sets in which triggers neovascularization. This process depends on VEGF and its two receptors, VEGFR1/FLT1 and VEGFR2/KDR. In consequence of the VEGF-receptors interaction, mitogenesis and chemotaxis are stimulated and morphology changes occur. Release of VEGF from extracellular matrix is possible due to local degradation of basement membrane mainly by MMP2 and MMP9 metalloproteinases. Moreover, hypoxia and angiogenesis are important factors influencing effectiveness of RT and CHT. Since they may modulate individual angiogenic and metastatic potential, polymorphisms of genes participating in angiogenesis and reconstruction of extracellular matrix have been found to be linked to tumor aggressiveness and unfavorable prognosis in many solid tumors. Our previous studies in lung cancer, for example, showed that certain functional variants of VEGFR2 and MMP2 genes may increase risk of progression and death in patients treated with RT and platinum-based CHRT. In HNC, the studies concerning the role of polymorphic variants of angiogenesis genes in therapy outcome are scarce and inconsistent.

Therefore, the objective of this study was to assess whether certain polymorphic variants of VEGF, VEGFR1, VEGFR2, MMP2 and MMP9 genes were associated with therapy outcome, including risk of local and regional recurrence and metastasis, overall (OS), disease-free (DFS) and relapse-free survival (RFS) in 522 patients with squamous cell HNC (HNSCC) treated with RT and cisplatin-based CHRT. Polymorphisms were identified using PCR-RFLP and TaqMan-MGB probes (Applied Biosystems). Survival curves were determined with Kaplan-Meier method. HRs and ORs (95% CI) were estimated using Cox proportional hazards and logistic regression models. The VEGF -634 CC was strongly associated with poor OS in uni- and multivariate analyses (P = 0.014 and 0.008). In both models, the VEGFR2 -271 T and MMP2 -1306 T variant carriers were at higher risk of local recurrence (P = 0.030 and 0.034). Finally, VEGF 634 CC as well as VEGFR2 -271 T and MMP2 -1306 T were identified as independent predictors of shorter OS and time to local recurrence, respectively.

Our preliminary results indicate that genetic polymorphism in key members of angiogenesis pathway, such as VEGF, VEGFR2 and MMP2, may be predictive of outcome in HNSCC patients treated with RT or CHRT.

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

[C-31] STUDY OF ETHANOL AND WATER VAPOUR PERMEATION THROUGH BIOPOLYMER BASED MEMBRANES IN THE PRESENCE OF METAL OXIDES

Małgorzata Gnus, Gabriela Dudek, Roman Turczyn

Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian university of Technology, 44-100 Gliwice, Ks. M. Strzody 9 Street, Poland

Many studies are conducted on developing high performance and selective homogeneous or heterogeneous membranes for dehydration applications. From the wide range of polymers, biopolymers enjoy the greatest interest. However, the possibility of using them as membrane, without prior modification, is quite limited. A useful strategy for improving separation efficiency of polymeric membranes is formation of hybrid materials through the incorporation of various inorganic materials, typically oxides or metal nanoparticles, into the polymer matrix. These membranes benefit in the selectivity of active components and the simplicity of polymeric membrane processing. The effectiveness of this kind of membranes depends mainly on the interaction between components and penetrants, their compatibility and homogeneous dispersion of inorganic component, into polymer matrix.

The aim of this work was to compare transport properties of membranes based on different biopolymers in ethanol and vapour permeation process. For this purpose three kind of mixed matrix membranes filled with metal oxides were used: epichlorohydrin crosslinked chitosan membranes, calcium and citric acid crosslinked alginate membranes. Permeation experiments were caried out at room temperature using as feed solution pure water, 99,8% v/v ethyl alcohol and 50% v/v ethyl alcohol solution in water. Then based on the total flux and GC analysis results several transport characteristic of investigated membranes was evaluated.

Based on the obtained transport parameters, it was observed that regardless of the polymer matrix, crosslinking agent and the type and amount of applied filler, the permeability coefficients determined for water as well as for ethanol are higher than the analogously determined parameters for the mixture of these components. It was shown that the used metal oxides differently affected the membrane's transport properties. The results show also how important is the selection of proper crosslinking agent, because not only does it affect the membrane selectivity coefficient, but can also substantially modify the effect of the used metal oxide.

The autors would like to thank the Silesia University of Technology for providing financial support under the project BKM/526/RCH4/2016.

[C-32] EVALUATION OF PROAPOPTOTIC AND GENOTOXIC ACTIVITY OF MELPHALAN DERIVATIVES

Agata Goczał[1], Arkadiusz Gajek[1], Małgorzata Łukawska[2],Violetta Cecuda- Adamczewska[3] Irena Oszczapowicz[2], Agnieszka Marczak[1]

[1]Department of Medical Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, 141/143 Pomorska St., 90-236 Lodz, Poland; [2]Department of Modified Antibiotics, Institute of Biotechnology and Antibiotics, 5 Staroscinska St., 02-516 Warsaw, Poland; [3]Department of Bioengineering, Institute of Biotechnology and Antibiotics, 5 Staroscinska St., 02-516 Warsaw, Poland

The major method of treatment of different types of cancers is chemotherapy intended to reduce the number of cancer cells. However, anticancer chemotherapy is related to numerous side effects and in many cases condemned to failure because of the multidrug resistance developed by cancer patients, often at the very beginning of the treatment.

The limitations in efficacy and safety of chemotherapy require development of novel treatment strategies often based on modification of existing drugs.

For this reason, the main aim of our investigation was to evaluate the profile of antitumor activity of newly synthesized melphalane derivatives and to select the most active compounds. The most interesting structural feature of melphalan molecule is the presence of two modifiable functional groups (amino and carboxyl) allowing extensive comparisons of derivatives simultaneously modified in both groups or only in one of them. We found that there may indeed be an actual structure-activity relationship within the investigated group of analogs.

In our research we have focused on the anti-proliferative activity and the value of apoptosis as well as DNA damage induced by the tested analogs.

To estimate the cytotoxicity of melphalan and its derivatives, and to choose appropriate concentrations for further analysis, resazurin viability assay was performed. To analyze phosphatidylserine (PS) externalization on the plasma membrane, double staining using fluorescence probes annexin V- Fluorescein isothiocyanate/propidium iodide was carried out. Additionally, the DNA degradation (alkaline version of comet assay) was determined.

The in vitro anticancer activity of the melphalane and its derivatives was evaluated against a human cancer cell line used as in vitro model for acute monocytic leukemia (THP1 cells). All investigated modified compounds were highly cytotoxic to this cell line, demonstrating half-maximal inhibitory concentrations (IC_{50}) in the low-micromolar range (0.04 μM to 0.69 μM) in comparison to melphalane drug (6.26 μM).

Moreover, the obtained results show that the newly synthesized compounds were considerably more genotoxic towards the investigated cell line than melphalane, the currently used lead drug. This effect was especially visible after longer times of incubation. Detailed studies indicate that modified derivatives induce programmed cell death (observed changes were characteristic for late apoptosis), detected at relatively late time points.

[B-14] DIFFERENTIAL EXPRESSION AND CELLULAR DISTRIBUTION PATTERN OF HSPA2 CHAPERONE PROTEIN IN HUMAN NORMAL BRAIN AND MALIGNANT ASTROCYTOMAS

Agnieszka Gogler-Pigłowska, Anita Strączyńska-Niemiec, Mykola Chekan, Damian Sojka, Urszula Bojko, Zdzisław Krawczyk, Dorota Ścieglińska

Maria Skłodowska-Curie Institute-Oncology Center, Gliwice Branch, Gliwice, Poland

HSPA2 chaperone protein is poorly characterized member of the HSPA (HSP70) multigene family. HSPA2 was originally described as a testis-specific and crucial for male fertility. Our original studies revealed that HSPA2 is also expressed in selected somatic tissues, including epidermis, and is involved in the control of keratinocyte differentiation process. Furthermore, HSPA2 has attracted increased interest due to its alleged supportive role in maintenance of the invasive phenotype of epithelial cancer cells derived from various non-testicular tissues. At present, the knowledge about expression of HSPA2 in CNS is limited.

The present study is the first descriptive tissue microarray-based immunohistochemical analysis of HSPA2 protein distribution in normal and pathological human brain. We have showed that HSPA2 is present in mature macroglial cells – oligodendrocytes and ependymal cells but not in astrocytes. Analysis of HSPA2 expression in gliomas – malignances derived from astroglia – revealed changes in the localization pattern of immunolabeling for HSPA2, compared to normal brain tissue. Dual immunofluorescent staining of the astrocytoma specimens showed that HSPA2 is localized in malignant astrocytes (GFAP+) and non-endothelial, perivascular cells (CD31-/αSMA+) of neoplastic blood vessels. Moreover, analysis of the intensity of HSPA2 immunohistochemical staining in astrocytomas revealed elevated level of the protein correlated with the increase in tumor histologic grade.

In summary, high HSPA2 expression in high-grade astrocytomas and change in its intratumoral tissue distribution may suggest the involvement of HSPA2 in growth and invasiveness of astrocytoma cells. Since HSPs are proposed as a novel attractive target for anticancer therapy, further studies are needed in order to clarify the functional significance of HSPA2 in gliomas development and/or progression.

The research has been supported by National Science Centre, grant no. 2013/09/B/NZ5/01815

[C-12] PHYSICAL AND BIOLOGICAL PROPERTIES OF GOLD (III) COMPLEXES WITH 2,2':6',2''-TERPYRIDINE

M. Golec[1,2], K. Czerwińska[3], M. Skonieczna[4,5], J. Palion-Gazda[3], D. Zygadło[1,2], A. Szlapa-Kula[6], S. Krompiec[6], A. Ratuszna[1,2], G. Kramer-Marek[7], B. Machura[3], A. Szurko[1,2]

[1]The August Chełkowski Institute of Physics, University of Silesia, 41-500 Chorzow, 75 Pulk Piechoty 1A, Poland, [2]The Silesian Centre for Education and Interdisciplinary Research (ŚMCEBI), 41-500 Chorzow, 75 Pulk Piechoty 1A, Poland, [3]Department of Crystallography, Institute of Chemistry, University of Silesia, 40-006 Katowice, Szkolna 9, Poland, [4]Silesian University of Technology, Center Biotechnology Bioengineering and Bioinformatics, Gliwice, Poland, [5]Silesian University of Technology, Institute of Automatic Control, Gliwice, Poland, [6]Department of Inorganic, Organometallic Chemistry and Catalysis, Institute of Chemistry, University of Silesia, 40-006 Katowice, Szkolna 9, Poland, 7The Institute of Cancer Research, London, UK

Chemotherapy is one of the most effective treatment method for cancer diseases. For many years the main chemotherapeutics have been compounds containing platinum, but the nagging side effects, as well as the tumor-cell resistance to these drugs are driving the research for new paths. In the recent years the metal complexes have been tested, included gold complexes. Published studies have shown that the biological activity of gold (III) complexes is better than the most commonly used drug – cisplatin. A certain limitation of the gold (III) in terms of their use in cancer therapy is their low stability in the physiological environment. Multidentate N-donor ligands, such as 2,2':6',2''-terpyridine, provide the possibility of obtaining a harvest of sufficient stability.

Two gold (III) complexes (Au5, Au14) with 2,2’:6’,2”-terpyridine have been characterized for their physical properties, as absorption, fluorescence and stability in different pH solutions. They have been tested also for their biological properties, as cytotoxicity for a different cancer lines (MCF7, MCF7/DOX, Me45, K562) and normal cell ones (HaCaT, GM07942). For more advanced analyses have been chosen cell lines and the compound with the best IC50. Cell cycle analysis have been done, mechanism of action have been tested by Western blot and the localization of Au14 by confocal microscopy. Also comparison of action of gold (III) complexes have been tested on MCF7 and MCF7 drug resistant cell line (MCF7/DOX).

The results shown that the gold (III) complexes with 2,2’:6’,2”-terpyridine are more cytotoxic compared to cisplatin. Received results confirm the similar mechanism of action of both compounds – cell cycle inhibition in G1 phase for Au14, for cisplatin in G2 phase. Cell cycle results were confirmed by analysis of cell cycle proteins with Western blot (p21, p53 and cyclin D). The physical measurements have shown that both gold (III) complexes characterized with fluorescence at 350-360 nm.

[B-48] MCPIP1 PROTEIN INFLUENCES ON THE PROCESS OF CARCINOGENESIS AND THE EPITHELIAL-MESENCHYMAL TRANSITION

Górka J., Jura J., Miękus K.

Department of General Biochemistry, Faculty of Biochemistry, Biophisics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, Kraków, Poland

Monocyte Chemotactic Protein-1 Induced Protein (MCPIP1) is a negative regulator of inflammation degrading of pro-inflammatory cytokines such as IL-1, IL-2 mRNA because of it is RNase activity. MCPIP1 is also known to reduce the activity of the transcription factor NFκB. Epithelial mesenchymal transition (EMT) is a key element in which epithelial cells acquire characteristics of mesenchymal cells. EMT has been shown to occur in wound healing, in organ fibrosis and in the initiation of metastasis for cancer progression. During EMT cells begin to express specific transcription factors: Slug, Snail, ZEB1, ZEB2 and Twist in order to reprogram gene expression in the cell. Epithelial markers such as E-cadherin which is hallmark of epithelial phenotype are reduced, while the expression of mesenchymal markers, N-cadherin and vimentin is increased. EMT process is believed to be a connection between inflammation and cancer progression.

This study was to investigate the correlation of the level of MCPIP1 protein and the process of EMT in normal kidney and cancer clear cell renal cell carcinoma. We evaluated the influence of MCPIP1 on the process of EMT.

Our results show that low expression of MCPIP1 protein increases the character of mesenchymal cells in tumor and normal cells. Downregulation of MCPIP1 suppressed E cadherin and upregulated β-catenin and vimentin. MCPIP1 silencing induced the expression of E-cadherin repressors and Snail. In contrast, overexpression of MCPIP1 causes a decrease of transcription factor Snail, vimentin and β-catenin and an increase of E-cadherin. MCPIP1 overexpression significantly decreases the motile activity in ccRCC. In addition, we observed changes in the level of focal adhesion kinase, which affects the migration and cell adhesion processes, affecting the levels of SRC, integrin, MMP2 and MMP9. The results indicate that the level of MCPIP1 is critical in the regulation of EMT and carcinogenesis.

Supported by grants: NSC 2013/09/D/NZ/00249, KNOW 35p/3/2015, BMN 1/2017

[C-21] CFD MODELLING FLOW AND MASS DIFFUSION OF DOXORUBICIN IN CELL CULTURE CHAMBERS OF MICROFLUIDICS CHIP

Maria Gracka[1], Ziemowit Ostrowski[1*], Marta Iwanaszko[2,3], Wojciech Adamczyk[1], Sebastian Student[2]

[1]Institute of Thermal Technology, Biomedical Engineering Laboratory, Silesian University of Technology, Poland, [2]Institute of Automatic Control, Silesian University of Technology, Poland; [3]Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, USA
**Corresponding author: ul. Konarskiego 22, 44-100 Gliwice, Poland;*
ziemowit.ostrowski@polsl.pl

In the last few decades, microfluidics systems relevance has significantly increased. Those techniques are widely used in various branches of science, ie chemistry, biology, genetics, pharmacology because of their advantages over standard analytical methods. Biologically, microfluidics seems to be particularly important as most processes involve micro-flow, from transport through cell walls, through oxygen diffusion into the lungs, to blood flow in the capillaries. Micro-scale analysis has many advantages, both from an analytical and economic point of view. One of the biggest advantages of miniaturization is the reduction of reagent consumption, leading to reduced waste and costs, as well as the ability to carry out more experiments. In addition, with long and narrow channels, the flows are laminar, thus ensuring that mixing is only by diffusion, in which case adjusting the appropriate flow conditions is particularly important for the success of the process [1].

Computer models offer many advantages over experimental methods, so in recent years, Computational Fluid Dynamics (CFD) has been widely used in many fields of engineering. Computer models can be used to perform virtual experiments that allow the simulations of processes that could be difficult to reproduce under laboratory conditions. Computer models allow a relatively easy description of phenomena and flow conditions in the analyzed microcircuits. The scope of this work was to develop a numerical model of flow and mass diffusion of drugs, for example, anti-cancer drugs like doxorubicin in cell culture chambers of microfluidics chip. The species transport model allows to simulate diffusion and mixing processes within the presented microchannels. Volumetric flow measured in laboratory condition was used to implement boundary conditions at the inlet of the main channel and supply channel of doxorubicin. Such simulation can be helpful in selecting the optimal channel geometry to provide full mixing process. ANSYS Fluent [2] commercial software (ANSYS Inc., USA) was used to develop the numerical model.

[1] Kolnik M et al. Lab Chip 2012 November 21; 12(22):4732-
[2] ANSYS Academic Research, Release 16.2, Help System, User Guide, ANSYS, Inc.

This research is supported by Innovation and Technology Transfer Centre CITT of Silesian University of technology within Inkubator Innowacyjności+ project and by Faculty of Energy and Environmental Engineering within Ministry of Science and Higher Education (Poland) statutory research funding scheme This help is gratefully acknowledged herewith. We thank Jeff Hasty and Krzsztof Wabnik from UCSD Biodynamics Laboratory for chip production help.

[B-47] INDUCED NEURAL STEM CELL BASED MODELS FOR ASSESSMENT OF IDH1R132H MUTATION EFFECTS

Dagmara Grot, Kamila Rosiak, Piotr Rieske

Department of Tumor Biology, Medical University of Lodz, Zeligowskiego 7/9, 90–752, Lodz, Poland

Diffusely infiltrating gliomas are one of the most frequent primary tumors of central nervous system. Gliomas are divided based on isocitrate dehydrogenase (IDH) gene mutation status. Mutation of IDH1R132H is detected in approximately 90% of the IDH-mutated tumors and lead to changes in activity of endogenous enzymes, affecting regulation of cell differentiation, proliferation and viability. Gliomas are generally thought to arise from the neural stem cells or the populations of progenitor cells. Due to the difficulties with establishing suitable culture conditions for primary glioma cells with endogenous expression of IDH1R132H, previous studies have been conducted on stable cancer cell lines, differentiated astrocytes and variety of stem cells of non-neural origin, giving contradictory results. Therefore, novel in vitro models are required to study the effects of IDH1 gene mutations.

The analyses were conducted on models obtained through differentiation of human induced pluripotent stem cells with an intermediate step comprising embryoid bodies formation. In preliminary studies, cells were subjected to stable transduction and constitutive expression of IDH1R132H was obtained. The effect of IDH1R132H on neuronal and astrocytic differentiation was assessed with Real-time PCR and immunocytochemistry analysis of Map2 and GFAP expression. Apoptosis was assessed with Western Blot (detection of PARP degradation) and caspase-3 activity assay. Analyses showed the inhibitory effect of constitutive expression of IDH1R132H on differentiation of iNSc. Results of Real-time PCR analysis showed a statistically significant decrease in GFAP mRNA levels. In the case of Map2, the level of mRNA did not reach a significant difference, which may be due to the fact that expression of this marker is also detected in non-differentiated neural stem cells. Analyses of the effect of IDH1R132H on iNSc viability showed increased caspase-3 activity and PARP protein degradation as compared to the control group. Additional model with inducible IDH1R132H expression was developed through transduction of iNSc obtained from iPSc with Tet-On 3G regulatory elements. Expression of Sox2 and Nestin as well as IDH1R132H after doxycycline induction was evaluated at different time points with immunocytochemistry. Analyses with specific antibodies confirmed neural stem cell phenotype, the correctness of transgene introduction and doxycycline-induced expression.

The improved experimental model with inducible expression of IDH1R132H will constitute a valuable platform for studies on molecular basis of glioma, and, thus, will contribute to broadening the knowledge of the genesis and biology of these tumors.

This study was financially supported by the National Science Center, grant no. 2012/05/B/NZ4/02623 and grant no. 2016/21/N/NZ3/03271.

[A-16] VASCULAR TREE GENERATION METHOD APPLIED TO MORPHOMETRIC ANALYSIS OF CIRCLE OF WILLIS

Anna Grzenik[1], Krzysztof Psiuk-Maksymowicz[1,2]

[1]Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 16, Poland,
[2]Biotechnology Centre, Silesian University of Technology, 44-100 Gliwice, ul. Krzywoustego 8, Poland

The circle of Willis is a cerebral arterial circle that supplies blood to the brain. It forms circulatory anastomosis in order to protect the brain from ischemia. There are many anatomical variants of the circle. Changes in the normal morphology of the circle may condition the appearance and severity of symptoms of cerebrovascular disorders, such as aneurysms, infarctions and other vascular anomalies. Cerebral aneurysm often occurs in specific regions of the circle of Willis.

The aim of this work was application of developed method for identification and skeletonization of the vascular network to the medical image data, in particular to capture the circle of Willis structure basic morphometric characteristics.

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

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

This work was financially supported by internal grant of Silesian University of Technology BK-213/Rau1/2016.

[C-3] THE POWER OF NATURE – BERBERINE AND RESVERATROL COMBINED THERAPY

Dorota Hudy[1,2], Aleksandra Poterala-Hejmo[1,2], Tomasz Hejmo[3], Arkadiusz Dziedzic[4], Rafal J. Buldak[3], Magdalena Skonieczna[1,2]

[1]Biosystems Group, Silesian University of Technology, Institute of Automatic Control, Akademicka 16, 44-100 Gliwice, Poland; [2]Biotechnology Centre, Silesian University of Technology, Krzywoustego 8, 44-100 Gliwice, Poland; [3]Department of Biochemistry, Medical University of Silesia, School of Medicine with the Division of Dentistry, Jordana 19, 41-808 Zabrze, Poland; [4]Department of Conservative Dentistry with Endodontics, Medical University of Silesia, Pl. Akademicka 17, 41-902 Bytom, Poland;
Corresponding author: magdalena.skonieczna@polsl.pl

In today's world conventional medicine more often tests natural based substances in many health treatments. These substances are often from plant sources like herbs, fruits etc. In our study these substances were berberine from Berberis plants and resveratrol from grapes. Both of them are known or believed to have positive effects on human health and are used in natural/alternative medicine.

Squamous carcinoma cells (SCC-25) were exposed to combined resveratrol and berberine therapy with different ratios between those two substances for 24 hours, followed by the MTS assay in four independent experiments.

The results revealed that combined berberine and resveratrol therapy may have an additive or antagonizing effects after 24 hours exposition to these substances. Additive effects were observed for doses lower than IC50 of berberine [IC_{50}=23 μg/mL], and resveratrol [IC_{50}=9 μg/mL], after MTS assays and isobologram analysis. The results suggest that combined administration of berberine, in the presence of resveratrol, could be decreased even by 50% (half of IC_{50} for berberine).

The work was supported by grant (M.S.) No. 02/010/RGH17/0092, founded by Rector of the Silesian University of Technology in Gliwice, Poland.

[B-23] DETECTION OF DNA REPLICATION ORIGINS BASED ON SOMATIC MUTATIONS

Roman Jaksik[1], Marek Kimmel[1,2]

[1]Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland; [2]Department of Statistics, Rice University, Houston, Texas, USA

DNA replication origins are selectively activated by a mechanism which is difficult to study in living cells due to its complexity and lack of precise experimental procedures. Additionally, the location of replication origins varies significantly between cell cycles and cells from the same population, making them difficult to map in the genome, and relate to its specific characteristics. In this work, we employed, at the genomic scale, the finding of Shinbrot et al. (2014) that the mutator phenotype associated with damaged polymerase ε can be used to identify genomic positions of the replication origins. We used whole genome sequencing data from The Cancer Genome Atlas project to carry out a genome-wide search for DNA replication origins which were later used to determine structural properties of the DNA in their vicinity.

Using data obtained from nineteen patients, that show specific damages in polymerase ε, and our custom detection algorithm, we were able to identify over three thousand replication origins that are located close to the origins obtained using other approaches. We than used them to determine the structural properties of DNA in their vicinity showing that chromatin structure might be the leading factor that affects their location. Additionally we were able to characterize the mutational landscape associated with damaged polymerase ε in the vicinity of replication origins.

This work shows that the specificity of cancer related processes might be successfully used to study basic intracellular mechanisms and that mutation-based detection of replication origins is a viable way of determining their activity and sequence features associated with their location.

This work was supported by the Polish National Science Center grant DEC-2012/04/A/ST7/00353 and BKM-506/RAU1/2016 t.46.. Calculations were carried out using the computer cluster Ziemowit (http://www.ziemowit.hpc.polsl.pl) funded by the Silesian BIO-FARMA project in the Computational Biology and Bioinformatics Laboratory of the Biotechnology Centre in the Silesian University of Technology.

[C-7] DENOSUMAB IN PATIENTS WITH GIANT CELL TUMOR AND ITS RECURRENCE: A SYSTEMATIC REVIEW AND META-ANALYSIS

Jamshidi Khodamorad[1], Gharehdaghi Mohamad[2], Hajialiloo Sami Sam[1], Mirkazemi Masoud[1], Ghaffarzadehgan Kamran[3], Izanloo Azra[3*]

*[1]Bone and Joint Reconstruction Research Center, Shafa Orthopedic Hospital, Iran University of Medical Sciences, Tehran, IR Iran; [2]Orthopedic Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; [3]Razavi Cancer Research Center, Razavi Hospital, Imam Reza International University, Mashhad, Iran; *corresponding author: Azra Izanloo, Razavi Cancer Research Center, Research and Education Department, Razavi Hospital, After Ghaem Bridge, Asiayi Highway, Mashhad, Iran. Email: a.izanloo64@gmail.com , Fax: +985136004359. ORCID ID: 0000-0002-2430-1591*

This study reviews the literature to determine the effectiveness and safety of Denosumab in reducing activity and skeletal- related events in patients with giant cell tumor of bone and its recurrence.

Primary outcomes associated with skeletal-related event, overall survival, secondary outcomes such as pain, quality of life and adverse events were evaluated.

The total population of this meta-analysis consisted of 686 patients. Of this population, only 55% had primary giant cell tumor of bone and the remaining 45% had giant cell tumor recurrence, with 2% experiencing secondary recurrence.

The results showed the effectiveness of Denosumab in neoadjuvant and adjuvant settings.

Key words: Giant Cell Tumor of Bone, Denosumab, Systematic Review, Meta-Analysis, Recurrence

[C-40] DYNAMICS OF SEASONAL CHANGES IN BACTERIAL BIODIVERSITY IN COASTAL LAKES

Marcelina Jureczko[1]*, Aleksandra Ziembińska-Buczyńska[1], Katarzyna Glińska-Lewczuk[2], Paweł Burandt[2], Szymon Kobus[2], Sylwia Lew[3], Krystian Obolewski[4]

[1]*Environmental Biotechnology Department, Faculty of Energy and Environmental Engineering, The Silesian University of Technology, Akademicka 2A Str., 44-100 Gliwice, Poland*
[2]*Department of Water Resources, Climatology and Environmental Management, University of Warmia and Mazury in Olsztyn, Plac Łódzki 2 Str., 10-719 Olsztyn, Poland*
[3]*Department of Microbiology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5 Street, 10-719 Olsztyn, Poland*
[4]*Department of Hydrobiology, University of Kazimierz Wielki in Bydgoszcz, Jana Karola Chodkiewicza 30 Str., 85-064 Bydgoszcz, Poland*
[*]*corresponding author: marcelina.jureczko@polsl.pl*

The Polish shore of the Baltic Sea is rich in brackish lakes of estuarine character. They are dynamic ecosystems with high and continuous amplitudes of physicochemical and biological parameters that result from changes in salinity, temperature, pH, oxygen and nutrient concentrations and organic matter content.

The brackish-water habitats are rich in both, marine and freshwater fauna and flora, according to their adaptive possibilities. The most abundant group of living organisms in the estuaries is a diverse bacterial microflora. They can be divided into: inland (freshwater, soil, sewage), marine and halotolerative (growing in a very wide range of salinity) bacteria. These microorganisms must resist the constant changes of salt concentration, which exerts a certain osmotic pressure on cells in the environment.

The aim of this work was to study seasonal changes in bacterial diversity in coastal brackish lakes: Sarbsko and Łebsko. Biodiversity was studied for salinity gradient (resulting from the distance of a given lakes and its individual zones from the Baltic Sea), as well as physicochemical and biological changes (caused by seasons and depth of the reservoir from which the test material was taken). To monitor the genotypic variation of individual microorganisms and estimate biodiversity of the bacterial community in the settlement and to estimate the genotype complexity of the samples PCR-DGGE method (polymerase chain reaction, combined with denaturing gradient gel electrophoresis) was used.

The results show that salinity could be the factor that models the bacteria's genotype structure. Temperature through the seasons could also influence the bacterial biodiversity, but probably there is no relation between bacterial community structure and biodiversity and oxygen concentration. During the experiment there were no changes in pH level and this parameter has not got influence on bacteria.

The research was supported by National Science Center project number UMO-2012/07/B/ST10/04359

OPTIMIZATION OF PROTOCOL FOR DETERMINATION OF METABOLOMIC PROFILE OF EXOSOMES RELEASED BY FADU CELLS EXPOSED TO IONIZING RADIATION

Jakub Idkowiak[1], Anna Wojakowska[2], Agata Abramowicz[2], Piotr Widłak[2], Monika Pietrowska[2], Łukasz Marczak[1]

[1]Mass Spectrometry Laboratory, Department of Biochemistry of Natural Products, Institute of Bioorganic Chemistry, Polish Academy of Sciences, 61-704 Poznan, Z. Noskowskiego str. 12/14, Poland; [2]Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Institute – Oncology Center, Gliwice Branch, 44-101 Gliwice, Wybrzeze Armii Krajowej str. 15, Poland

Exosomes are cell-derived membrane vesicles of endocytic origin and diameter size range reaching 30 – 150 nm. Nearly all cell types release exosomes and therefore they are frequently found in numerous body fluids such as blood, urine, bronchoalveolar lavage fluid, saliva, synovial fluid or breast milk. Among bioactive molecules composing these vesicles nucleic acids, proteins, but also wide group of metabolites can be distinguished. Additionally, crucial biological function of exosomes has been previously revealed in terms of cellular signaling under normal and diseased conditions consequently leading to rapid growth of interest in their clinical application. In order to use exosomes for cancer therapy and prognosis of disease-progression precise characteristic of their composition is needed. Mass spectrometry-based techniques in combination with modern extraction protocols have been found as powerful, accurate and high-throughput tools for metabolomic screening and investigations. Here we present optimized protocol for complete metabolome profiling of exosomes by wide range of mass spectrometry techniques.

Exosomes isolated from FaDu cell cultures by ultrafiltration and size-exclusion chromatography on mini-SEC were used in experiments. Lipid fraction of vesicles was separated by "in-house" optimized liquid two-phase MTBE extraction method previously published and developed by V. Matyash and total primary metabolites were extracted according to protocol reported by A. Wojakowska. High-resolution Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled to TriVersa NanoMate nano-ESI ion source (Advion Biosciences) was used to perform lipid screening and GC-QqQ (Thermo Fisher Scientific) to total metabolite profile determination. Lipid profiles were further proceeded with use of LipidXplorer software (freely available, Max Planck Institute of Cell Biology and Genetics in Dresden, Germany) and primary metabolite profiles obtained by application of GC-MS were analyzed by ChromaTOF software (LECO), respectively.

The developed analysis techniques allowed identification of more than 300 lipid species and over 400 other metabolites forming exosomes.

This work was supported by the National Science Centre, Poland, Grant 2013/11/B/NZ7/01512.

[C-33] PHARMACOLOGICAL PLATFORM TO TEST THERAPEUTIC MOLECULES - IN SEARCH OF THE MECHANISM UNDERLYING NEGATIVE *IN VITRO* SELECTION OF TUMOR CELLS WITH ONCOGENE AMPLICONS

Karolina Janik[1,2], Cezary Treda[1,2], Wojciech J. Stec[2], Kamila Rosiak[1,2], Piotr Rieske[1,2], Marcin Ciechanowski[1], Ewelina Stoczynska-Fidelus[1,2]

[1]Department of Tumor Biology, Medical University of Lodz, 90-752 Lodz, ul. Zeligowskiego 7/9, Poland; [2]Research and Development Unit, Celther Polska Ltd., 93-193 Lodz, ul. Milionowa 23, Poland

Complex process of oncological drug development requires in vitro models reflecting in vivo heterogeneity of tumor cells. However, there is a limited availability of stable cell lines, commonly applied as testing platforms, meeting this requirement, especially in regard to the presence of oncogene amplifications (e.g. EGFR, PDGFR, MET) and their mutated variants (e.g. EGFRvIII).

In vivo occurrence of these alterations is frequent, while even in primary cultures, negative in vitro selection of cells with amplicons is observed. Using glioblastoma (GB) model we previously demonstrated that senescence is the main reason of the observed stabilization failure, however, the mechanism underlying this phenomenon remains to be elucidated.

Real-time PCR at DNA level and FISH analyses revealed statistically significant differences in amplicons presence between in vivo and in vitro conditions - GB primary cultures stabilized in our laboratory preserved alterations such as CDKN2A deletion or chromosome 7 polysomy, while oncogene amplifications or EGFRvIII mutation were not retained. Our data implies that it may result from mitotic catastrophe or mitotic cell death as these phenomena were observed in primary

GB cultures, possibly due to random separation of extrachromosomal amplicons during mitosis. It is also possible that amplicon-containing cells stabilize under in vitro conditions, but following amplicon loss, as we detected nuclear amplicon extrusion (so called nuclear buddings) in primary cultures derived from tumors with EGFR amplification. Moreover, we established an unique model for functional analyses of cells with oncogene amplifications - DK-MG cell line with subpopulation of cells characterized by endogenous EGFRvIII amplicons. Interestingly, our results indicate that although EGFRvIII-positive cells are characterized by higher proliferation rate than the negative subpopulation, stable percentage of EGFRvIII-positive cells is maintained in culture. Additionally, FISH analysis of DK-MG cells revealed asymmetric EGFR amplicon division and asymmetric distribution of EGFR protein expression was detected in these cells in immunocytochemical analysis.

Our analyses clearly identified two possible mechanisms responsible for the inability to maintain cells with amplification of oncogenes under in vitro conditions. However, which of these phenomena is observed in cells with amplicons and what mechanisms triggers this process under cellular stress conditions caused by in vivo-in vitro transfer needs to be determined. Further functional studies and analysis of paracrine interactions between cells with and without amplicons are required. Results of these analyses may contribute not only to establishment of in vitro platforms reflecting the heterogeneous nature of glioblastoma but also to the development of future therapeutic solutions.

This study was supported by the National Science Centre grant no. 2016/21/D/NZ3/02616.

[B-29] INVOLVEMENT OF ESTROGEN RECEPTOR ALPHA IN HSF1 ACTIVATION

Patryk Janus, Agnieszka Toma-Jonik, Wieslawa Widlak, Natalia Vydra

Maria Sklodowska-Curie Institute - Oncology Center, Gliwice Branch, 44-101 Gliwice, ul. Wybrzeze Armii Krajowej 15, Poland

HSF1 is primary transcription factor responsible for stress-induced activation of HSP genes. Additionally, HSF1 is involved in the regulation of many other genes associated with multiple cellular processes including cell signaling, development, fertility, cell death, and metabolism. In turn, estrogens (e.g. E2), recognized by the specific receptors (especially by ERα) are the primary female sex hormones which play an essential role in sexual and reproductive development. Clinical studies implicate both HSF1 and estrogens are risk factors for developing and metastasis breast and endometrial tumors. Our original studies revealed, that both cell signaling pathways can interfere each other, with the participation of ERα.

We noticed that E2 treatment led to rapid phosphorylation of HSF1 on S326 (activation site) in ERs-positive mammary breast adenocarcinoma MCF7 cells, but not in ERα-negative normal breast MCF10A and MCF12A cells nor in ovarian ERα-negative cancer cell lines, such as ES2 or OVCAR3. We stated, that the effect of E2 treatment on S326 HSF1 phosphorylation was independent of the status of other estrogen receptors, ERβ and GPER1. HSF1 activated by E2 was transcriptionally potent, since it could bind to the chromatin, but we did not observe a significant increase in expression of HSP genes. Interestingly, ERα-mediated phosphorylation of HSF1 was observed also as a result of cell stimulation with other estrogen-like compounds, such as bisphenol A (BPA) or agonist of ERα, propylpyrazoletriol (PPT). To investigate the mechanism of HSF1 phosphorylation under E2 treatment, we examined the activation of several important kinases using Human Phospho-Kinase Arrays. We observed phosphorylation of ERK1/2, JNK1/2/3, CREB, and mTOR kinases in response to E2. EGFR-PI3K-mTOR pathway involvement in HSF1 phosphorylation after E2 stimulation was confirmed by validation experiments using specific inhibitors of these enzymes. We assume that planned genomic analyses would explain the role and biological consequences of E2 and HSF1 cooperation in tumor growth as well as neoplastic transformation.

This work was supported by grants no 2014/13/B/NZ7/02341 and 2015/17/B/NZ3/03760 from the Polish National Science Centre.

[A-7] NOVEL SMALL-MOLECULE MDM2 INHIBITORS: A POTENT ANTI-CANCER THERAPEUTICS

Sabina Jeleń, Maria Mazur, Joanna Jaszczewska, Wojciech Lewandowski, Iwona Kalinowska, Jakub Witkowski, Katarzyna Woś-Latosi, Piotr Rózga, Małgorzata Teska-Kamińska, Marcin Feder

Department of Research and Development, Adamed Group, 05-152 Czosnów/Warsaw, Pienków 149, Poland

A growing number of preclinical and clinical investigations has been recently presented concerning small molecule inhibitors targeting the MDM2-p53 interactions dedicated as potent therapeutic agents against various cancer types. In almost half of the cancer patients TP53 gene is mutated or deleted leading to defective p53 protein expression and subsequent deregulation of cell cycle checkpoints promoting malignant cell growth. The remaining patients with wild-type TP53 gene have functional p53 protein, which is however, rapidly degraded by aberrantly expressed regulatory protein such as MDM2. Thus, molecules interrupting MDM2-p53 interactions are an attractive clinical approach to restore anti-tumor activity of p53 in patients bearing wild-type p53 gene. Encouraged by the therapeutic potential, we have developed a series of self-designed highly potent MDM2 inhibitors.

We have generated an extensive library of derivative molecules sharing a common core structure. The molecules have been tested according to the following pattern of consecutive studies: in vitro efficacy (MTT assay), receptor binding (fluorescence polarization test), in vitro ADME studies including microsomal stability (MS), drug permeability in Caco-2 cells monolayer, CYP450 inhibition, LogD, plasma protein binding, in vivo mouse pharmacokinetic (PK) study including exposure (AUC), determination of bioavailability (F), drug clearance (CL), half-life (T1/2), and in vivo efficacy in mice models.

Based on the aforementioned parameters, 15 precandidates have been selected and submitted to the ongoing mouse in vivo efficacy studies. Additionally, the basic mechanism of action of the selected molecules has been verified. Upon selection of the lead compound toxicological studies on relevant species will be performed.

We have generated meticulously designed MDM2 inhibitors with very well understood structure-activity relationship (SAR) characterized by high in vitro and in vivo efficacy, lower toxicity as well as favorable ADME and PK profile. We believe that our lead compounds represent a new generation of highly effective anti-cancer therapeutics.

[C-4] CYTOSTATIC PHARMACEUTICALS AS ENVIRONMENT CONTAMINANTS - A THREAT TO HUMAN HEALTH AND OTHER ENTITIES

Marcelina Jureczko, Joanna Kalka

Environmental Biotechnology Department, Faculty Of Energy And Environmental Engineering, Silesian University of Technology, Akademicka 2A Street, 44-100 Gliwice, Poland

Cytostatic drugs have become one of the greatest environmental threats. Investigations of consumption trends at a global scale show an increase in the use of anticancer drugs. It leads to increased levels of cytostatics released in the environment. Furthermore, due to the changes in therapy models and rise of anticancer home treatments, majority of the prescribed cytotoxic agents are now available in town pharmacies. Consequently, hospital effluents are no longer the main expected entry route of anticancer drugs into the aquatic environment. It is therefore necessary to continue investigating their presence in surface waters and in wastewater treatment plant (WWTP) effluents. The concentration of cytostatics in the aquatic environment depends on the location, matrix and specific drug, however, their concentrations comes up to a quarter million ng/L. These compounds are recalcitrant in natural waters and they are not effectively removed during wastewater treatment processes.

Cytostatics are known to be carcinogenic, mutagenic and toxic for reproduction. They interfere with the DNA, and therefore non-target organisms are likely to be affected. In this light, obtaining comprehensive ecotoxicity data is becoming crucial to determine their actual impacts on the ecosystem. There is also a lack in physico-chemical data for forecasting the chemodynamics of cytostatics in natural waters along with its human metabolites and environmental transformation products. Concerning ecotoxicological risks, current knowledge remains insufficient to support a definitive conclusion. Risk posed by cytotoxic molecules is still not well documented and it is not possible to conclude on their long-term effects on non-target organisms.

[B-15] CHANGES IN GENE EXPRESSION IN HCT116 AND ME45 CELL LINES

Sylwia Kała, Dorota Hudy, Roman Jaksik, Joanna Rzeszowska-Wolny

Institute of Automatic Control, Silesian University of Technology, Gliwice. Center of Biotechnology

Every cell line can be characterized by expression of a specific set of genes. This is a normal process which is followed by cells performing particular roles in organism (different environmental conditions) and responding to distinct signals.

Here we compared ionizing radiation-induced changes of gene expression in two cancer cell lines, Me45 (Human Melanoma cells) and HCT116 (Human Colorectal Carcinoma) focusing attention on genes linked to the redox state of cells. Both cell lines respond differently to ionizing radiation, suggesting changes in the activity of redox genes. Identifying these genes' expression levels in both cell lines suggests that every cell line can respond differently to the same stimulus which implies the existence of different mechanisms activated for a particular cell line. A distinct mechanism connected with regulation of free radicals may influence, for example, proliferation, cell survival and apoptosis. The range of genes overexpressed in HCT116 in comparison to Me45 cells and in Me45 in comparison to HCT116 cells is wide and includes also genes linked to calcium, NF-κB, and collagen production, as well as proliferation, apoptosis and cell cycle progression and mitochondrial homeostasis.

Complete data from Affymetrix microarrays were analyzed using RMAExpress software. All results were normalized and data for irradiated cells were compared to unirradiated controls, then with the other cell line.

This work was supported by the Polish National Science Center Grant 2015/19/B/ST7/02984 (J.RW), BKM- 508 /RAU1/2017 (S.K., D.H.)

[A-17] NON-INVASIVE IN VIVO LUMINESCENCE SPECTROSCOPY DIAGNOSIS SYSTEM FOR SKIN CANCER RESEARCH

Piotr Kałużyński[1*], Daria Kogut[2]

[1]*Department of Optoelectronics, Silesian University of Technology, Krzywoustego 2 Str, Gliwice, Poland;*
[2]*Institute of Automatic Control, Silesian University of Technology, Akademicka 2A Str, Gliwice, Poland*
**Mail: piotr.kaluzynski@polsl.pl, daria.kogut@polsl.pl*

Cancer diagnostics makes use of numerous methods. According to the type of tissue, scientists and physicians can use most suitable methods, like biopsy, mammography, endoscopy, etc.. Despite their effectiveness, all of these methods are invasive. Consequently, many biologists, optoelectronic engineers and scientists, focus on developing non-invasive methods for early diagnosis of cancer. Diagnosticians, in order to interfere as little as possible with human body, began to search for novel methods of non-invasive detection of carcinoma e.g. using visible light, based on naturally occurring photosensitizers e.g. HpD (hematoporphyrin derivatives), which occur in human blood. Literature data point to increased levels of HpD in tumor tissues, with noticeable differences in comparison to healthy tissues. According to spectral analysis, tumor tissue (by the presence of HpD), excited by near UV light (λ=405 nm), gives specific peaks of emitted spectrum in a different range (620 to 680 nm) of visible light than do healthy tissues (500 to 550 nm). There is also a difference in fluorescence intensity in emitted spectra between different types of skin cancer (e.g. SCC, BCC).

In this report we propose a novel design of skin cancer optical diagnostic system (Pic. 1), which uses a non-invasive optical method of detection, and which can be adapted to visualize malignancies based on luminescence spectroscopy analysis of given tissues. The detection of cancerous tissues can be increased by using differential analysis for comparing red and green peaks in given spectra. The proposed “optical biopsy” method and given fluorescence spectra show that such system could be used as one of the criteria in skin cancer diagnosis.

[A-1] SENSITIVITY ANALYSIS OF SIGNALING PATHWAY MODELS IN THE FREQUENCY DOMAIN FOR SIMULTANEOUS PARAMETER CHANGES

Małgorzata Kardyńska, Jarosław Śmieja

Institute of Automatic Control, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland.

Sensitivity analysis methods have been in use for over half a century. They 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]. In recent years these methods have been applied also to perform analysis of biological systems models. While they proved to be helpful in analysis of various pathway models whose simulation results units were fixed, they may lead to false conclusions when the model is based on relative measurement data. For this reason, it is necessary to develop methods which take into account specificity of such experimental data.

Here we propose a new method of sensitivity analysis based on frequency distribution of a system time response, which takes into account specificity of biological experimental data. Since frequency distribution provides valuable information about qualitative and quantitative system behavior, it can be used to analyze models based on absolute and relative measurements (frequently encountered in molecular biology). Furthermore, the proposed method allows to study the influence of simultaneous changes in various parameters.

To test the procedure, we performed sensitivity analysis of a model combining two signaling pathways: HSF and NF-κB. The model explains the inhibition of the NF-κB pathway after a heat shock, which may constitute one of the goals in anticancer therapies [2] and therefore its analysis may be important for cancer research. The possible interactions between the HSF and NF-κB pathways taken into account include creation HSP|IKK complexes, temperature-dependent inactivation of proteins located upstream of IKK activation and inhibition of NF-κB import to the nucleus under heat shock condition. Sensitivity analysis was performed for simultaneous changes of two parameters and the results were presented in the form of heatmaps, illustrating the effect of changing various parameter pairs on the NF-κB response.

[1] Saltelli A et al. 2008. Global Sensitivity Analysis: The Primer. Wiley.
[2] Zanotto-Filho A et al. 2011. Biochem. Pharmacol. 81(3), s. 412–424.

This work was financially supported by internal grant of Silesian University of Technology BKM-508/RAU1/2017/t.9 (02/010/BKM17/0083). Calculations were performed on the Ziemowit computational cluster (http://www.ziemowit.hpc.polsl.pl) created in the POIG.02.01.00-00-166/08 project (BIO-FARMA) and expanded in the POIG.02.03.01-00-040/13 project (Syscancer).

[A-18] AN INFLUENCE OF DIFFERENT TUMORS AND ARTERIOVENOUS MALFORMATION IN FUNCTIONAL IMAGING

Ilona Karpiel[1], Zofia Drzazga[1], Patrycja Mazgaj[1], Aldona Giec-Lorenz[2]

[1]Department of Medical Physics, Institute of Physics, University of Silesia, ul. Uniwersytecka 4, 40-007 Katowice, Poland; [2]Helimed Diagnostic Imaging Sp. z o.o., Laboratory of Magnetic Resonance Imaging, Katowice, Poland

fMRI is a modern, non-invasive diagnostic method which can be used in diagnosing preoperative patients in order to obtain important information for surgical intervention. Mapping brain activity is possible due to changes in hemoglobin forms in blood vessels.

The aim of our study was to evaluate the functional areas of motor cortex adjacent to the different type of tumors and arteriovenous malformation (AVM). Motor area are very important from the point of view patients quality life.

In our study we have focused on impact of different types of tumor as well as arteriovenous malformations (AVM) on fMRI images performed during normal clinical work at MRI center. Analyses were obtained in SPM package for selected parameters (Gaussian kernel, significance level and cluster-level extent threshold).

We observed that brain tissue retains its functionality in the case of venous angioma unlike neoplastic changes. It seems to be associated with the fact that presence of tumor lesions changes brain parenchyma substantially causing disappearance of functional areas dependent on the stage of disease.

[A-19] APPLICATION OF FULLY CONVOLUTIONAL DEEP NEURAL NETWORKS AS AN EFFICIENT TECHNIQUE FOR AUTOMATED SEGMENTATION OF GLIOMAS

Szymon Kocot, Franciszek Binczyk

Silesian University of Technology, Institute of Automatic Control, Data Mining Group 44-100 Gliwice, ul. Akademicka 16, Poland

The aim of the work was to implement and conduct quality evaluation of an automated brain tumor segmentation technique based on Tiramisu architecture.

Since 1990s first methods for automated segmentation of brain tumors have been published, but all of them were validated on small, private datasets, until 2012, when MICCAI organized a Multimodal Brain Tumor Image Segmentation Challenge (BraTS) releasing publicly available dataset, consisting 4 modalities of MRI images: T1, T1 with contrast (gadolinium), T2 and FLAIR with manually segmented tumors.

Recent improvements in neural networks training performance by taking advantage of thousands cores of GPGPU and ReLU activation function have led to usage of convolutional neural networks for sematic segmentation. The examples of successfully used architectures are: 2015 U-Net [1] and 2016 100-layer Tiramisu DenseNet [2]. Especially, Tiramisu has shown great performance on sematic segmentation of urban scene benchmarks. It contains up-paths and up-paths, but also Dense blocks with skip-paths include Concatenation of feature maps from output of Convolutional layer along with its input. Each hidden layer uses ReLU activation and Batch Normalization and L2 regularization. Different variants of Tiramisu consist of various number of layers per Dense block (usually 4÷5, up to 12) and growth factor which defines how fast number of feature layers growths after each Concatenation (usually k=12÷15). Rise of these parameters causes rapid increase of trainable parameters.

We here propose a variant of Tiramisu Densenet with k=8 and number of layers per Dense Block = [4,5,7,10,12,15] with 9.3 mln of trainable parameters build in Keras with Tensorflow backend. Training dataset have been prepared by cropping each slice to 192x160 pixels, to eliminate redundant background reducing amount of needed memory and standardized by Z-Score. Network have been trained with categorical cross-entropy loss and RMSprop optimizer with learning rate 1e-3. Training on Nvidia GTX1080 took approximately 2 days.

Resulting internal measure of average Dice score on training dataset is exemplary: 0.890 for Whole Tumor, 0.798 for Tumor Core and 0.801 for Enhancing Tumor. NN have been also tested on whole BraTS validation dataset and its median Dice scores are: 0.882 for WT, 0. 751 for TC and 0.778 for ET. We will continue our research to improve segmentation quality, that is already at the level of the state of the art methods. We recently discovered corruption of considerable amount of images due to improper registration process, that could cause some underperformance of NN training and in effect in final segmentation. To correct bias fields, we plan to apply N4ITK algorithm. There is probably also a huge margin for hyper-parameter tuning, which we plan to apply in the coming future.

[1]. Ronneberger O et al. U-Net: Convolutional Networks for Biomedical Image Segmentation, Medical Image Computing and Computer-Assisted Intervention (MICCAI), Springer, LNCS, Vol.9351: 234--241, 2015
[2]. Jégou S et al. IEEE Conference on Computer Vision and Pattern Recognition Workshops, 2017

This work was financed by grant no. 02/010/BK17/0060/9; BK-204/RAU1/2017/9. Additionally, FB was financed by project granted by Narodowe Centrum Nauki: UMO-2015/19/B/ST6/01736.

[C-23] SYNTHESIS OF 8-HYDROXYQUINOLINE GLYCOCONJUGATES USING AZIDE-ALKYNE CYCLOADDITION AND PRELIMINARY EVALUATION OF THEIR ANTICANCER ACTIVITY

Monika Krawczyk[1,2], Gabriela Pastuch-Gawołek[1,2], Magdalena Skonieczna[2,3], Robert Musioł[4]

[1]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, 44-100 Gliwice, ul. Krzywoustego 4, Poland; [2]Biotechnology Centre, Silesian University of Technology, ul. Krzywoustego 8, 44-100 Gliwice, Poland; [3]Biosystems Group, Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 16, Poland; [4]University of Silesia, Institute of Chemistry, 40-006 Katowice, ul. Szkolna 9, Poland

8-Hydroxyquinoline (8-HQ) scaffold is a privileged structure used in designing new active agents with therapeutic activity. Unfortunately, the compounds designed on the core of quinoline also have undesirable effects, such as toxicity, poor bioavailability or lack of selectivity in targeting drugs to the specific site of action, resulting in side effects of drugs on healthy tissues [1]. Only modifications of this structure aim to improve its biological and pharmacological parameters. An interesting opportunity for their derivatization is to connect them with the sugar unit. Glycoconjugates of 8-HQ derivatives exhibit a wide spectrum of biological activity [2,3]. Particularly in recent years there has been an increase in interest in their anticancer activity, which is due to their ability to chelate the metal ions that are necessary for cancer growth [4].

Results previously reported in the literature indicate that the presence in the glycoconjugate structure an additional aromatic or heteroaromatic fragments is important for their biological activity [5,6]. Therefore, the introduction of 1,2,3-triazole ring, received by the click chemistry reaction, into quinoline glycoconjugates seems to be an interesting idea. New glycoconjugates were synthesized by the connection 1-azido sugars derivatives of D-Glucose or D-Galactose with propargyl derivatives of 8-hydroxyquinoline or 8-hydroxyquinaldine as well as propargyl β-O-glycosides with azides 8-HQ derivatives. For the above mentioned conjugation copper(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition was applied.

The obtained quinoline glycoconjugates were tested for their anticancer activity (HCT-116, HeLa) as well as their ability to inhibit β-1,4-Galactosyltransferase. It was found that the used glycoconjugation strategy influenced the activity and improved bioavailability of 8-HQ derivatives. The results of the initial assessment of the biological activity of these compounds will be presented.

[1] Song Y et al. Med. Chem. Commun., 2015, 6, 61-74.
[2] Calvaresi EC et al. Chem. Sci., 2013, 4, 2319–2333.
[3] Oliveri V et al. Dalton Trans., 2012, 41, 4530-4535.
[4] Ding WQ et al. IUBMB Life, 2009, 61, 1013-1018.
[5] Pastuch-Gawołek G et al. Eur. J. Med. Chem., 2016, 112, 130-144.
[6] Areti S. Et al. ACS Omega, 2016, 1, 626-635.

[B-9] ANTIOXIDANT AND DNA REPAIR GENE POLYMORPHISMS INFLUENCE THERAPY OUTCOME IN SQUAMOUS CELL CARCINOMA OF HEAD AND NECK (HNSCC)

Małgorzata Krześniak[1], Agnieszka Gdowicz-Kłosok[1], Tomasz Rutkowski[2], Iwona Matuszczyk[1], Iwona Domińczyk[1], Monika Pietrowska[1], Krzysztof Składowski[2], Dorota Butkiewicz[1]

[1]Center for Translational Research and Molecular Biology of Cancer, [2]I Radiation and Clinical Oncology Department, M. Skłodowska-Curie Institute-Oncology Center, Gliwice Branch, 44-101 Gliwice, Poland

Head and neck cancer accounts for 5-6% of all malignancies in Poland. Almost 75% of the cases are diagnosed at advanced stage and the treatment of choice for these patients are radiotherapy (RT) and cisplatin-based chemoradiotherapy (CHRT). However, the treatment outcome is unsatisfactory with more than 50% rate of failure and local recurrence. IR and platinum analogs generate reactive oxygen species (ROS) that cause DNA strand breaks and oxidative damage leading, in consequence, to cancer cell death. The activity of antioxidant enzymes (e.g. GPX4, CAT, NQO1 and GSTs), as well as DNA repair proteins, such as NBS1, protects cells from this damage. Glutathione peroxidase family member GPX4 catalyzes reduction of hydrogen peroxide, catalase CAT converts hydrogen peroxide to water and oxygen and thereby mitigates the toxic effects of hydrogen peroxide, while dehydrogenase NQO1 reduces quinones to hydroquinones and prevents the production of radical species. Glutathione S-transferases (GSTs) are a large family of enzymes involved in detoxification of products derived from oxidative stress and exposure to carcinogens including therapeutic drugs, e.g. cisplatin. NBS1 protein, in turn, is a member of the MRN complex participating in DNA double-strand break repair pathways. Individual capacity of DNA repair and antioxidant systems plays an important role in modulation of sensitivity to DNA damage-inducing anticancer therapies. Some polymorphic variants of genes coding for above mentioned proteins may affect the gene/protein expression, cancer risk and treatment efficiency.

The aim of our study was to examine the effects of functional polymorphisms in GPX4, CAT, NQO1, GSTM1, GSTT1 and NBS1 genes on clinical outcome in 522 HNSCC patients treated with RT and cisplatin-based CHRT. PCR-RFLP and multiplex PCR were used for genotyping. Survival curves were determined with Kaplan-Meier method. HRs and ORs (95% CI) were estimated using Cox proportional hazards and logistic regression models. The GPX4 718 CC genotype and NBS1 185 C variant were associated with shorter relapse-free survival (RFS) in uni- and multivariate analyses ($P = 0.044$ and 0.049). Carriers of the combination of the GPX4 CC and NBS1 C allele showed significantly increased risk of local recurrence compared to other patients ($P = 0.015$ in uni- and 0.021 in multivariate model). The NBS1 CC homozygotes were also at higher risk of treatment failure (OR 1.87, 95% CI 1.13-3.10).

These preliminary results suggest that common rs713041 and rs1805794 polymorphisms in GPX4 and NBS1 genes may be potential prognostic factors in HNSCC patients subjected to RT and CHRT.

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

[B-49] HYPERACTIVATION OF p53 IS ASSOCIATED WITH UPREGULATION OF STING, NLRX1, IFIT1 AND IFIT3 GENES INVOLVED IN INNATE IMMUNITY

Małgorzata Krześniak, Artur Zajkowicz, Agnieszka Gdowicz-Kłosok, Iwona Matuszczyk, Marek Rusin

Center for Translational Research and Molecular Biology of Cancer, M. Skłodowska-Curie Institute-Oncology Center, Gliwice Branch, Poland

The transcription regulator, p53 modulates the expression of wide set of genes. Hyperactivation of p53 in response to a strong stress leads to the induction of pro-apoptotic genes. A marker of hyperactivated p53 is a phosphorylation on serine 46. We observed that in various cell lines co-treatment with actinomycin D and nutlin-3a (A+N) synergistically hyperactivated p53 pathway. Actinomycin D stimulates p53 by activating kinases that phosphorylate this protein, while nutlin-3a inhibits a negative regulator of p53 – MDM2 protein. Surprisingly, in A549 cells, the A+N co-treatment induced cell death which morphologically resembled not apoptosis but a kind of necrosis named pyroptosis. Pyroptosis is executed by activation of caspase 1 – a protein, which is upregulated by p53.

A general aim of our study was testing the hypothesis that activation of p53 by A+N co-treatment induces pyroptosis in A549 cells. Using various antibodies for Western blotting, in cells treated with A+N we did not detect activated caspase 1 – a marker of pyroptosis. However we found that p53 hyperactivated by A+N could stimulate genes coding for proteins of innate immunity, which have not been so far found as p53 targets. For investigation we selected STING, NLRX1, IFIT1 and IFIT3 genes coding various elements of defense system to bacterial or viral pathogens.

The cells in culture were treated with stress-causing substances: actinomycin D, nutlin-3a and camptothecin. The protein expression was examined by Western blotting and changes in the levels of mRNA were measured by semi-quantitative real-time PCR of RNA samples isolated from treated cells. The influence of p53 on the gene regulatory regions of investigated genes was measured using dual-luciferase reporter assay system. For this purpose, the gene regulatory regions with the potential p53 binding sites were cloned into pGL3-Basic reporter vector into the restriction sites generated by PCR primers.

We found strong induction of STING, NLRX1, IFIT1 and IFIT3 genes following co-treatment with actinomycin D and nutlin-3a, what was associated with phosphorylation of p53 on Ser46 and Ser392. Moreover, we demonstrated that expression of examined innate immunity genes was attenuated by p53 knock-down, what confirms that these genes are regulated in p53-dependent fashion. The luciferase reporter assays showed that ectopically expressed p53, to various extent, upregulated promoters of examined genes.

Based on our results we conclude that innate immunity genes STING, NLRX1, IFIT1 and IFIT3 are newly discovered p53 targets strongly upregulated by hyperactivated p53.

This work was supported by grants no. 2013/11/B/NZ5/03190 and 2014/15/D/NZ5/03410 from the National Science Centre, Poland (NCN).

[B-16] THE FATE OF MELANOMA CELLS EXPOSED TO UVA DEPENDS ON THEIR CROSS-TALK WITH NON- IRRADIATED NEIGHBORS

Aleksandra Krzywoń, Maria Wideł, Krzysztof Fujarewicz, Magdalena Skonieczna, Joanna Rzeszowska-Wolny

Institute of Automatic Control, Silesian University of Technology, Akademicka 16 Street, 44-100 Gliwice, Poland

UVA, which accounts for ca. 95% of solar spectrum, is harmful and potentially carcinogenic for dermal tissue chronically exposed to sunlight. Dermal tissue comprises different cells: keratinocytes, melanocytes and fibroblasts. An increasing number of *in vitro* studies indicates that UVA can damage dermal cells (keratinocytes, fibroblasts) not only directly, but also by inducing damage in neighboring cells *via* molecular signaling. Furthermore, the coexistence of cells exposed to UV and non-exposed neighbors can modulate the results in both groups due to reciprocal communication. In the current project we studied the fate of malignant melanoma cells (Me45 line) exposed to low dose of UVA (20 kJ/m^2; one tenth of the dose causing erythema) under co-culture with non-exposed cells of the same line or with normal dermal fibroblasts (NHDF line). For directly exposed melanoma cells the applied dose of UVA has low toxicity, expressed as decreased viability and increased apoptosis. However, toxic effects increase greatly when exposed cells are co-incubated with unexposed melanoma cells. In contrast, bystander fibroblasts sent rescue signals causing a protective effect on viability and apoptosis of UVA-exposed melanoma cells. Non-irradiated fibroblasts also led to a decline in ROS and superoxide that were effectively generated in UVA-exposed melanoma cells. These effects pronounced by fibroblasts were liked to secretion of interleukin 6 and 8. Interestingly, we did not observe similar protective effect of non-irradiated fibroblasts towards UVA-irradiated fibroblasts; also, non-irradiated melanoma cells did not exert protection towards irradiated fibroblasts. All these findings indicate that fibroblasts, which are localized in dermis, may modify direct action of UVA through bystander-like signaling and that interleukins secreted by them appear to be the main players in this system.

Supported by the grant BKM/506/RAU1/2016 from Silesian University of Technology.

[C-24] ANTICANCER ACTIVITY OF NOVEL STYRYLQUINOLINE ANALOGUES

Michał Kuczak[1,2], Ewelina Spaczyńska[1], Anna Mrozek-Wilczkiewicz[2], Robert Musioł[1]

[1]University of Silesia, Institute of Chemistry, Szkolna 9 Street, 40-006 Katowice, Poland; [2]University of Silesia, A.Chełkowski Institute of Physics and Silesian Centre for Education and Interdisciplinary Research,75 Pułku Piechoty 1 Street, 41-500 Chorzów, Poland

Privileged scaffolds in chemistry are definied as a core structure with binding potential to distinct targets leading to modification of functional groups. One of well-known and important heterocyclic privileged structure is quinoline. Styryl analogues of this pharmacophore are interesting class representing wide spectrum of properties and functions. Compounds based on styrylquinoline moiety possess broad especially ranges of biological activity, such as antifungal [1], antibacterial [2] and HIV replication inhibitors [3]. Recently, some quinoline-based compounds have been synthesized and reported as potent antitumor agents [4].

Series of new compounds based on quinoline scaffolds were obtained according to Scheme 1. A group of styrylquinoline derivatives were synthesized obtained in a condensation reaction from appropriate methyl-quinoline derivatives and aldehydes. Two step method provided pathway to different substitution pattern with hydroxyl and acyl groups at quinolone core. The structures of newly synthesized compounds were confirmed by 1H NMR and 13C NMR spectroscopy. The lipophilicity of the compounds was calculated by ChemSketch. These data correlated with activity of studied compounds.

All the studied compounds were tested for their *in vitro* antitumor activity. We used HCT116 cell culture model; HCT116 with wild type p53 (p53 +/+), and with a deletion of TP53 gene (p53 -/-). The most active compounds were also tested on NHDF non-tumor cell line.

[1] Musioł R et al. Bioorg Med Chem 2006, 14, 3592-3598.
[2] Palit P et al. Eur J Med Chem 2009, 44, 845-853.
[3] Zouhiri F et al. Tetrahedron Lett 2005, 46, 2201-2205.
[4] Musioł R et al. Bioorg Med Chem, 2007, 15, 1280-1288.

[C-25] EFFECT OF SALTS ON PHASE TRANSITION OF pH- AND THERMORESPONSIVE POLYMERS AS POTENTIAL DRUG DELIVERY SYSTEMS

Maria Kupczak[1], Katarzyna Homarowicz[1], Anna Mielańczyk[1], Marcin Lemanowicz[2], Wojciech Bogacz[2], Dorota Neugebauer[1]

[1]Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland, [2]Department of Chemical Engineering and Process Design, Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland

Smart polymers due to their sensitivity to stimuli such as pH, temperature, light, ionic strength, etc. are of great interest because of their ability to be used in drug delivery systems. The usage of such polymers as carriers for drug molecules will affect both the place and the rate of their release.[1] The phase transition of pH- and thermoresponsive polymers dissolved in the salt solutions is dependent on the interaction between ions of the salts and polymeric chains, and on the interaction of salts with solvent molecules. The higher charge density of ions on their surface, the greater the interaction between water molecules and salt ions.[2]

In our research we have focused on the impact of the type of salton the phase transition temperature of polymers possessing UCST i.e. poly(methacrylic acid) (PMAA), poly(acrylic acid) (PAA) or LCST, that is poly(N,N-dimethylaminoethylmethacrylate) (PDMAEMA), differing in concentration (1% vs 0.1%) and topology (linear vs star-shaped). The cloud point temperatures (TCP) of polymers solutions were determined in the 4.5M KCl and 0.01M PBS at pH 7.4. Additionally, hydrodynamic diameters (Dh) and zeta potential values of these polymeric solutions in salts were measured at room temperature and TCP.

The results show that in the case of increased concentration of polymer, a sharp decrease of transmittance occurred for PDMAEMAs whereas in the case of polyacids (PAA, PMAA), it caused a sharp increase of transmittance. The concentration of the polymer solutions have an influence on the size of their particles, which was indicated by the DLS analysis.

[1] H. Almedia et al. J. Appl. Pharm. Sci., 2012, 2, 01–10
[2] F. Eeckman et al. J. Pharm., 2001, 222, 259–270

Authors are grateful for financial support by Marcin Lemanowicz BKM 531/RCH3/2016

[A-26] MATHEMATICAL MODELING CYTOSINE METHYLATION AND DEMETHYLATION

Karolina Kurasz[1], Dorota Hudy[1], Magdalena Skonieczna[1], Ewelina Zarakowska[2], Krzysztof Fujarewicz[1], Joanna Rzeszowska-Wolny[1]

[1]Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland; [2]Department of Clinical Biochemistry, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Karlowicza 24, 85-092 Bydgoszcz, Poland

The aim of this study was to propose a mathematical model of methylation and demethylation of cytosine forms, which would be able to predict amount of different cytosine forms based on biological data.

The second aim of this work was selection of the model structure and, on its basis, explanation of the contribution of the TET family proteins. To find the optimal structure of the model 343 possible combinations of parameters have been analyzed. In the process of DNA methylation DNA methyltransferases are involved. DNMT1 is mainly responsible for DNA methylation during replication process where the daughter strand should get the same methylation pattern. DNA demethylation is believed to involve the successive oxidation of 5 methylcythosine (5-mC) to 5-hydroxymethyl- (5-hmC), 5-formyl- (5-fC) and 5-carboxy- (5 caC) cytosine in a process that involves the TET (ten-eleven translocation) family of enzymes including TET1, TET2 and TET3.

The model is described by six ordinary differential equations. To calculate parameters of the models we used Nonnegative Linear Least Squares which compute a nonnegative solution to a linear least squares problem, and the predictive ability of the model was assessed based on leave-one-out cross validation which is a model validation technique for evaluating how the results of a statistical analysis will generalize to an independent data set.

Our research was focused on places where TET proteins work. The best model structures suggests that in the process of transformation 5-mC to 5 hmC TET3 proteins are not involved and between 5-hmC and 5-fC TET2 proteins are not involved.

This work was funded by the Silesian University of Technology under grant BKM/508/RAU1/2017/12

[A-2] A SIMULATION STUDY OF THE IMPACT OF THE CELL CYCLE PHASE ON DOUBLE STRAND BREAKS REPAIR PATHWAY CHOICE

Monika Kurpas, Krzysztof Puszyński

Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 16, Poland

Double-strand breaks (DSBs) are repaired with the use of several distinct mechanisms. The most important of them are non-homologous end joining and homologous recombination (HR). These mechanisms have different requirements and are characterized by different repair kinetics. Moreover, HR is restricted to S and G2 phases of the cell cycle, when sister chromatid serves as a template to rebuild damaged DNA strand.

In this work we studied the impact of cell cycle phase on repair pathway choice. We also tried to examine how cell cycle phase influences DSBs formation and DNA damage detection process. We developed a stochastic mathematical model, which we used to investigate the dependencies between these two important cellular pathways.

Our results confirm that availability of sister chromatid as a DNA repair template is not the only requirement for HR occurrence. We show how changes in activity of mediatory proteins during the cell cycle influence probability of HR repair. Our results show also that percentage of lesions repaired by HR should be gradually increasing during S phase of the cell cycle.

This project was funded by BKM- 508 /RAU1/2017, project 10

[B-1] ULTRASTRUCTURAL CHANGES IN CARDIOMIOCYTES OF MOUSE AFTER RADIATION AND DOXORUBICIN INDUCTION

Małgorzata Łysek-Gładysińska[1], Anna Wieczorek[1], Teodora Król[1], Karol Jelonek[2], Anna Walaszczyk[2], Dorota Gabryś[3], Ronald Kulik[3], Monika Pietrowska[2]

[1]Department of Cell Biology and Electron Microscope, Institute of Biology, University of Jan Kochanowski, Swiętokrzyska 15, 25-406, Kielce, Poland, [2]Center for Translational Research and Molecular Biology of Cancer, [3]Radiotherapy Department, Maria Sklodowska-Curie Institute-Oncology Center, Gliwice Branch Wybrzeże Armii Krajowej 15, 44-101 Gliwice, Poland,
e-mail:mglad@ujk.kielce.pl

Doxorubicin (Dox) is an effective and frequently used chemotherapeutic agent for various malignancies including breast cancers. Its major adverse effect is cardiotoxicity, which may limit its use. Redox injury and interference with protein synthesis are considered to play a significant role in Dox cardiotoxicity. Conditions that exacerbate free-radical formation may enhance Dox cardiotoxicity. According to a lot of studies, metallic ions have an important role in Dox- mediated damage. Doxorubicin interacts with metallic ions, especially iron, which results in the formation of Dox-iron (III) complex. Iron independently plays an important role in generation of harmful free radicals with potential deletorious effects on the myocardium. Cardiovascular damage has also been reported as a long-term toxicity in breast cancer survivors previously treated with radiotheraphy (IR). Little is known about the influence on the combination of chemotherapy and radiotherapy on the morphology changes in the mouse cardiomiocytes.

The aim of this study was to investigate the short- and long-term effect of applying doxorubicin and ionizing radiation in different combination on the ultrastructure of the mouse cardiomiocytes.

The experiment was carried out on male C57Bl/6J mice (weight 20 – 22 g). The animals were kept in room temperature (21º) in naturally controlled ratio of light and dark 12 : 12 and were given laboratory chow ad libitum. The mice were divided into one control group and three experimental groups. The mice were treated with doxorubicin in dose 8 mg/kg (Dox) alone or in combination with irradiation in dose 8Gy (Dox+IR) and irradiation in dose 8Gy in combination with doxorubicin in dose 8 mg/kg (IR+Dox) Those experimental animals were irradiated with the help of therapeutic linear accelerator Clinac 2300 and underwent heart positioning. Doxorubicin was given twice a week at a dose of 2 mg/kg for 2 weeks. After 10 weeks and 40 weeks since the chemotherapy and radiotherapy, the animals were killed by cervical dislocation and animal livers were immediately taken for morphological studies.

Heart specimens were fixed with 3% glutaraldehyde in 0.1M cacodylic buffer (ph 7.4). After an overnight rise in 0.1 M cacodylic buffer they were postfixed in 2% OsO4 in 0.1M cacodylic buffer for 1h, dehydrated in a graded alcohol and propylene oxide series and embedded in Epon 812 (Marzella & Glaumann, 1980). Ultra-thin sections (40-60 nm) were cut on a Reichert-Jung ultramicrotome and double stained with uranyl acetate and lead citrate. Evaluation of ultrastructure was performed using a transmission electron microscope Tesla BS-500 with Frame Transfer-1K-CCD-Camera (TRS, Germany).

The results have shown damage in ultrastructure of mouse cardiomiocytes. Loss of myofilaments, discontinued membranes as well as swelling and discontinued cristae of mitochondria cardiomiocytes were observed. The observed changes were dependent on the type of test agent.

Acknowledgements : This work has been supported by National Science Centre, research grant NCN no. NN402685640

[B-19] OPTIMIZATION OF PROTOCOL FOR DETERMINATION OF METABOLOMIC PROFILE OF EXOSOMES RELEASED BY FADU CELLS EXPOSED TO IONIZING RADIATION

Łukasz Marczak[1], Anna Wojakowska[2], Agata Abramowicz[2], Jakub Idkowiak[1], Piotr Widłak[2], Monika Pietrowska[2]

[1]Mass Spectrometry Laboratory, Department of Biochemistry of Natural Products, Institute of Bioorganic Chemistry, Polish Academy of Sciences, 61-704 Poznan, Z. Noskowskiego str. 12/14, Poland; [2]Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch, 44-101 Gliwice, Wybrzeze Armii Krajowej str. 15, Poland

Exosomes are cell-derived membrane vesicles of endocytic origin and diameter size range reaching 30-150 nm. Nearly all cell types release exosomes and therefore they are frequently found in numerous body fluids such as blood, urine, bronchoalveolar lavage fluid, saliva, synovial fluid or breast milk. Among bioactive molecules composing these vesicles nucleic acids, proteins, but also wide group of metabolites can be distinguished. Additionally, crucial biological function of exosomes has been previously revealed in terms of cellular signaling under normal and diseased conditions consequently leading to rapid growth of interest in their clinical application. In order to use exosomes for cancer therapy and prognosis of disease-progression precise characteristic of their composition is needed. Mass spectrometry-based techniques in combination with modern extraction protocols have been found as powerful, accurate and high-throughput tools for metabolomic screening and investigations. Here we present optimized protocol for complete metabolome profiling of exosomes by wide range of mass spectrometry techniques.

Exosomes isolated from FaDu cell cultures by ultrafiltration and size-exclusion chromatography on mini-SEC were used in experiments. Lipid fraction of vesicles was separated by "in-house" optimized liquid two-phase MTBE extraction method previously published and developed by V. Matyash and total primary metabolites were extracted according to protocol reported by A. Wojakowska. High-resolution Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled to TriVersa NanoMate nano-ESI ion source (Advion Biosciences) was used to perform lipid screening and GC-QqQ (Thermo Fisher Scientific) to total metabolite profile determination. Lipid profiles were further proceeded with use of LipidXplorer software (freely available, Max Planck Institute of Cell Biology and Genetics in Dresden, Germany) and primary metabolite profiles obtained by application of GC-MS were analyzed by ChromaTOF software (LECO), respectively.

The developed analysis techniques allowed identification of more than 300 lipid species and over 400 other metabolites forming exosomes.

[A-20] NEW METHOD FOR EFFICIENT FILTERING OF 2D GEL ELECTROPHORESIS IMAGES

Michal Marczyk

Data Mining Group, Institute of Automatic Control, Silesian University of Technology, ul. Akademicka 16, 44-100 Gliwice, Poland

In modern molecular biology it is important to accurately characterize proteins and compare variations in their expression levels between phenotypes. A high-throughput measurement technique used to analyze single proteins in a complex sample is 2D gel electrophoresis. A single sample measurement is a grayscale image with light background and dark spots whose intensity represents the amount of the given protein in the analyzed sample. The technique is cheap and sensitive, but due to the multi-stage preparation of the sample and the uncertainties in image acquisition process, the overall quality of the gel image is reduced. The gel image may contains different random artifacts, inhomogeneous background and high level of noise. Also, the proteins can form streaks or clusters in which spots are hardly recognizable. Image processing is the first and very important step of the data analysis. The successful noise reduction can significantly improve the estimation of true spots.

The main idea of the proposed algorithm is to use matched filtering, a technique created to distinguish objects of known pattern in a noisy signal. However, due to complex nature of 2D gel electrophoresis images, direct application of the method is not possible. Thus, the following steps are performed to overcome these problems. Application of mathematical model of spot shape based on spot diffusion in gel is done to distinguish spots of varying shape, size and intensity. Division of image intensity and analysis of each fragment separately increases the dynamical range of spot detection. Splitting each fragment into regions leads to better estimation of spot model parameters. The last step is correction of saturated areas. The proposed method was compared to combination of three background correction methods and six image filtering methods. Two fragments of real gel image with existing annotation were analyzed and two fragments of gel done on human leukemic cells from the peripheral blood were chosen and manually annotated. Protein spots were detected by application of Gaussian mixture modeling with 2DGMMgel software to processed gel images. The evaluation of image processing methods was made by measuring quality of spot detection by sensitivity, false discovery rate and F1 score, and quality of spot location estimation by accuracy and precision.

The presented results show that efficient mixture modeling of 2D gel electrophoresis images requires the use of proper image processing methods. The proposed filtering method leads to the best overall performance of spot detection. Important outcome is that false discovery rate was low in all analysis. In most comparisons the quality of spot location estimation was the best after using matched filtering. In conclusion, the proposed solution seems to be the most efficient and flexible method for 2D gel electrophoresis image filtering.

This work was financially supported by internal grant of Silesian University of Technology for young researchers 02/010/BKM17/0083 t.40. All calculations were carried out using GeCONiI infrastructure funded by project number POIG.02.03.01-24-099/13.

[B-25] RNASE ACTIVITY OF MCPIP1 IS CRUCIAL IN THE PROCESS OF CLEAR CELL RENAL CELL CARCINOMA VASCULARIZATION

Paulina Marona, Judyta Górka, Zofia Mazurek, Jolanta Jura, Katarzyna Miękus

Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

Formation of new blood vessels is a critical step during tumorigenesis and metastatic spread. Recent reports indicate an important role of inflammation and angiogenesis in clear cell renal cell carcinoma (ccRCC) development. Monocyte Chemotactic Protein-1 Induced Protein (MCPIP-1) mediates inflammatory processes by regulating the stability of transcripts coding for proinflammatory cytokines such as IL-6 or IL-8, which are also engaged in controling angiogenesis. Our results show, that MCPIP1 protein level decreases during the process of renal cancer progression. Moreover, proangiogenic transcripts vary depending on the stage of the disease. We identify that low MCPIP1 level correlates strongly with increased proliferation in vitro, tumor growth in mice and enhanced vascularity of emerging tumors. We found that MCPIP1 silencing increases chemotaxis of microvascular endothelial cells due to increased levels of VEGF, IL-8 and IL-6. VEVO and IHC stainings, demonstrate a significant increase in the volume of functional blood vessels in tumors formed by cells with downregulation of MCPIP1. In addition, we have observed that the transcript level of IL-6 and IL-8 in cells with D141N point mutation, which abolishes ZC3H12A RNase activity, is similar to control cells whereas MCPIP1 upregulation led to a decrease in the expression of these cytokines. Moreover, tumors formed by cells with D141N mutation are significantly bigger than with MCPIP1 upregulation. Furthermore, MCPIP1 may affect endothelial cells functions. We showed that reduction of MCPIP1 protein level in ccRCC cells is crucial for tumor growth and blood vessel formation. These observations make MCPIP1 a plausible target in ccRCC therapy.

Supported by grants: NSC 2015/19/B/NZ5/01405, KNOW BMN 09/2016.

[B-50] ROLE OF P2X7 RECEPTOR IN GLIOMA C6 CELLS

Damian Matysniak[1,3], Vira Chumak[1,2], Natalia Nowak[1], Lilia Lehka[2], Dorota Scieglinska[3], Pawel Pomorski[1]

[1]Nencki Institute of Experimental Biology of PAS, Warsaw, Poland; [2]Institute of Cell Biology of NAS, Lviv, Ukraine; [3]Center For Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch, Poland

P2X7 is the ionotrophic nucleotide receptor located on the cell surface and activated by extracellular ATP. Many studies reported that activation of P2X7 is crucial for pathological states like chronic inflammation and cancer. Gliomas are the most common and most aggressive tumors of central nervous system with high metastatic potential and resistance to chemotherapy and radiotherapy. Interestingly, many aggressive glioma cell lines are characterized by increased P2X7 expression.

The aim of the presented work was to investigate the role of P2X7 receptor in rat glioma C6 cells. After P2X7 stimulation with its synthetic, selective agonist 2'-&3'-O-(4-benzoylbenzoyl)-ATP (BzATP) we observed increased level of reactive oxygen species (ROS) measured by DCF-DA molecular probe and decreased level of mitochondrial membrane potential (JC-1). Further experiments showed that these P2X7-dependent effects were accompanied with increased p38 MAPK phosphorylation and elevated expression of HSPA1 and HSPA2 chaperone proteins. Stimulation of P2X7 in C6 cells led to increased cells viability and decreased the toxic effect of anticancer drugs doxorubicin and carmustine. P2X7 activation also stimulated release of ATP into culture medium. In vivo studies, performed on C57BL/6 mice with subcutaneously injected glioma C6 cells, have shown that administration of Brilliant Blue G, a selective inhibitor of P2X7 effectively reduced tumor growth and formation of metastases in animals.

The obtained data suggest that activation of P2X7 in C6 glioma cells results in change of redox potential and reduced mitochondrial activity. Moreover, it seems that activated P2X7 can be engaged in shaping tumor microenvironment through regulation of cancer-associated inflammation via p38 MAPK signaling pathway and increased ATP release.

This work has been supported by National Science Centre research grant no. 2015/17/B/NZ3/03771

[C-8] CIRCULATING HPV16 DNA IN THE BLOOD OF HEAD AND NECK CANCER PATIENTS

Agnieszka M. Mazurek[1], Urszula Bojko[1], Krzysztof Składowski[2], Tomasz Rutkowski[2]

[1]Center for Translational Research and Molecular Biology of Cancer; [2]I Radiation and Clinical Oncology Department,, Maria Skłodowska-Curie Institute –Oncology Center, Gliwice Branch, Wybrzeże Armii Krajowej 15, 44-101 Gliwice, Poland

A viral origin of some head and neck squamous cell carcinomas (HNSCCs) is evident. Many studies have documented the role of human papillomavirus (HPV) in the pathogenesis of HNSCC. HPV16 is the most prevalent genotype in HNSCCs, found in up to 90% of HPV-positive cases. Standard HPV diagnosis for viral etiology of head and neck cancers is currently based on histopathological analysis of tumor tissues. HPV detection using blood can be advantageous compared to its detection using tumor tissue. The advantages include relatively easily obtainable material, rapid acquisition of results, and ability to perform repeated analyses during and after the completion of therapy.

Consecutive patients treated definitively with radiotherapy (RT) or radiochemotherapy (ChRT) for HNSCC between 2011 and 2013 at the Maria Skłodowska-Curie Institute-Oncolog Center, Gliwice Branch (Poland) were included in the study. Blood samples of all 187 patients were collected before the therapy was initiated. Circulating cell-free DNA (cfDNA) was extracted from plasma. In order to measure the total cfDNA concentration in blood plasma, amplification of TERT (human telomerase reverse transcriptase) was performed. For detection of HPV16 and HPV18 DNA, the primers and probes were used.

A significantly higher level (p=0.001) of the total cfDNA (circulating cell-free DNA) was found in patients with oropharyngeal squamous cell carcinoma (OPSCC) (10.02±6.45 ng/ml) in comparison with other HNSCC (7.59±4.35 ng/ml). A level of cfDNA in patients with clinical N2-N3 disease (9.28±6.34 ng/ml) was significantly (p=0.015) higher than in patients with a clinical N0-N1 disease (7.50±3.69 ng/ml).

Analysis of the HPV16/18 in plasma revealed that 14% of patients were HPV-positive. Plasma samples from most HPV-positive patients showed the presence of HPV16 (96.4%), while only 0.6% showed the presence of HPV18. Twenty-four out of 26 cHPV16-positive patients (92.6%) had oropharyngeal cancer, and one patient each had nasopharyngeal and salivary gland cancers. The probability of finding cHPV16 DNA in the plasma was significantly higher for the OPSCC patients than for the non-OPSCC patients (OR = 37.5, $p < 0.0001$). Multiple regression analysis between cHPV16 DNA detection and clinical parameters (T classification, N classification, sex, cigarette consumption, age, alcohol consumption) in OPSCC patients (n = 62) revealed that the most important predictors for cHPV16 DNA detection were no smoking (beta coefficient = –0.441, $p = 0.0001$) and advanced N disease (beta coefficient = 0.355; $p = 0.0015$).

Our results prove diagnostic potential of plasma-based HPV cfDNA tests for HPV16-positive OPSCC. Development of biomarker analysis using the circulating HPV DNA (cHPV) methodology is a challenge for noninvasive cancer diagnosis.

[A-3] NUMERICAL MODEL OF WHITE BLOOD CELLS TRANSPORT IN ARTERIES BASED ON DISCRETE PHASE MODEL APPROACH

Bartlomiej Melka

Institute of Thermal Technology, Biomedical Engineering Laboratory, Silesian University of Technology, Konarskiego 22, 44-100 Gliwice, Poland,
e-mail: bartlomiej.melka@polsl.pl, www.itc.polsl.pl/bmelka

Computational fluid dynamics (CFD) is entering more and more intensively the biomedical application field, especially the blood flow investigations. CFD technique becomes already very valuable if applied in the early stage diagnostics of the blood vessel pathological changes. This diagnostic tool could help estimate the importance of pathological change and its influence on the blood flow distribution and in consequence motivate decision on surgical intervention. Despite these potential profits, CFD technique is not yet commonly used as an additional package to the medical imaging devices. The main barrier to implement this concept is limited automatization of discretization process from medical imaging to the numerical mesh used in the CFD calculations. Additional discouraging feature to use CFD in the wider scale is the time-consuming calculations. Nevertheless, it is expected that in the nearest future this technique will be improved in order to commonly implement and combine it with medical devices.

The reasonable results can be obtained by testing various modelling approaches. The complex phenomena of blood flow being a multiphase mixture of solid and fluid components has to be investigated at many levels. The behaviour of specific components of blood can be determined by their properties and interaction with the blood vessel. Therefore, simulation of blood flow as a multiphase medium can be considered useful for future simplified models.

The presented research has focused on White Blood Cells (WBC) transport model in CFD simulations using discrete phase model based on Lagrange tracking of particle groups. Assuming that volume concentration of WBC is on the level of 1%, the WBC could be transported by the continuous phases without influence on the flow path. Application of this approach allows to estimate position and velocity of WBC in the calculation domain. The described approach was implemented in the coronary artery prepared on the base of medical imaging from real geometry. Obtained results show reasonable WBC flow paths in the numerical domain. The main solid phase of blood representing Red Blood Cells (RBC) was modelled according to a previously tested model describing continuous medium in Euler approach [1]. Plasma constituted 55% of volume and was modelled as a primary liquid phase. Implementation of Lagrange technique is a novelty in the simulation of coronary artery flow.

[1] B. Melka et al. Heat Mass Transf., pp. 1–9, Aug. 2017.

This research is supported by National Science Centre, Poland within project No 2014/13/B/ST8/04225. This help is gratefully acknowledged herewith.

[A-11] ANALYSIS OF DATA FROM IMMUNOPHENOTYPING OF PATIENTS UNDERGOING LOW DOSE RADIATION THERAPY

Justyna Mika[1], Udo Gaipl[2], Joanna Polańska[1]

[1]Data Mining Group, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, Gliwice, Poland, [2]The Radiation Immunobiology Group, Department of Radiation Oncology, Universitätsklinikum Erlangen, Erlangen, Germany

Introduction: Immunophenotyping is a method for cell identification based on the type of marker or antigen presented on its surface. Here immunophenotyping of blood cells was performed to analyze at the systemic level the eventual modulation of the different immune system cell populations induced by radiation exposure.
Materials: Immunophenotyping was performed on blood samples from 20 patients with benign painful shoulder or elbow syndrome locally treated with low-dose RT (single fraction of 0.5Gy and total dose of 3Gy in 3 weeks). The blood samples were collected in two time-points: before and after therapy. The established flow-cytometry-based assay allowed the examination of immune cell subsets, common activation markers on all immune cells as well as PD-1 & CTLA-4 and its binding partners on T cells and dendritic cells. Altogether 112 features for 20 patients were detected.
Methods: Imputation of missing values was performed due to incompleteness of data. It was based on k-NN algorithm for 2 nearest neighbours calculated by correlation distance. Final dataset consisted of 74 features and 40 observations (2 time-points x 20 patients). Paired t-test was performed for each feature separately. Storey method was used to control false discoveries due to multiple testing. Additionally correlation between cells and activation markers was calculated, considering separately measurements before irradiation, measurements after irradiation and the difference between the two time-points.
Results: The results allowed for identification of activation markers that differ significantly in the expression level before and after irradiation. It was shown that CD25 and HLA-DR are down-regulated on monocytes (MO) in response to low dose radiation therapy.

The work was supported by SUT grant BKM/508/RAU1/2017t.27 (JM) and BK/213/Rau1/2016/10 (JPo). Calculations were carried out using infrastructure of GeCONil (POIG.02.03.01-24-099/13)

[A-8] THE OPENING AND CLOSING MOVEMENTS OF LOOP REGULATING THE ACCESS TO THE ACTIVE SITE

Karolina Mitusińska[1,2], Tomasz Magdziarz[1], Artur Góra[1]

[1]Tunneling Group, Biotechnology Centre, Silesian University of Technology, Krzywoustego 8, 44-100 Gliwice, Poland [2]Faculty of Chemistry, Silesian University of Technology, Strzody 9, 44-100 Gliwice, Poland

The loop is the most unorganised protein secondary structure. As such it can undergo numerous conformational changes that can provide important functionality of the system. Unfortunately high flexibility might cause difficulty in determination of electron distribution in X-ray experiments and results in uncompleted structures deposited in Protein Data Bank (PDB). In such situation, we can benefit from computational tools that could rebuild missing part of protein and analyse their movement during molecular dynamics (MD) simulations. In our work we aim to provide a suitable pipeline for analysis of the conformational changes of the fragments that were rebuilded, which requires careful procedure and detailed verification of delivered data.

Epoxide hydrolases provides their catalytic mechanism in the active site that is burried inside the enzyme's core and connected with the surroundings by tunnels. Every spatial model of Aspergillus niger's epoxide hydrolase structure (PDB ID: 1QO7, 3G0I and 3G02) submitted to Protein Data Bank (PDB) database is missing the important information about the position of 320TASAPNGAT328 loop. This loop is located near the entrance to the active site and could have impact on the access to the active site. All mentioned properties render this enzyme as an ideal candidate for our study.

We have rebuilt the missing loop using homology modelling and described it using geometrical and potential energy-related parameters. Then we have chosen three representative loop models and run MD simulations with them. After that we studied loop movements dependency on the starting model with aforementioned parameters and with metastable states detection tools.

This work is supported by National Science Centre Poland (www.ncn.gov.pl) grant SONATA-BIS 2013/10/E/NZ1/00649.

[B-37] HSF1-DEPENDENT REGULATION OF lncRNA TRANSCRIPTION IN RESPONSE TO HEAT SHOCK

Katarzyna Mrowiec[1,2], Agnieszka Toma-Jonik[1], Joanna Korfanty[1], Roman Jaksik[2], Patryk Janus[1], Marek Chadalski[1], Natalia Vydra[1], Wieslawa Widlak[1]

[1]Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, 44-101 Gliwice, Wybrzeze Armii Krajowej 15, Poland; [2]Instituite of Automatic Control, Faculty of Automatic Control, Electronics and Computer Sciences, Silesian Un[iversity of Technology, Gliwice, Poland

Long noncoding RNAs (lncRNAs), arbitrarily defined as being longer than 200 nucleotides, are the largest family of noncoding transcripts. Authentic biological roles have been proven only for a small fraction of lncRNAs and most of them remain uncharacterized. To elucidate if they could participate in heat shock response, we examined how heat shock and HSF1 are involved in the regulation of lncRNAs transcription.

First we looked for HSF1 consensus binding sites (Heat Shock Elements, HSEs) in promoters (–1 500 to +500 in relation to all transcription start site variants) of 118 777 human lncRNAs annotated in LNCipedia 4.0 database. To do this, we used a GenomicFeatures package in order to download lncRNAs promoter sequences (from either the UCSC Genome Browser or a BioMart database). HSEs motif as a position frequency matrix was loaded from the JASPAR CORE database using TFBSTools. We created position weight matrixes (PWMs) for basal and inverted HSE and used Biostrings software to localize HSEs in lncRNA promoters. All analyses were performed using the R computing environment. We found 3 561 transcript variants containing basal HSE in the promoter and 3 817 containing inverted HSE.

To verify if HSEs found in lncRNA promoters can be occupied by HSF1 we explored data from ChIP-Seq analyses in control and heat shocked U2OS cells. We found binding of HSF1 induced by heat shock to approximately 80 lncRNA sequences. Then, we performed RT-PCR to check if HSF1 binding following heat shock is correlated with the transcription upregulation in U2OS cells. We have chosen lncRNAs with a high score of HSF1 binding (i.e. LINC00304, NEAT1, FLJ37453, TTC28-AS1, MORF4L2-AS1, PAX8-AS1, LINC00963, and MALAT1) and analyzed their transcript levels up to 24 hours recovery from heat shock. In contrast to highly activated HSP genes, the transcript levels of the above lncRNAs were not considerably increased after treatment. However, looking for alternatively spliced isoforms in RNA-Seq data we noticed some new FLJ37453 and MORF4L2-AS1 transcript variants following heat shock. These new transcript variants started in annotated FLJ37453 and MORF4L2-AS1 sequences about 1.1 to 1.4 kb downstream of HSF1 binding but were terminated far away from them (about 22-23 kb regions were skipped). In case of MORF4L2-AS1, it led to the creation of a fusion transcript with TMEM31. We confirmed by RT-PCR the creation of such alternative transcripts in several cell lines. Additionally, we postulate that their formation is HSF1-dependent since it is diminished after HSF1 silencing in MCF7 cells.

To conclude, about 6% of human lncRNAs possess HSE in the promoter thus can be potentially regulated by HSF1. However, even strong, real HSF1 binding to some of these HSEs does not correlate with significant activation of the transcription in response to heat shock. Interestingly, some new lncRNA transcript variants are generated as a result of heat shock treatment, possibly in a HSF1-dependent manner. In silico predictions of the binding propensity of protein-RNA pairs using the catRAPID algorithm revealed that such new variants lost the ability to interact with selected proteins (encoded by genes located in the opposite direction to these lncRNA genes). The exact mechanism and biological significance of above observations need further studies.

This work was supported by grant no 2014/13/B/NZ3/04650 from the Polish National Science Centre.

[A-21] SPECTRE – A WEB SERVICE FOR EFFICIENT TUMOR MOLECULAR HETEROGENEITY ASSESSMENT IN MALDI MSI DATA

Grzegorz Mrukwa[1,2], Dariusz Kuchta[1,2], Sebastian Pustelnik[1,2], Michał Wolny[1,2], Daniel Babiak[1,2], Michał Gallus[1,2], Wojciech Wilgierz[1,2], Maciej Gamrat[1,2], Roman Lisak[1,2], Joanna Polańska[1]

[1]Data Mining Division, Silesian University of Technology; [2]Spectre Team, Data Mining Division, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100, Gliwice, Poland

Introduction: Recently a number of brand-new algorithms for analysis of Mass Spectrometry Imaging datasets, dedicated to different stages of molecular data processing, was developed. Implementations of these algorithms are usually prototype ones, they work in different environments and with different data formats. There is missing connection between these algorithms, forming a pipeline to be consistently applied on exploding amounts of MSI data. We provide a web platform with easy-to-use, simple interface wrapping cutting-edge science in the field of MSI. To demonstrate its potential, a comparison study on proteomic and lipidomic molecular images with respect to capturing tumor tissue heterogeneities was performed.
Material: Tissue sections were subjected to peptide and lipid imaging with the use of MALDI TOF/TOF mass ultrafleXtreme spectrometer in the ranges of 800-4000 Da and 300 1200 Da respectively. In total, 7520, 10945, 11692 and 4464 spectra were measured in 109568 mass channels for peptides; and 8426, 13957, 13013, and 5466 spectra respectively in 54272 mass channels for lipids.
Methods: During signal preprocessing step, the adaptive algorithm with flexible frame width was applied for baseline detection and removal, PAFFT algorithm for data alignment, and Total Ion Count technique for spectra normalization. Gaussian Mixture Modelling (GMM) algorithm was used to construct adjusted peak models, which were then used for estimation of peptide/lipids abundance by spectrum & model convolution. To capture tumor tissue heterogeneity, DiviK algorithm with intelligent stop criterion was applied. Analysis was partially carried out using Spectre framework, as its development is still ongoing.
Results: Four sections were analyzed together in both lipid and peptide domains. Data was compressed to heterogeneity-preserving representation of 0.25% and 0.42% of initial size. Mean cluster size was 7.64 and 7.10 spectra per cluster at depth of up to 22 splits. Greedy approximation (GA) of pathologist ROI exhibited that 43.21% of molecularly homogenous clusters were linked to tumor region (as compared to 39.16% of spectra) for peptides, and 34.18% for lipids (29.53% of spectra). Obtained Dice similarity of pathologist defined ROI and GA based tumor area was 82.87% in peptides and 65.77% in lipids. Results were consistent when analysis has been carried out for each pair of preparations separately, and with results reported using MATLAB implementations.
Discussion: Heterogeneity of tumor area is higher than its surrounding tissue, independently of imaging domain. Number of homogeneous clusters necessary to cover tumor region increases in both domains, however similarity to pathologist ROI (as measured by Dice index) of such representation is much lower for lipids. Developed Spectre software allows to analyze MSI data and provides results identical to its MATLAB-based origins.

This study has been financially supported by NCN grant BITIMS no 2015/19/B/ST6/01736 (JP, GM), and PO WER NzN! 2.0 SPECTRE project, (0303400-00-P009/16). Computations were carried out using the GeCONiI infrastructure (grant POIG.02.03.01 24 099).

[C-26] NEW CLASS OF 2-STYRYLQUINAZOLINE TYROSINE KINASES INHIBITORS

Jacek Mularski[1,4], Katarzyna Malarz[1,4], Marcin Pacholczyk[2], Tomasz Magdziarz[3], Robert Musioł[1,4]

[1]Institute of Chemistry, University of Silesia in Katowice; [2]Institute of Automatic Control, Silesian University of Technology, [3]Biotechnology Center, Silesian University of Technology; [4]Silesian Center for Education and Interdisciplinary Research, University of Silesia in Katowice

Tyrosine kinases and their inhibitors as anticancer agents seem to be a particularly well explored field of medicinal chemistry. Kinases participate in many signaling pathways, including those involved in cell proliferation, growth, metabolism, apoptosis, and differentiation. Their abnormal activation or functional mutations can be found in a number of disorders including cancers but also development or cardiovascular diseases. The numerous small-molecule inhibitors that have been developed recently represent a major drug discovery efforts in this important field. The main goal of these efforts is a recipe for targeted therapy, with low side effects and better tolerability. On the other hand however, the emerging drug resistance become a barrier that prevents straightforward approach for targeted therapy. There are two important factors triggering new search for active inhibitors: emerging resistance and vast area of kinases with potential as therapeutic targets. 2-Styryl substituted quinazoline are relatively new candidates, however their unique substitution pattern can be found in various preclinical structures. CP-31398 is a prototype p53 reactivator effective in some cancers with specific mutation of this suppressor gene. However its mechanism of action is more complex as induction of p53-dependent and p53-independent cell death was reported. Having strong experience in design and synthesis styrylquinoline and styrylquinazolines as antifungal, 5–89 antiretroviral 10–12 and anticancer 13–15 agents, we decided to explore this interesting field further on. In our approach we designed more rigid analogs of KIN series and itch compound that combine some structural features of older 4-anilino inhibitors.

Compound IS-20 reached highest activity level among all tested compounds with nearly 95% (0.5 μM) against ABL1. At the same time it is the most active as in six out of eight kinases induce high inhibitory effect.

New compounds were subject to molecular docking studies. We used Schrodinger Glide XP (Schrodinger Suite 2017-3). Docking to prepared crystallographic structures of kinases confirmed inhibitory potential of the studied compounds. IS-20 occupies ATP binding pocket in conformation characteristic of type-I kinase inhibitors.

[B-51] THE EFFECT OF NOVEL OLEANOLIC ACID DERIVATIVES ON THE EXPRESSION AND ACTIVITY OF TRANSCRIPTION FACTORS NRF2 AND STAT3 IN HEPG2 CELLS

Maria Narożna, Violetta Krajka-Kuźniak, Hanna Szaefer, Wanda Baer-Dubowska

Department of Pharmaceutical Biochemistry, Poznan University of Medical Sciences, 60-781 Poznań, ul. Święcickiego 4, Poland

Naturally occurring triterpenoids such as oleanolic acid (OA) have been shown to possess cytoprotective, anti-inflammatory and anti-tumorigenic activities in experimental models and thus may be considered promising chemopreventive and/or chemotherapeutic agents. However, their low bioavailability prompts to modification of the chemical structure of OA and synthesis of its analogs with improved pharmacokinetic parameters. Some of these analogs were shown to be powerful inhibitors of cellular inflammatory processes and extremely potent inducers of phase 2. enzymes. The expression of these enzymes is a part of ARE-Nrf2-Keap1 signaling pathway activation.

In this study the effect of 4 new synthetic analogs of OA: 3-succinylmyloxymanine-12-ene-28 acid (A), 3-succinylmyloxymin-12-ene-28-methyl ester (EM), 3-succinylmylamino-12-en-28-benzyl ester (EB) and 3-succinylmyloxymino-12-en-28 morpholide (M) on the expression and activation of Nrf2 and STAT3 transcription factors was evaluated. The latter mediates the expression of a variety of genes and plays a key role in cellular processes such as cell growth and apoptosis.

HepG2 cells, derived from hepatocellular carcinoma, were incubated with OA analogs at the doses of 10 and 20 μM for 24 h. The specific genes expression was evaluated by RT PCR and Western blot in nuclear and cytosolic cell fractions. Treatment with OA analogs increased the expression of Nrf2 and its translocation from cytosol to nucleus. As result of Nrf2 activation increased transcript and protein levels of GSTA, NQO1, SOD1, involved in cell protection against electrophilic and ROS insult, were observed. STAT3 gene expression was decreased after incubation with all of tested derivatives. The expression of STAT3-controlled genes MYC and BCL-XL was also decreased, but enhanced Bax mRNA and protein level was observed as result of treatment with all tested compounds. However the most pronounced effect observed after treatment with OA analog EB and M.

These results indicate that new derivatives of OA may affect the expression of genes, which products are involved in cell protection, cell cycle progression and apoptosis. The detailed mechanism of this activity, particularly potential pro-apoptotic, requires further studies.

This work was supported by grant no 2016/21/B/NZ7/01758 from the National Science Centre, Poland.

[B-10] ANALYSIS OF SNP G34A ON THE ABCG2 GENE AND POTENTIAL SUSCEPTIBILITY TO MULTIPLE MYELOMA

Katarzyna Niebudek, Ewa Balcerczak, Agnieszka Jeleń, Marta Żebrowska

Laboratory of Molecular Diagnostics and Pharmacogenomics, Department of Pharmaceutical Biochemistry and Molecular Diagnostics, Interfaculty Cathedral of Laboratory and Molecular Diagnostics, Medical University of Lodz; Muszynskiego 1, 90-151 Lodz, Poland

Multiple myeloma (Lat. myeloma multiplex; MM) is a malignant tumor of blood cells coming from the bone marrow. The cancer is derived from a single clone of tumor cells, usually plasmocytes that produce monoclonal protein (protein M). The etiology of the disease is not fully explained. Multiple myeloma accounts for 14% of haematological malignancies. There has been an increase in the incidence of the disease noted in the epidemiological studies since the 1990s. Multiple myeloma, despite the constant development of science and medicine, remains an incurable disease with an average of 4-5 years of patient survival. The risk of developing a first-degree relative is 3.7 times higher supports the idea of the involvement of genetic factors in the development of the disease.

The aim of our study was to assess polymorphism at position G34A of ABCG2 gene in the group of patients with multiple myeloma.

Material for the study included DNA isolated from nucleus cells of peripheral blood patients diagnosed with multiple myeloma (investigated group N=187) and from healthy people (control group N=102). The research of the polymorphism was conducted with applying the PCR-RFLP technique. [Consent of Bioethics Committee of Medical University of Lodz No: RNN/88/16/KE; RNN/285/13/KE].

In the group of patients with multiple myeloma wild genotype - GG - demonstrated the dominance with 100 %. No differences were found between the investigated group and the control.

The examined polymorphism seems not to correlate with the development of the multiple myeloma. However, the obtained results require confirmation in further research on the greater group of patients as well as confirmation using sequencing.

Research supported by statutory funds of Department of Pharmaceutical Biochemistry and Molecular Diagnostics, Medical University of Lodz 503/3-015-02/503-31-001; and Grant UMED 564/3-000-00/564-20-013

[C-9] THE PHENOMENON OF MEMBRANOUS/CYTOPLASMIC MIB-1 IMMUNOPOSITIVITY IN BREAST CANCER

Joanna Niemiec[1], Agnieszka Adamczyk[2], Aleksandra Ambicka[1], Anna Mucha-Małecka[3], Wojciech M. Wysocki[4], Kaja Majchrzyk[2], Janusz Ryś[1]

[1]Department of Tumor Pathology, [2]Department of Applied Radiobiology, [3]The Oncology Clinic, [4]Department of Surgical Oncology; Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Cracow Branch, 31-115, ul. Garncarska 11, Poland

Applying the MIB-1 clone of anti-Ki-67 antibody (which is currently endorsed for assessment of the Ki-67 labeling index), in some neoplasms a membranous/cytoplasmic staining pattern is observed. According to current recommendations, the above-mentioned staining, if observed in tumor cells, should be ignored while creating a Ki-67 index. For pathologists, a recommendation on how to proceed in such cases should be valuable. Therefore the pattern of immunonopositivity of MIB-1 and BGX-Ki-67 clones; as well as their labelling inexes were compared.

In a group of 156 patients with invasive ductal breast cancer based on immunohistochemistry with MIB-1 and BGX clones; both labeling index (MIB-1LI; BGXLI) and pattern of immunopositivity were evaluated both in primary tumors (T) and synchronous lymph node metastasis (LNM).

In addition to nuclear MIB-1 staining, membranous/cytoplasmic labeling was found in 23 of 145 primary tumors and 19 of 144 T LNM. In these cases, BGX-Ki-67 showed exclusively nuclear labeling and presented significantly higher labeling index. Survival analysis revealed that, high BGXLI(T) was a significant independent negative prognostic factor for disease-free survival. Moreover, based on BGXLI(T)/BGXLI(LNM), patients with high MIB-1LI(T) were stratified into low- and high-risk carriers.

In carcinomas with membranous/cytoplasmic MIB-1 staining, additional assessment of BGXLI may be recommended. It could help in defining breast cancer subtype and in selection of individuals at risk who, despite appropriate therapy, would benefit from more frequent controls aimed at earlier implementation of second-line treatment.

The study was supported by grant number NN 401 096 137 from the Polish Ministry of Science and Higher Education.

[B-26] THE EPIGENETIC INHIBITORS INFLUENCE ON THE CDH1 ACTIVITY AND IN VITRO MIGRATION OF BREAST AND OVARIAN CANCER CELLS

Ewa Nowak, Dominik Bieg, Daniel Sypniewski, Magdalena Wasińska, Ilona Bednarek

Department of Biotechnology and Genetic Engineering, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland

The epithelial to mesenchymal transition (EMT) consist in the transdifferentiation of epithelial cells into motile mesenchymal ones, and refers to a series of biochemical changes occurring in cells, during embryogenesis, organ development and tissue regeneration. Changes in cell phenotype are also observed in pathological processes associated with cancer progression and metastasis. A hallmark of EMT is the downregulation of E cadherin (encoded by CDH1) to reinforce the destabilization of adherent junctions. These alterations are based on genetic changes observed within DNA sequence as well as epigenetic modifications such as DNA methylation which is mediated by DNA methyltransferases. DNA methylation status and are therefore very valuable agents for cancer prevention.

The aim of this study was to evaluate the influence of decitabine (DNA methyltransferase inhibitor, DNMTi) and trichostatin A, TSA (histone deacetylase inhibitor, HDACi) on the CDH1 activity and change in the migration speed of breast adenocarcinoma cells (MCF7 cell line, ATCC HTB-22) and ovarian carcinoma cells (A2780 cell line, ECACC 93112519) in vitro.

Cells were cultured in RPMI-1640 medium with L-glutamine, 10 % FBS and 10 μg/ml gentamycin, in a humidified atmosphere containing 5 % CO2, at 37˚C. Cells were treated with decitabine in concentrations 25 μM and 12,5 μM and TSA in concentrations 12,5 nM and 6,25 nM for 72 hours. The motility of cells were analysed with wound healing assay (WHA). The CDH1 expression level was estimated by Real-time™ RT-PCR in relation to TATA expression level (housekeeping gene encoding the TATA Box Binding Protein).

The results of WHA showed statistically significant ($p<0.001$) reduction in MCF7 cells migration in vitro after 48 and 72 hours of decitabine usage and after 24, 48 and 72 hours of TSA usage, both compared to control cells. Similar results were observed in A2780 cells. The higher the concentration of decitabine and trichostatin A, the lower in vitro cell migration potential. Real-time™ RT-PCR show significant ($p<0.001$) increase of CDH1 expression in MCF7 cells treated with both concentration decitabine and TSA in reference to control (untreated) cells. Similar results were observed in A2780 cells but TSA in concentration 6.25 nM had no statistically significant influence on the CDH1 expression increasing.

Presented data suggest that decitabine and trichostatin A inhibit the migration and motility of MCF7 and A2780 cells, depending on the concentration used.

Treatment with both epigenetic inhibitors increased the level of CDH1 expression in both cell lines, and therefore the low level of E-cadherin expression in cancer cells may be caused by epigenetic mechanisms.

The presented results are part of the PhD dissertation Epigenetic modifications of expression of genes involved in epithelial to mesenchymal transformation. The study was supported by the funds of Medical University of Silesia: KNW-2-B10/N/6/K, KNW-2-B20/N/7/N, KNW-1-090/N/7/B.

[A-4] IDENTIFICATION AND MODELLING OF THE PULSATILE BLOOD FLOW IN SECTION OF ELASTIC LARGE BLOOD VESSEL

Marcin Nowak

Institute of Thermal Technology, Silesian University of Technology, ul. Konarskiego 22, 44-100 Gliwice, Poland
marcin.nowak@polsl.pl

Constant development of the blood flow dynamics modelling can improve the prevention and medical treatment of the cardiovascular diseases in non-invasive way. The state-of-the-art numerical model should include the behavior of vessels, pulsatile blood flow, multiphase approach and the outlet boundary condition based on the electrical analogy [1].

In the research a real geometry obtained by Magnetic Resonance Angiography (MRA) was analyzed [5]. The subject was 8-year old female patient with a moderate thoracic aortic coarctation. Due to the lack of the detailed data concerning arterial wall, the geometry of the vessel walls was created with the variable thickness, as 10% of the local effective vessel radius [2].

Assumption of rigid blood wall decrease the correctness of results, especially when the vessel undergo quite large displacements [4]. During cardiac cycle, the fluid flow induce forces from the time-varying blood pressure and wall shear stress. These forces causes strains of elastic vessels, which results in modification of the flow area. That was the motivation of using Fluid-Structure-Interaction (FSI) method.

The simulation was performed with 2-way iteratively implicit approach of FSI. This approach is connected with two applications: ANSYS Mechanical (Finite Element Method) and ANSYS Fluent (Finite Volume Method). After obtaining the solution from Mechanical solver, the information about forces and displacements are transferred to the Fluent solver. The last one calculate same variables and send them back. Discussed sequence is repeated within each time step, until the results between two different fields are converged [3].

Pulsatile flow profile was implemented via User Defined Function (UDF) to mimic the cardiac cycle. This UDF consist of several polynomials created of twenty measuring points [5]. A blood flow was modelled as single-phase, using non-Newtonian Carreau viscosity model.

[1] Formaggia L et al. Cardiovascular Mathematics. Modeling and simulation of the circulatory system, Springer, 2009
[2] Bazilevs Y et al. Computational fluid-structure interaction. Methods and applications, Wiley series in computational mechanics, John Wiley & Sons, 2013
[3] ANSYS® Academic Research, Release 16.2, Help System, UDF Manual, ANSYS, Inc.
[4] P. Crosetto et al. Computers & Fluids 43 (2011) 46–57
[5] Source of medical data: http://www.vascularmodel.org/ (accessed October 25th 2016)

This research is supported by National Science Centre, Poland, within project No 2014/13/B/ST8/04225 and No 2014/15/D/ST8/02620 (usage of GeoMagic Design X software). This help is gratefully acknowledged herewith.

[A-27] DIVERSITY OF CELL POPULATION BEHAVIOUR RESULTED FROM RANDOM GENE ACTIVATION

Magdalena Ochab, Krzysztof Puszyński, Andrzej Świerniak

Institute of Automatic Control, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 16

The behaviour of single cell is closely connected with its internal state. Activation or deactivation of intercellular processes depends on the concentration of regulatory molecules, such as transcription factors and enzymes. Randomness plays an important role in determining cell behaviour and describing characteristics of the single cell. In case of processes that incorporate a huge number of molecules, influence of reactions randomness is negligible. However, there are a variety of the intercellular processes including a small number of substrates. The most typical example can be protein production, which is based on active gene alleles. Typically, two alleles of the gene are present in the cell, so randomness of the change between its active or inactive state has a significant influence on protein number. However great majority of the protein networks models are deterministic and diminish the random character of the processes.

This paper presents results of simple protein production models with regard to the randomness of the gene activation and deactivation processes and compares them with deterministic model results.

In order to examine the influence of the stochastic gene activation, we create two simple models of protein regulatory networks. Both models consist of two variables: mRNA and protein. The number of the active gene is included in the model directly by the mRNA production rate, which depends on the protein number. Consequently, the model is piecewise linear and the parameter values change steeply. The first model included the negative feedback loop: increase of the protein number induces deactivation of the gene and step decrease of the mRNA production rate. The positive feedback loop in the second model is created analogously: increase of the protein number induces gene activation and leads to step increase of the mRNA production rate.

To examine influence of randomness of the gene activation/deactivation process, we conduct the computer simulation, where the probability of the gene state change is described by the Hill function. The applied algorithm is deterministic (precisely Runge-Kutta) with the stochastic events of the change of the mRNA production parameter value. We checked the model's responses to three different inputs that increase the protein degradation, imitating drug administration in the purpose of decreasing protein concentration. The input values are fitted to achieve different types of the system behaviour.

The analysis and simulation indicate significant differences between fully deterministic and the stochastic approach. In the case without any input (the protein number is higher than the assumed threshold for gene activation/deactivation) in the model with negative feedback loop mean protein level in the stochastic simulations is higher than in deterministic solution. Just the opposite situation is in the model with positive feedback, where the stochasticity results in decreased protein level compared with the deterministic solution. In the contrary high values of the input results in decreasing the protein number below the threshold value. In the model with negative feedback the mean of the stochastic solution is lower than the deterministic one, however in the model the positive feedback the mean stochastic solution is higher than deterministic. Such result suggests that the stochasticity weakens the feedback in the analysed models, both positive and negative. The results for medium values of the input presents the averaging of the deterministic solutions by introducing stochastic behaviour into the systems. In the model with positive feedback, the bistability observed in deterministic solution is less probably to achieve, because of the random switches between these two states. In the model with negative feedback oscillation in stochastic results are greater than in deterministic results, because the switch of the mRNA production rate is stiffly connected with the threshold value.

Summing up the results, it can be concluded that the influence of the stochasticity cannot be easily omitted without a loss of the model precision. Deterministic models are faster and easier to analyse, but the achieved results can be biased. As in the presented example, the selected drug dose can have lesser influence on the biological system due to the existence of the feedback loops.

The research was supported by the National Science Centre in Poland granted with decision number DEC-2016/23/B/ST6/03455 (for KP), DEC-2014/13/B/ST7/00755 (for AS) and BKM- 508 /RAU1/2017 t. 6 (MO).

[C-27] STAR-SHAPED ANIONIC POLYELECTROLYTES AS CARRIERS FOR CONTROLLED DRUG RELEASE

Justyna Odrobińska, Dorota Neugebauer

Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology, 44-100 Gliwice, ul. M. Strzody 9, Poland

Delivery systems (DDS) with controlled release of cancer and anti-inflammatory drugs, e.g. indomethacin [1,2,3], quercetin [1,4] and doxorubicin [5], encapsulated in the micelles of amphiphilic copolymers are widely described in the literature.

Four series of star copolymers with pentaerythritol core and polymethacrylate, poly(methacrylate-co-acrylate) or polyacrylate arms containing tert-butyl groups (protecting acid units removed by acidolysis as post-polymerization modification) were obtained. The use of atom transfer radical polymerization (ATRP) enabled control of the content and distribution of acid units in the chain by selecting the initial comonomer concentration with appropriate relative reactivity. The relative reactivity ratios of the monomers for each system have been determined by Jaacks method. In the case of a pair of acrylate or methacrylate monomers with similar reactivity, statistical copolymers were obtained, while the varied reactivity of methacrylate vs acrylate monomers led to the gradient copolymers. Amphiphilic star-shaped copolymers were self-assembled to give a micellar superstructures of 100 – 200 nm, and critical micellar concentration (CMC) ranged from 0.018 to 0.074 mg/mL. The effectiveness of indomethacin encapsulation (DLE = 6-86%) and kinetics of its release (10 – 85% within 140 h) was investigated for the prepared polymer systems.

Studies indicate that the pentaerythritol based star-shaped (meth)acrylate copolymers functionalized with acidic groups might be applied as micellar nanocarriers with adjustable characteristics of drug delivery depending on the therapy.

[1] K. Bury et al. Macromol Biosci 13, 2013, 1520
[2] K. Bury et al. In. . Pharm 460, 2014, 150
[3] D. Neugebauer et al. New J Chem 40, 2016, 10002
[4] M.R. Nabid et al. Polymer, 52, 2011, 2799
[5] G. Ma et al. Mater Sci: Mater Med 27, 2016, 17

[B-11] SNPs VARIATIONS WITHIN 3'UTRs OF XMETs GENES AND ITS POTENTIAL EFFECTS ON THE CHEMOTHERAPY IN BREAST CANCER PATIENTS

Jolanta Pamuła-Piłat, Karolina Tęcza, Magdalena Mazur, Magdalena Kalinowska, Joanna Łanuszewska, Lucyna Ponge, Iwona Domińczyk, Ewa Grzybowska

Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Institute-Oncology Center, Gliwice Branch, 44-101 Gliwice,
ul. Wybrzeże Armii Krajowej 15, Poland

Breast cancer is common cancer among women in the world and is one of the leading causes of cancer death among women. Chemotherapy is a critical therapeutic strategy for patients resistant to endocrine therapy or advanced stage patients. Our breast cancer treatment model is based on FAC regime, which combines 5-fluorouracil (5-FU), doxorubicin and cyclophosphamide. These drugs on the cellular level are responsible for genetic material damage leading to cell cycle checkpoints activation and cell death. Xenobiotic metabolizing enzymes and transporters (XMETs) are involved in biotransformation, detoxification and transport of therapeutic drugs. Genetic variations in the XMETs genes may modulate an activity of anticancer drugs. Single nucleotide polymorphisms (SNPs) in the regulatory sequences of genes (3'UTR) may be the cause of the individual variation in treatment response in breast cancer patients and could lead to drug-resistance/drug sensitivity.

We examined the eleven 3'UTR SNPs in nine genes in 324 breast cancer patients treated with FAC first-line chemotherapy. In this study we analyzed genes encoding proteins involved in FAC drugs transport (ABCA1, ABCC4, ABCC1), metabolism (CYP1A2, CYP2E1, GSTM3, TYMS) and drug-induced damage repair (ERCC1, ERCC4). Only functional polymorphisms in 3'UTRs of genes were selected for the analysis.

The impact of polymorphism on therapeutic toxicity was based on multivariate models based on 12 symptoms of toxicity. Preliminary results showed the effect of 3'UTR polymorphisms of the ABCC1 and ERCC1 genes on hematological therapeutic toxicity. The preliminary results of this study confirm the value of the previously developed by our team multifactorial model of therapeutic response predictions. Genetic variants identified in 3'UTRs of XMETs genes may contribute to interindividual variability in treatment response and toxicity associated with FAC chemotherapy in breast cancer patients.

This work was financially supported by National Science Centre grant no. 2016/21/D/NZ5/01913 and grant no 2012/07/N/NZ5/00026

[B-42] LICHEN-DERIVED DEPSIDES AND DEPSIDONES MODULATE THE NRF2 AND NF-κB SIGNALING PATHWAYS IN COLORECTAL CANCER CELLS

Katarzyna Papierska[1], Jarosław Paluszczak[1], Robert Kleszcz[1], Elżbieta Studzińska-Sroka[2], Violetta Krajka-Kuźniak[1]

[1]Department of Pharmaceutical Biochemistry, Poznan University of Medical Sciences, 60-781 Poznań, ul. Święcickiego 4, Poland; [2]Department of Pharmacognosy, Poznan University of Medical Sciences, 60-781 Poznań, ul. Święcickiego 4, Poland

Lichens are symbiotic organisms consisting of fungus and a photosynthetic symbiont, which can be an alga or a cyanobacterium. Lichens produce characteristic and unique secondary metabolites including depsides and depsidones which possess important antioxidant, antibacterial, anti-inflammatory and cytotoxic activity. The anticancer effects of lichen extracts were shown in many types of tumors, including colorectal cancer (CRC). Several studies revealed that these compounds may modulate cell function by affecting signaling pathways. In this regard, it has been recently shown that atranorin and lecanoric acid may inhibit AhR-XRE-dependent gene transcription. However, the molecular mechanisms of the activity of lichen-derived compounds remains largely unknown.

Thus, the aim of our study was to evaluate the effects of depsides (atranorin, lecanoric acid, squamatic acid) and depsidones (physodic acid, salazinic acid) and a poly-carboxylic fatty acid – caperatic acid, on the Nrf2 and NF-κB signaling pathways in colorectal cancer cell lines. These pathways are crucial in the regulation of cell survival and proliferation, and their deregulation significantly contributes to carcinogenesis.

The compounds were extracted from samples of several lichen species and their purity was verified. HCT116 and DLD-1 colorectal cancer cell lines were treated with the lichen compounds at the concentration of 50 μM or 25 μM (physodic acid). Gene transcript and protein level was assessed by RT-PCR and Western blot, respectively.

The results of the study demonstrate that caperatic acid, atranorin, lecanoric acid, squamatic acid, salazinic acid (50 μM) and physodic acid (25 μM) significantly increased the level of Nrf2 transcript in HCT116 and DLD-1 cells. Moreover, Nrf2 protein was accumulated in the nucleus after the exposure to all the studied compounds, however the highest level of nuclear Nrf2 was observed in DLD-1 cells after treatment of physodic acid, atranorin and salazinic acid. Furthermore, all the tested compounds increased GSTP and SOD expression and protein level in both studied cell lines, however the effects in HCT116 cells were much weaker. In order to establish the potential mechanism of the anti-inflammatory action of the tested compounds we evaluated the cytosolic and nuclear content of NF-κBp50 and NF-κBp65. Our studies demonstrated that all lichens decrease the expression of NF-κB and COX-2 in both tested cell lines. Physodic acid and salazinic acid were the most effective inhibitors of NF-κB and COX-2 expression.

These results indicate that lichens, especially physodic and salazinic acids, are potent modulators of Nrf2 and NF-κB pathway in colorectal cancer cells. Further studies are necessary in order to explain the mechanism of their modulation of this signaling pathway.

The present study was supported by research funding from Poznań University of Medical Sciences, Poznań, Poland.

[A-12] DROSOPHILA MELANOGASTER RNA-SEQ ANALYSIS BY K-MEANS CLUSTERING WITH ADAPTIVE INITIAL CONDITIONS

Anna Papiez, Joanna Zyla, Franciszek Binczyk, Joanna Polanska

Data Mining Group, Institute of Automatic Control, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland

Aim: The aim of the study was to investigate leading factors responsible for sample differentiation obtained via an RNA-Seq experiment of complex design (different: sex, fly strain and biological replicates) using k-means clustering technique with adaptive initial conditions and feature selection.
Material & methods: The material under investigation consists of 304 biological samples from Drosophila Melanogaster, where for each RNA-Seq measurements were performed. The flies belong to 7 different strains of both sexes, and in each 3 biological experiment replicates were performed (Environment effect). The RNA-Seq counts quantification was performed using Kallisto package while the normalization was performed with DESeq procedure. The zero read counts were removed, and the final number of features equaled 30,407. Next, the first division with the usage of k-means algorithm was performed on the group of all features using adaptive initial conditions. They were based on finding the first centroid in the largest aggregation of points in each dimension separately. The next is found by maximization of a distance between first centroid and other data points, third to the line, fourth to plane spanned by found centroids and so forth, up to the defined number of clusters. Afterwards, in each k groups separately, the features with the highest non-parametric coefficient of variation were selected by Gaussian Mixture Model signal decomposition. Using the obtained features, k means algorithm with adaptive initial conditions was performed. The described procedure was continued till no clusters were observed. In all divisions Dunn index was used to set the number of clusters. The preselected features were then used in separate sex groups for a dimension reduction algorithm using Partial Least Squares variable selection with Environment groups as a covariate. The identified transcripts were analysed comparatively, and moreover, for Gene Ontology biological process associations.
Results: Based on the 304 samples of Drosophila Melanogaster and 30,407 measured transcripts, first division clustered samples according to sex factor (class1:147 M, 8F, class2: 149 F). Next, in each subgroup non-parametric coefficient of variation selected 2,666 features in class1 and 2,396 in class2 for further clustering. The next clustering step, showed differences between biological replicate 1 vs. others. Also 2 strains of flies showed differences compared to the rest. No further divisions were observed. The Partial Least Squares procedure yielded the most discriminative non-redundant features for males and females in terms of Environment grouping: 29 for males, 32 for females, out of which 2 are common. The common transcript proved to be linked with phagocytosis, regulation of imaginal disc-derived wing size, regulation of cell cycle and carbohydrate metabolic processes.
Conclusion: K-means algorithm with adaptive initial conditions is an efficient tool for analysis of sequencing data of complex design. The main differentiating factor appears to be sex, followed by environment. This may serve as a guideline for future studies planning that tailored data mining techniques are necessary for conclusive investigating other traits outweighed by strong factors, such as sex.

This work was financed by grant no. BKM/508/RAU1/2017/t.24 (AP), DWM.ZWB.183.243.2015 (JZ, JP) and BK-204/RAU1/2017/9 (FB). All calculations were carried out using GeCONiI infrastructure funded by NCBiR project no. POIG.02.03.01-24-099/13.

[C-15] SYNTHESIS OF URIDINE GLYCOCONJUGATES CONTAINING 1,2,3-TRIAZOLE LINKER AND THEIR BIOLOGICAL ACTIVITY

Gabriela Pastuch-Gawołek[1,2], Magdalena Skonieczna[2,3], Monika Krawczyk[1,2], Sonia Ratajczyk[1]

[1]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, 44-100 Gliwice, ul. Krzywoustego 4, Poland; [2]Biotechnology Centre, Silesian University of Technology, ul. Krzywoustego 8, 44-100 Gliwice, Poland; [3]Biosystems Group, Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 16, Poland

Glycosyltransferases (GTs) belong to the group of enzymes that are responsible for the formation of the glycosidic bond in living system [1]. They are responsible for the synthesis of glycoconjugates that play major role in recognition or signaling events, cell adhesion, cell differentiation, glycoprotein folding, targeting organelles and bacterial/viral infections [2].

In recent years intensive research on the design of new effective GTs inhibitors has been conducted. The design of the structure of GTs inhibitors are generally based on similarity to their natural substrates: donor type (NDP-sugars) and acceptor type or on their analogies to the components of the transition state. Designing of donor type analogues is generally based on the modification of one of three structural part: carbohydrate part, the diphosphate linkage or the nucleoside moiety. In donor-type substrate the pyrophosphate moiety interacts with a bivalent metal cation present in an enzyme active site. Analogues of such compounds have an anionic character, which prevents their entry into cells through the phospholipid bilayer. The solution to this problem, especially for in vivo biological applications, may be achieved by the preparation of GT inhibitors containing a neutral diphosphate surrogate which would interact with metal bivalent cation [3].

Recently, a convenient strategy toward GTs inhibitors synthesis was the application of click-chemistry approach. Taking this into account, a series of uridine derivatives was designed in which diphosphate bridge was replaced with a linker containing 1,2,3-triazole unit and/or amide bond, which connects the sugar and uridine moiety [4].

The obtained uridine glycoconjugates were tested for their ability to inhibit β-1,4-Galactosyltransferase as well as for their cytotoxicity toward selected cancer lines (HCT-116, HeLa). The results of the assessment of the biological activity of these compounds will be presented.

[1] K.H. Jung et al. in: C.-H. Wong (Ed.), vol. 2, Wiley-VCH Verlag, Weinheim, 2003, 609–659.
[2] K. Hosoguchi et al. J Med Chem 2010, 53, 5607–5619.
[3] S.Wang et al. Chemistry 2013, 19, 15346-15357.
[4] G. Pastuch-Gawołek et al. Bioorg Chem 2017, 30, 80-88.

[B-38] HSF1-MEDIATED HEAT SHOCK RESPONSE REGULATES CYTOKINE-SPECIFIC NF-κB SINGLE- CELL DYNAMICS

Anna Paszek[1,2,3*], James Bagnall[3], Wieslawa Widlak[1], Michael RH White[3], Pawel Paszek[3], Marek Kimmel[2]

[1]Maria Skłodowska-Curie Institute-Oncology Center, Gliwice Branch, 44-101 Gliwice, ul. Wybrzeże Armii Krajowej 15, Poland [2]Institute of Automatic Control, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, Gliwice, Poland [3]Faculty of Biology, Medicine and Health, University of Manchester, Oxford Road, Manchester M13 9PT, UK

Heat shock affects key cellular processes. However, specific mechanisms linking the Heat Shock Response (HSR) pathway to other major cellular signalling systems are not fully understood. Here we used single-cell microscopy approaches to quantitatively analyse the crosstalk mechanisms involved in the heat shock-dependent signalling of the Nuclear Factor κB (NF-κB) system, a master regulator of cell proliferation and inflammation. The activity of the NF-κB system can be oscillatory, where the transcription factor exhibits multiple rounds of cytoplasmic-to-nuclear translocations to encode target gene expression. The cellular response to heat-shock (HS) includes the Heat Shock Factor 1-dependent transcription of genes encoding heat shock proteins (HSPs) as a part of an internal repair mechanism. In MCF7 cells stably expressing human p65-EGFP fusions, TNFα or IL1β treatment alone led to rapid nuclear translocation of the NF-κB. Over a long time-course (up to 10 h) some of the single cells exhibited oscillations in p65-EGFP translocation in response to TNFα but not IL1β. However, exposure to 1 h of HS at 43°C immediately before cytokine treatment led to the attenuation of NF-κB activation. This was manifested by abolished response in a subset of cells, or delayed and dampened p65-EGFP response in other subsets. This response strongly depended on the HS recovery time. The amplitude of the first peak increased to the level comparable with the untreated steady-state in response to IL1β after 4 h of recovery, however in response to TNFα was still attenuated. Changes in NF-κB dynamics during the heat shock response were mimicked by the regulation of p65-Ser536 phosphorylation and transcription of target genes (NFKBIA, TNFAIP3). A possible mechanism involved in HS-dependent NF-κB dynamics may be related to HSR activation, manifested as transcriptional activity of HSF1. siRNA knockdown of HSF1 led to stronger attenuation of NF-κB signalling after 1 h of HS and 4 h of recovery in response to TNFα but, interestingly not to IL1β. This was exhibited on the single-cell level and regulation of p65-Ser536 phosphorylation. This study suggests that elevated temperature might attenuate cytokine-induced NF-□B system responses, resulting in complex and unintuitive single cell behaviour. Considering the oncogenic associations of the NF-κB pathway, the crosstalk mechanisms are important for the understanding of chemo- and radioresistance to treatment. However, more but similar, quantitative studies are required to ultimately change currently used therapy protocols.

[B-30] DETECTING THE HARDLY DETECTABLE: THE BEST APPROACH TO ANALYSE EPIDERMAL GROWTH FACTOR RECEPTOR VARIANT III (EGFRvIII)

Joanna Peciak[1,2], Karolina Janik[1,2], Marta Popeda[2], Wojciech J Stec[2], Cezary Treda[1,2], Kamila Rosiak[1,2], Ewelina Stoczynska-Fidelus[1,2], Piotr Rieske[1,2]

[1]Department of Tumor Biology, Medical University of Lodz, Zeligowskiego 7/9, 90-752 Lodz, Poland; [2]Department of Research and Development, Celther Polska, Ltd., Milionowa 23, 93-193 Lodz, Poland

EGFRvIII is the most common mutation of epidermal growth factor receptor (EGFR), detected only in tumor cells. In frame deletion of exons 2-7 of EGFR gene results in the formation of abnormal receptor variant, lacking functional extracellular domain. Despite its incapability of binding any known ligand, low-level of EGFRvIII constitutive signaling is often detected, possibly due to reduced internalization and downregulation of this receptor. Mutated variant is reported to be involved in tumor progression and associated with poor prognosis. Nevertheless, the presence as well as the potential role of EGFRvIII in tumors other than glioblastoma remains a controversial subject, with many contradictory data published.

Despite a wide range of analytical methods applied, there are still many challenges in EGFRvIII detection in human tissues, especially since the false positive rate of some methods ranges from 4 to 100%. It is mostly due to factors such as availability (patents and licences), specificity or detection limits of applied methods. Therefore, the main purpose of this study was to determine the reliable methodology of EGFRvIII detection. Various techniques have been analyzed and compared using tissue specimens from the following malignancies: glioblastoma, prostate, breast and colorectal cancer as well as unique stable cancer cell lines showing endogenous EGFRvIII expression. Results indicated that immunocytochemistry, Western Blot and MLPA are not suitable for this purpose, as the former technique is limited by specificity and availability of EGFRvIII-dedicated antibodies, while the last two methods require high percentage of EGFRvIII-positive cells in analyzed sample to be efficient. In case of Sanger sequencing, it is difficult to design universal primers, as deletion breakpoints in intron 1 tend to differ among patients. We demonstrated that real-time qRT-PCR should be considered the most sensitive method for EGFRvIII detection, keeping in mind that the presence of EGFRvIII transcripts does not necessarily correlate with protein levels. Importantly, this was the first study comparing relative and absolute EGFRvIII expression level between different tumor types.

This study undoubtedly demonstrates that EGFRvIII detection must be performed with emphasis on technical limitations of analytical methods. In order to obtain the most reliable and valid data two complementary techniques should be used in future experiments: real-time qRT-PCR for detection of EGFRvIII transcripts together with at least one of protein-based methods.

This study was supported by Innovative Economy Operational Programme grant no. POIG.01.04-00-10-037/11-00 as well as Medical University of Lodz grant No. 503/0-166-01/503-01-002 and 503/0-166-01/503-01-003.

[C-5] ISOLATION AND CHARACTERIZATION OF BIOSURFACTANTS PRODUCING MICROORGANISMS

Anna Pieprzyca[1,2], Aleksandra Ziembińska-Buczyńska[1]

[1]The Silesian University of Technology, Faculty of Power and Environmental Engineering, Environment Biotechnology Department, Akademicka 2 str., 44-100 Gliwice, Poland; [2]Biotechnology Students' Scientific Committee, Krzywostego 8 2, 44-100 Gliwice, Poland

The aim of this study was to isolate and characterize biosurfactants producing bacteria from soil contaminated with crude oil products (including oil and its derivatives). Surfactants are surface-active compounds capable of reducing surface and interfacial tension at the interfaces between liquids, solids and gases, thereby allowing them to mix or disperse readily as emulsions in water or other liquids. Production of biosurfactants is crucial. Traditionally produced biosurfactants may bio-accumulate and their production, processes and products can be environmentally hazardous. Sharpened environmental regulations resulted in an increasing interest in biosurfactants as possible alternatives to chemical surfactants. These substances have many advantage compared with synthetic surfactants. Firstly, they are biodegradable, have low toxicity and better functionality under extreme conditions. Moreover, biosurfactants can be obtained with the use of agro-industrial waste as substrate, which helps reduce overall production costs.

Biosurfactants production performed by the environmental bacterial strains was examined in an indirect way by blood agar lysis, oil spreading and drop collapsing tests. Moreover, isolated strains were characterized in a macro and microscopic way. In sample collected from contaminated soil the average number of microorganisms (total number of bacteria and spore-forming bacteria) was analysed. Biosurfactants production was measured on Diesel Oil (Statoil Miles), were the positive control was SDS (Sodium Dodecyl Sulfate) and negative was distillated water.

This study demonstrated that isolation of strains able to produce biosurfactants from contaminated soil is possible. Two out of twenty-one strains were able to produce biosurfactants, which was confirm by hemolytic assay, drop-collapse and oil-spreading tests. All of the isolated strains are Gram-positive and some of them have haemolysin enzyme, so additionally to the biosurfactants production they could be pathogenic. Strains AP_5 and AP_12 produce biosurfactants, so they can be potentially used in different fields of industry, for example in bioremediation or enhanced oil recovery.

Keywords: biosurfactants, microorganisms, bacteria, drop-collapse, oil-spreading, haemolytic assay

[B-2] INHIBITION OF KEY CANCEROGENESIS PROTEOLYTIC ENZYMES – METALLOPROTEINASES IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA BY IONS OF SELECTED METALS

Jacek Pietrzak, Radosław Reczulski, Damian Wodziński, Rafał Świechowski, Agnieszka Jeleń, Marek Mirowski

Department of Pharmaceutical Biochemistry and Molecular Diagnostics, Faculty of Pharmacy, Medical University of Lodz, 90-151Lodz, Muszyńskiego 1, Poland

Acute myeloid leukaemia (AML) is the most common haematological malignant neoplasm in human population. It is characterized by infiltration of pathologically changed cells in bone marrow, blood and other tissues. This disease is associated with low cure ratio, about 30 - 40% in patients under 60 years of age and dramatically decreases to 5-15 % when they are older than 60. Treatment is still based on chemotherapy or transplantation of haematological stem cells and is related to high mortality. It seems to be important to look for new substances which can help in treating patients with AML.

Metalloproteinases (MMPs) form a broad group of proteolytic enzymes the activity of which depends on the presence of zinc ion in active site. This protein family consists of 23 members which are divided into five groups by their structure and ability to digest some protein compounds. The role of these enzymes in carcinogenesis is widely proven. They participate in every stage of this process. They are able to damage genetic material, release many biological active cytokines, growth factors. They are involved in formation of vessels or space for growing tumor. One subgroup of metalloproteineses – gelatinases (MMP-2, MMP-9) seems to play a key role in cancer development. Gelatinases digest gelatin and this feature is used to detect different form of MMP-2 and MMP-9 in many biological material as a serum, cell culture, urine.

In the present study we analysed sera obtained from patients suffering from acute myeloid leukaemia by zymography technique which is based on polyacrylamide gel electrophoresis polymerization with gelatin as a substrate. Results are presented as clear bands derivated from MMPs enzymatic activity on a blue background. Effect of selected metal ions was tested by one-hour gel pre-incubation after electrophoresis in iron, zinc, magnesium and copper solutions. Comparison of metal ions' impact was performed by densitometry gel analysis.

The results show significant inhibition of metalloproteinases activity after use of all investigated ions. Zinc ions at different concentrations and iron ions at 1mM concentration caused complete inhibition. Magnesium ions were inhibiting gelatinolytic activity in a broad range (10% - 63%). The level of inhibition was determined by concentration of metal ions. Only Fe^{3+} ions at 0.001mM concentration caused increase of gelatinolytic activity. Iron ions showed positive impact on complex preform of MMP-9 with lipocalin.

[A-25] APPLICATION OF ONCOMINETM BRCA RESEARCH ASSAY AND ION TORRENT PERSONAL GENOME MACHINE NGS SYSTEM TO DETECT GENETIC VARIATIONS IN PATIENTS WITH FAMILY HISTORY OF BREAST AND OVARIAN CANCER IN BRCA1 AND BRCA2 GENES

Wojciech Pigłowski[1,3], Artur Zajkowicz[1], Marta Nycz-Bochenek[2], Jolanta Pamuła-Piłat[1], Lucyna Ponge[1], Anna Fiszer-Kierzkowska[1]

[1]Center for Translational Research and Molecular Biology of Cancer, [2]Genetic Outpatient Clinic, [3]Tumor Pathology Department; Maria Skłodowska-Curie Institute-Oncology Center, Gliwice Branch, 44-101 Gliwice, ul. Wybrzeże Armii Krajowej 15, Poland.

Deleterious mutations in BRCA1 and BRCA2 genes increase the risk of breast and ovarian cancer up to 80% over a lifetime. Detection of pathogenic BRCA1 and BRCA2 mutation allows personalized cancer treatment (for example application of PARP inhibitors). Detection of germline mutation provides the basis for genetic counselling. The most popular and cost-effective method of BRCA1 and BRCA2 mutation testing in Poland involves PCR-based detection of common founding mutations. This strategy allows detection of the majority of families carrying the pathogenic mutation.

We performed NGS sequencing of all exons of the BRCA1 and BRCA2 genes in 60 patients with family history of breast/ovarian cancer who were negative for selected mutations common in the Polish population (BRCA1: c.68_69delAG, c.181T>G, c.3700_3704delGTAAA, c.4035delA, c.5266dupC; BRCA2: c.5946delT, c.9403delC).

We applied OncomineTM BRCA Research Assay and Ion Torrent Personal Genome Machine (PGM) semiconductor massively parallel sequencing system together with a cloud-based analysis platform Ion ReporterTM Software v.5.6 (Thermo Fisher Scientific) for nucleotide sequence analysis of all BRCA1 and BRCA2 exons. The applied strategy allowed us to detect and identify known pathogenic variants and novel deleterious changes in BRCA1 and BRCA2 genes. In total, we found 16 pathogenic mutations and one variant of unknown significance. Detected pathogenic variants where: stop-gains, frameshifts, missense mutations, splicing mutations and one exon deletion. We confirmed all deleterious variants by Sanger sequencing.

NGS substantially improved the detection rates of mutations in our group of patients. The heterogeneity of the detected mutations confirms the necessity of NGS analysis of the complete sequence of BRCA1 and BRCA2 genes in all patients with a strong family history of breast and/or ovarian cancer. In our opinion sequencing and analysis strategy applied by us is a powerful diagnostic tool for detection of genetic variations in BRCA1 and BRCA2 genes.

[B-3] M2-POLARIZED MACROPHAGES ENHANCE ANGIOGENESIS IN MURINE MODEL OF HINDLIMB ISCHEMIA AFTER ADSC INJECTION

Ewelina Pilny[1,2], Ryszard Smolarczyk[2], Magdalena Jarosz-Biej[2], Łukasz Krakowczyk[3], Stanisław Szala[2], Tomasz Cichoń[2]

[1]Department of Organic Chemistry, Biochemistry and Biotechnology, Silesian University of Technology, Gliwice; [2]Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Institute -Oncology Center, Gliwice Branch, Wybrzeże Armii Krajowej Street 15, 44-101 Gliwice, Poland; [3]Department of Oncologic and Reconstructive Surgery, Maria Sklodowska-Curie Institute -Oncology Center, Gliwice Branch, Wybrzeże Armii Krajowej Street 15, 44-101 Gliwice, Poland

Adipose derived stromal cells (ADSC) 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-polarized macrophages are known to play an important role in angiogenesis and wound healing. Liposomes containing clodronate (Clodronate Liposomes) are used to deplete macrophages in the muscle. Macrophages, as phagocyting cells, absorb these liposomes by phagocytosis. Intracellular phospholipases release then clodronate causing cell apoptosis.

Unilateral femoral artery ligation was performed on males of the C57BL/6NCrl strain (8-10 weeks of age). One hour after ligation, an aliquot of 10^6 ADSC in 100μL of PBS was administered into the femurs of studied mice. The control mice were injected with 100μL of PBS. Clodronate Liposomes or PBS-Liposomes were injected (i.v. (1mg/mL) and/or IM (1mg/mL)) twice: 2 days before and 1 hour prior the ADSC or PBS injection (to ensure depletion of resident macrophages) and every 2-3 days after (to deplete new infiltrating macrophages). On 7^{th} day of the experiment muscles were isolated, fixed in liquid nitrogen and stained by immunohistochemistry for the presence of F4/80 (mature macrophages) and CD206 (the M2 macrophage marker) to confirm macrophage depletion. 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). The endothelial cells, where the presence of CD31 was confirmed, were counted at 40× magnification in 10 fields from 8-10 mice/group.

Immunohistological assessment of the gastrocnemius muscle extracted on day 7^{th} showed that capillary density was significantly increased in ADSC-treated group, as compared to untreated control (PBS). Capillary density in the muscle was significantly reduced in ADSC with Clodronate Liposomes-treated group compared to ADSC group. After injection, the influx of F4/80+CD206+ macrophages was reduced in ADSC with Clodronate Liposomes group, as compared to ADSC group.

Liposomes containing clodronate effectively deplete F4/80+ and F4/80+CD206+ cells in mouse gastrocnemius muscles in vivo. Our data suggest that infiltrating macrophages are necessary for the formation of new blood vessels after ADSC injection.

This study was financed by Grant UMO-2014/15/B/NZ4/00696.

[C-28] ANTILEUKEMIC ACTIVITY OF NOVEL ADENOSINE DERIVATIVES

Anastazja Poczta[1], Aneta Rogalska[1], Małgorzata Łukawska[2], Irena Oszczapowicz[2], Agnieszka Marczak[1]

[1]Department of Medical Biophysics, Institute of Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland; [2]Department of Modified Antibiotics, Institute of Biotechnology and Antibiotics, Staroscinska St., 02-516 Warsaw, Poland

In this study we investigated the effect of cladribine and six of its derivatives containing formamidino group at the position 6, such as CLA-FDM [6-deamino-6-(N',N'-dimetyloformamidino)] cladribine, CLA-FPAZ [6-deamino-6 N,N-3'methylase-1',5' pentamethyleneformamidino)] cladribine, CLA – FPAZ [6-deamino-6-(N ', N'-methylase-3'-1', 5'-pentamethyleneformamidine)] claribine, CLA – FPIR [6-deamino-6- (N ', N'-l', 4'-tetramethyleneformamidino)] cladribine, CLA – FPIP [6-deamino-6- (N ', N'-1', 5'-pentamethylene formamidino)] cladribine, CLA - FHEX [6-deamino-6- (N ', N'-1', 5'-pentamethyleneformamidino)] cladribine, CLA – FMOR [6-deamino-6- (N ', N'-3'-oxa-1', 5'-pentamethyleneformamidine)] cladribine on T-cell lines originally derived from a patient with T-cell acute lymphoblastic leukemia (MOLT-4). We have made chemical modifications to the cladribine molecule by converting the amino group at position 6 to the amidino group. It may lead to the formation of new/highly active analogues of this undoubtedly important group of biologically active compounds.

We have focused on the cytotoxic and genotoxic activity induced by tested analogues. We investigated also the role of kinase ATR in deoxycytidine kinase (dCK). All probes were analyzed in both the presence and absence of the selective ATR kinase inhibitor VE-821.

The following methods were used: spectrophotometric assay with XTT (2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt) and alkaline comet assay with additional variant of this method including post-treatment with proteinase K.

The results demonstrated that all new derivatives of cladribine were effective against leukemic cells. The most cytotoxic analog of cladribine was CLA – FMOR. The least potent was CLA - FPAZ. Significant increase in DNA fragmentation in leukemic cells was observed predominantly after CLA-FMOR treatment. It has been proved that only CLA-FMOR exhibits the ability to induce DNA–protein cross-links. Our results demonstrate also that ATR kinase might controls dCK activity in response to synthetic derivative of deoxyadenosine analogue.

[A-5] IDENTIFYING OPTIMAL RADIATION DOSE TO INDUCE ROBUST SYSTEMIC ANTI-TUMOR IMMUNITY

Jan Poleszczuk[1], Heiko Enderling[2,3]

[1]Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, 02-109 Warsaw, ul. Ks. Trojdena 4, Poland; [2]Department of Integrated Mathematical Oncology, [3]Department of Radiation Oncology, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612, USA

Synergy of radiation and immune system is currently receiving significant attention in oncology as numerous studies have shown that cancer irradiation can induce strong antitumor immune responses. It remains unclear, however, what are the best radiation fractionation protocols to maximize the therapeutic benefits of this synergy.

In this work we developed a novel mathematical model that can be used to predict and dissect the complexity of the immune-mediated response at multiple tumor sites after applying focal irradiation and systemic immunotherapy. We successfully calibrated the proposed framework with published experimental data, in which two tumors were grown in mice at two spatially separated sites from which only one was irradiated using various radiation fractionation protocols with and without concurrent systemic 9H10 immunotherapy.

Model simulations suggest that the optimal radiation doses per fraction to maximize antitumor immunity are between 10-13 Gy, at least for the experimental setting used for model calibration. This work provides the framework for evaluating radiation fractionation protocols for radiation-induced immune-mediated systemic antitumor responses.

[C-34] PHARMACOGENOMIC PLATFORM BASED ON COMPREHENSIVE MOLECULAR CHARACTERIZATION AS A PROMISING TOOL TO SELECT NOVEL ANTI-CANCER POLYTHERAPIES

Marta Popeda[1], Karolina Janik[1,2], Kamila Rosiak[1,2], Maciej Smolarz[1], Roza Pawlowska[1], Joanna Peciak[1,2], Wojciech J. Stec[1], Marcin Ciechanowski[1,2], Ewelina Stoczynska-Fidelus[1,2], Piotr Rieske[1,2]

[1]Research and Development Unit, Celther Diagnostics, Ltd., Inwestycyjna 7, 95-050 Konstantynow Lodzki, Poland; [2]Department of Tumor Biology, Medical University of Lodz, Zeligowskiego 7/9, 90-752 Lodz, Poland

Recent advances in translational research have provided some powerful tools for personalized medicine. Cancer treatment is also evolving towards targeted therapy and molecular diagnostics is increasingly affecting treatment decision. This tendency is observed particularly in tumour types lacking effective therapeutic options, such as glioblastoma with median survival of 12-14 months following diagnosis.

EGFRvIII, a truncated version of the receptor expressed in 25-30% of glioblastomas, is correlated with a resistance to EGFR-targeted therapies. DK-MG, a unique glioblastoma cell line with endogenous EGFRvIII expression, constitutes a suitable model for research on the, yet not developed, effective treatment for EGFRvIII-positive tumours. In this study DK-MG cells were subjected to a comprehensive DNA analysis aimed at identification of potential therapy targets as well as promising anticancer polytherapies.

Next Generation Sequencing (NGS) was applied to determine variants in sequences of 409 genes relevant for cancer biology. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was performed to detect copy number alterations of 27 genes that are frequently lost or gained in tumours, particularly glioblastoma. Based on the obtained results along with literature data, small-molecule inhibitors and combinations thereof were selected for subsequent analysis.

Real-time impendence-based analysis of normal cells was employed to exclude cytotoxic synergy of inhibitors administered alone and in combination. Subsequently, anticancer efficacy of mono- and polytherapies was assessed on DK-MG cells with real-time cell culture observation system.

Out of 8 analysed combinations of inhibitors, two presented additive anti-cancer effect on EGFRvIII-positive glioblastoma cells, namely MK2206 (Akt inhibitor) administered with JSH-23 (inhibitor of NF-kB subunit nuclear translocation) and afatinib (EGFR inhibitor) administered with flavopiridol (CDK inhibitor).

The study demonstrated in vitro effectiveness of two novel targeted polytherapies selected for EGFRvIII-expressing glioblastoma cells. Molecular characterization enabled to identify genes related to EGFRvIII signalling that were altered in DK-MG cell line. Subsequent analysis on pharmacogenomic platform verified anti-cancer efficacy of inhibitors of these gene products under in vitro culture conditions. Therefore, on the example of EGFRvIII-positive glioblastoma, it was proven that complex preclinical analysis, including both pharmacogenomic platform and comprehensive molecular characterization, is a promising tool to select therapy for a tumour type lacking effective treatment options.

This study was supported by Smart Growth Operational Programme grant no. POIR.01.02.00-00-0035/15.

[B-17] P53-DEPENDENT REGULATION OF ANTIOXIDANT SYSTEMS

Aleksandra Poterala-Hejmo, Dorota Hudy, Joanna Rzeszowska

Silesian University of Technology, Institute of Automatic Control, Systems Engineering Group, Akademicka 16, Gliwice 44-100, Poland;

Cellular homeostasis strongly depends on redox balance understood as the equilibrium between the generation of reactive oxygen species (ROS) during mitochondrial oxidative metabolism, cellular response to radiation, xenobiotics or cytokines, and the cell's ability to eliminate them by an effective antioxidant response.

The protein p53 is known to play a role in the regulation of oxidation-reduction systems and cell responses to oxidative stress, but the precise mechanism remains unknown. The best-studied interaction of p53 with antioxidative systems is that with superoxide dismutase.

Here we present the analysis of gene ontology features in microarray data that reveal numerous genes involved in regulation of the redox potential which can be p53-dependent (including peroxiredoxin 3 and 4, glutaperoxiredoxin 4, glutathione S-transferase P1, NAD(P)H Quinone Dehydrogenase 1). We also present results indicating that p53 affects the redox potential by regulation of the total level of the non-enzymatic antioxidant glutathione. The level of glutathione in human colon cancer cells lacking p53 protein (HCT116 p53-/-) is lower than in wild type cells both in control conditions and upon radiation-induced oxidative stress.

Our results suggest that p53 plays a role in regulation of several enzymatic and nonenzymatic antioxidants.

This work was supported by grants no.: BKM-508/RAU1/2017 t. 5 (A.P.H), UMO-2015/19/ST7/02984 (J.R.)

[A-9] VALIDATION OF THE USABILITY OF VARIOUS CLUSTERIZATION AND TRIMMING METHODS FOR OBJECTS DISPERSED SPHERICALLY IN THREE DIMENSIONAL SPACE – EXTENSIVE ANALYSIS OF WATER FLOW THROUGH PROTEIN CORE

Agata Raczyńska[1,2], Tomasz Magdziarz[1], Karolina Mitusińska[1], Artur Góra[1]

[1]Tunneling Group, Biotechnology Centre, Silesian University of Technology, ul. Krzywoustego 8, 44-100 Gliwice, Poland, [2]Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, ul. Akademicka 1,
44-100 Gliwice, Poland

In the course of a molecular dynamics simulation thousands of single molecules pass through the active site of a protein. In order to fill the gap between tools which search for tunnels and advanced tools employed for accelerated water flux investigations, AQUA-DUCT [1] was developed. It is an easy-to-use tool which enables to analyze the flow of solvent molecules that enter any selected region of the macromolecule during molecular dynamic simulations. AQUA-DUCT [1] performs analysis of exits/inlets data and groups identified tracks into clusters according to the chosen method of clusterization. The tool also provides various definitions of the protein surface, thus allows to differentiate the location of trajectory exits/inlets more precisely.

The aim of this study was to provide detailed description of water flow, through the active site of murine soluble epoxide hydrolase structure and explore the features offered by AQUA-DUCT [1]. The study was performed based on 10 ns simulation of murine soluble epoxide hydrolase structure (PDB ID: 1CQZ). Combination of various clusterization and trimming options was used to validate previously described pathways in murine epoxide hydrolase [2] and explore water throughput through particular tunnels. The results provide selection of the best methods, which can be potentially useful for analysis of objects dispersed spherically in three dimensional space.

[1]. T Magdziarz et al. Bioinformatics 2017. 33 (13): 2045-2046. doi: 10.1093/bioinformatics/btx125
[2]. Argiriadi MA et al. 1999 Proc. Natl. Acad. Sci. USA 96: 10637-10642.

This work is supported by National Science Centre Poland (www.ncn.gov.pl) grant SONATA-BIS 2013/10/E/NZ1/00649.

[B-54] REGULATION OF INNATE IMMUNITY GENES BY P53 TUMOR SUPPRESSOR PROTEIN

Marek Rusin, Artur Zajkowicz, Małgorzata Krześniak, Agnieszka Gdowicz-Kłosok

Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch,44-101 Gliwice, ul. Wybrzeże Armii Krajowej 15, Poland

The p53 protein coded by the major tumor suppressor gene TP53 is best studied in the context of cancer formation. It is not surprising because TP53 is the most frequently mutated gene in human cancers. P53 is a pleiotropic protein, which acting as a transcription factor, can up- and down-regulate the expression of hundreds of genes involved in control of cell cycle, apoptosis, metabolism, DNA repair and other cellular functions. Interestingly, p53 was discovered because it was able to form a tight complex with a large T antigen - a protein coded by an oncogenic virus SV40. Other tumor viruses also code for proteins, which bind and inactivate p53, e.g. the notorious oncogenic papillomaviruses (HPVs) induce p53 degradation by E6 protein. Surprisingly, p53 is also blocked by viruses, which are not carcinogenic, e.g. SARS coronavirus. Moreover, p53 is activated when a cell is infected by some viruses. Hence, there is apparently a strong antagonism between p53 and viruses. The cells have many antiviral proteins at their disposal. The antiviral effector proteins interfere with life cycles of viruses at every stage, from virus entry into cells to the formation of progeny viral particles. These proteins are a part of innate immunity, a system that defends cells against infectious agents, including viruses. This part if immune system was formed during millions of years of evolutionary arms race between cells and pathogens. Many antiviral effectors are up-regulated by interferons - signaling proteins secreted by some infected cells. Interestingly, it was found that many genes stimulated by interferons were also up-regulated by p53. The mechanism of this phenomenon is poorly studied. It is known that p53 directly up-regulates the transcription of some interferon-stimulated genes, e.g. IFI16, ISG15. Moreover, p53 also positively regulates the expression of transcription factors, e.g. IRF5, IRF7, which directly stimulate the transcription of interferon genes. We found that treatment of cells simultaneously with actinomycin D and nutlin-3a (A+N) strongly stimulated p53. Actinomycin D activates p53 by a mechanism, which is not well understood but it apparently involves activation of some kinases phosphorylating p53. Nutlin-3a activates p53 by inhibiting its negative regulator - MDM2. There is strong synergy between these two substances in inducing p53 phosphorylation on key amino acid residues like serine 46 and serine 392. Consequently, many known p53-target genes were synergistically induced. Our analysis of RNA-seq results revealed that approximately 500 transcripts were up-regulated at least 10-fold by A+N in A549 cells. The bioinformatic analysis of gene expression data showed that in addition to regulators of apoptosis, the A+N treatment preferentially activated antiviral genes. We have selected few of them, which were not previously identified as p53 targets, and we performed experiments strongly indicating that these genes were directly regulated by p53. One of these genes is a master regulator of interferons, coding for a protein triggered by viral cytosolic DNA molecules. Moreover, we found that p53 phosphorylation following A+N treatment was inhibited by C16, a compound believed to be a specific inhibitor of PKR kinase - an antiviral protein coded by interferon-stimulated gene. However, the cells with knocked-down expression of PKR were still sensitive to C16, what indicated that other enzyme was involved in p53 activation leading to the induction of innate immunity genes.

This work was supported by the Polish National Science Center NCN (Grants no.2013/11/B/NZ5/03190 to MR, 2014/15/D/NZ5/03410 to AG-K)

[B-12] SINGLE NUCLEOTIDE POLYMORPHISM OF TERT GENE IN A GROUP OF EXTREMELY SENILE PATIENTS

Karol P. Ruszel[1], Sylwia Krawczyk[1,2], Agnieszka Wilińska[1], Alicja Petniak[1], Monika Czuba[1], Dariusz Polański[1], Janusz Kocki[1]

[1]Department of Clinical Genetics, Medical University of Lublin, 11 Radziwiłłowska Str., 20-080 Lublin, Poland;
[2]Undergraduate course in cosmetology student, Pharmaceutical faculty
To whom correspondence should be addressed: darpol2@onet.eu, janusz.kocki@umlub.pl

Research on mechanisms of human senescence is becoming more and more important for societies in developed countries due to negative changes in age structure of their populations (increase of number of aged persons). Moreover, knowledge of molecular and environmental causes of aging will contribute to alleviation of quality of life at it end period.

The hTERT (human Telomerase Reverse Transcriptase) protein has 127 kDa and has enzymatic activity of RNA dependent DNA polymerase. It is important in maintenance of chromosome structure and its activity is correlated with aging process.

Presented research focuses on one single nucleotide polymorphism (SNP) in TERT gene encoding hTERT protein. 20 persons, both men and women, of the age 88-110 years donated peripheral blood samples. As control, umbilical cord blood obtained from random births was used. DNA was isolated from 0,5 ml of whole blood. Subsequently one locus was genotyped and nucleotide polymorphism was evaluated.

The results clearly show that the considered SNP is more frequent in group of extremely senile persons than in control group.

The authors acknowledge support by the Medical University in Lublin (grant No DS 222/17).

[C-35] THE CHARACTERIZATION OF THE MODIFIED POLY(2,6-DIMETHYL-1,4-PHENYLENE OXIDE) HYBRID MEMBRANES WITH MAGNETIC FILLERS AND THEIR POTENTIAL USAGE IN AIR SEPARATION

Aleksandra Rybak[1], Waldemar Kaszuwara[2]

[1]Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, The Silesian University of Technology, Strzody 9, 44-100 Gliwice, Poland; [2]Faculty of Materials Science and Engineering, Warsaw University of Technology, Woloska 141, 02-507 Warszawa, Poland

Nowadays, the major goal of separation technology is the development of polymer materials to obtain gas separation membranes with high permeability, selectivity and resistance. This goal could be achieved via chemical and/or physical modification of existing polymers, especially commercial one. Another way to improve membrane's gas transport properties is the incorporation of inorganic materials (carbon molecular sieves, carbon nanotubes, silica nanoparticles, zeolites, metal organic framework, etc.) into a polymer matrix. All these operations provide materials which due to their unique properties have many potential medical and industrial applications (biomedical, air separation, CO2 removal, hydrogen recovery, aerospace, sensors, photocatalysis, magnetic devices, electrical-magnetic shields and microwave absorption materials, coatings, powder metallurgy, etc.).

The aim of the presented work (a continuation of our earlier research), was to prepare and characterize new inorganic-organic hybrid membranes based on modified polymer matrices NaSPPO or PrSPPO filled with magnetic particles.

The synthesized homogeneous and magnetic hybrid inorganic-organic membranes have been characterized in terms of their chemical, gas transport, magnetic, mechanical and rheological properties. The magnetic matrix of PrSPPO membranes influenced the introduced magnetic particles, resulting in membranes with improved gas transport and magnetic properties. The higher permeability and selectivity of PrSPPO membranes could be explained by the fact that they were more hydrophilic and trivalent Pr3+ counterion was a better cross-linking agent causing the increase in density and decrease in free volume of polymer. The results showed that the introduced modification caused mainly the increase in gas permeability and diffusivity. However, a slight increase in selectivity and solubility was also noted. It was found that the mechanical and rheological parameters and gas transport properties of magnetic membranes were improved by the increase of magnetic particle filling, decrease in powder particle size and selection of appropriate type of polymer matrix.

[B-27] IMPACT OF ADIPOSE DERIVED STEM CELLS ON THE LIMBAL EPITHELIAL STEM CELLS UNDER INFLAMMATION CONDITIONS

Bartosz Sikora, Aleksandra Skubis, Joanna Gola

Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine, Medical University of Silesia in Katowice, Jedności 8, 41-200 Sosnowiec

Limbal stem cells deficiency (LSCD) is the most common disease leading to cornea opacification. In case of LSCD the corneal epithelium is invaded by surrounding tissues, especially by conjunctiva. Major cause of such problems are chemical burns, mechanical damage or bacterial infection. To treat this disorder, the transplantation of corneal limbus from healthy donors is considered. The lack of donor limbus forces the search for alternative treatment methods.

Mesenchymal stem cells (MSC) are promising opportunity in the regeneration and restoration of damaged tissues. Adipose derived stem cells (ADSC) are one of the most simple in insulation MSCs which could be obtained from patient's fat tissue. This source of autologous stem cells makes it a grate material in the aspect of biosafety and exclude the need for determining bio and immune compatibility.

The presence of inflammation may change the potential of limbal epithelial stem cells' (LESC) regeneration. Thus the aim of the study was to examine the influence of ADSC on LESC in the presence of pro-inflammatory factor. Here we present the results of co-culture of ADSC with LESC under inflammation conditions induced by LPS.

For the experiment we used adipose derived stem cells line (Lonza) at passage 3. LESC were provided by the courtesy of Department of Microbial Biotechnology and Cell Biology, University of Debrecen, Hungary.

LESC were treated with LPS for 24h to induce the inflammation. The dose of LPS used for inducing the inflammation was set by MTT assay results and literature data. After the incubation, cells were passaged to new dishes. The expression of IL-6, IL-2, IFNγ and IL-10 was confirmed by ELISA tests t. Non-stimulated cells were used as a control.

Simultaneously, ADSC were cultured in separate dishes on Transwell© system wells. The co-culture of both types of cells was set for 24h. Another examined group was LPS-induced LESC treated by conditioned medium collected from ADSC culture, conducted in T25 flasks. Each examined group had a control.

All analyzed proteins were present in the tested media. The results showed differences in the expression of IL-6.

[B-31] EXPRESSION OF MELATONIN RECEPTORS GENES IN COLORECTAL ADENOCARCINOMA TISSUES

Klaudia Simka[1], Martyna Bednarczyk[1], Teresa Kokot[1], Aleksandra Skubis[2], Bartosz Sikora[2], Justyna Szota-Czyż[2], Celina Kruszniewska-Rajs[2], Janusz Wierzgoń[3], Urszula Mazurek[2], Ewa Ziółko[1], Małgorzata Muc-Wierzgoń[1]

[1]Department of Internal Medicine, Medical University of Silesia in Katowice; [2]Department of Molecular Biology, Medical University of Silesia in Katowice; [3]Department of Oncologic and Reconstructive Surgery Maria Sklodowska-Curie Institute-Oncology Center, Gliwice Branch

The activity of melatonin in suppressing tumour development is based on its inhibition of processes such as tumour transformation, angiogenesis, and metastasis. The antiproliferative and immunomodulatory effects of melatonin have been investigated in various types of neoplasms including melanoma, breast, prostate, ovarian, hepatocellular and colorectal cancer (CRC). In humans and other mammals, two G-protein-bound specific high affinity receptors (MT1 and MT2) mediate most of the melatonin physiological and pharmacological actions. Numerous studies have focused on the role of MLT and its receptors in prevention, diagnosis and treatment of CRC.

In the present study, we have studied the difference in melatonin receptors genes expression in colorectal adenocarcinoma tissues in relation to clinical stage of cancer.

A total of 24 pairs of surgically removed tumoral and healthy (marginal) tissues samples from colorectal cancer patients at clinical stages I-II and III-IV were collected. The patients were aged between 55-71 years and had not received any chemotherapy or radiotherapy treatment. Healthy control tissue specimens (marked K2) were obtained from an area 10 mm outside of the histologically negative margin. As second control, ten normal samples (K1) were taken from subjects whose large intestine tissues were reported as non-tumoral after colonoscopy. The tumor specimens were divided into two groups according to the 7th edition of the AJCC/UICC staging system of CRC: stages I and II (LGC) and III and IV (HGC). Expression of mRNA genes was studied by microarray HG-U133A analysis. The analysis of genes expression profile was performed using commercially available oligonucleotide microarrays of HG-U133A.

The profiling analysis identified differences in MT1 and MT2 gene expression levels between each of the groups. Expression of MT1 mRNA was the lowest in normal samples, increasing linearly in K2 and LGC specimens. The highest signal of the MT1 transcript was found in samples from patients at clinical stages I-II. The MT2 mRNA expression increased slightly from K2 to HGC samples. Analysing two control groups only, we observed the high expression of MT2 mRNA in normal tissues (K1) which decreased in marginal samples (K2).

The study group showed the differences in terms of age, gender, clinical and nutritional status. All summarized factors affect the activity of the melatonergic system. Furthermore, the microenvironmental regulation of colorectal cancer growth, invasion and metastasis play an important role in melatonin receptor expression. Monitoring the expression levels of genes that are related to melatonin receptors may offer a strategy to anticipate tumour development and estimate the molecular changes that occur during carcinogenesis.

[B-32] THE INFLUENCE OF DIABETES MELLITUS TYPE II AND INSULIN RESISTANCE ON ADIPOSE-DERIVED STEM CELLS

Aleksandra Skubis[1], Agnieszka Witkowska[2], Bartosz Sikora[1], Joanna Gola[1], Monika Paul-Samojedny[3], Urszula Mazurek[1]

[1]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland; [2]Department of Internal Medicine, Diabetology and Nephrology, Medical University of Silesia, Zabrze, Poland; [3]Department of Medical Genetics, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland

Mesenchymal stem cells (MSCs) are somatic, multipotent cell which can be derived from different tissue. One of the richest source of MSCs is adipose tissue (ADSC, adipose derived stem cells). There are many various experiments which show advantages of using mesenchymal stem cells in regenerative medicine. ADSC have a natural ability to differentiate into osteoblast and they can be used in treatment of bone disorders. Unfortunately diabetes mellitus may effect ADSC vital functions.

In the present study we investigated the influence of diabetes mellitus type 2 on proliferation and differentiation potential of adipose derived stem cells.

The fat from subcutaneous abdominal adipose tissue was acquired by lipoaspiration from 23 participants. All participants were divided into three groups: T2D (type 2 diabetes) group of 9 patients, IR (insulin resistance) group of 6 patients and C (control) group of 8 healthy participants. All patients took part in project "Healthy life with diabetes". They were informed about the research and consents were obtained from all participants. The study protocol was approved by Bioethics Committee of the Medical University of Silesia - KNR/0022/KB1/82/II/15/16.

Analysis consisted of two steps. First step included proliferation assays of cells, molecular analysis of proliferation markers and flow cytometry identification of proteins characteristic for mesenchymal stem cells (CD73, CD90, CD105). Next step consisted of analysis of osteogenesis differentiation potential of cells based on the expression of genes specific for osteoblasts (RUNX2, SPP1, ALP, BGLAP) and Alizarin red staining.

The results show that diabetes mellitus changes some parameters of adipose derived stem cells. Moreover, we observed differences in the proliferation potential of ADSC in the group of insulin-resistant patients.

[C-16] COMBINATION OF ANTIVASCULAR AGENT – DMXAA AND INHIBITOR OF HIF-1α – DIGOXIN INHIBITED MELANOMA TUMOR GROWTH

Ryszard Smolarczyk[1], Tomasz Cichoń[1], Ewelina Pilny[1,2], Magdalena Jarosz-Biej[1], Aleksandra Poczkaj[1], Natalia Kułach[1,3], Stanisław Szala[1]

[1]Center for Translational Research and Molecular Biology of Cancer Maria Sklodowska-Curie Institute - Oncology Center, Gliwice Branch, Wybrzeże Armii Krajowej Street 15, 44-101 Gliwice, Poland, [2]Department of Organic Chemistry, Biochemistry and Biotechnology, Silesian University of Technology, Gliwice, [3]Department of Animal Physiology and Ecotoxicology, University of Silesia, Bankowa 9 St., Katowice, Poland

One of the targets of anticancer therapy may be blood vessels, both the already existing ones (antivascular therapy) and the newly emerging (antiangiogenic therapy). Vascular disrupting agents as DMXAA inhibit tumor growth only for a short period of time after which rapid tumor growth is observed. Among others, hypoxia and appearance of transcription factor HIF-1α are responsible for tumors regrowth. HIF-1α activates several mechanisms responsible for tumor growth, such as angiogenesis. Tumor angiogenesis improves blood supply and thereby stimulates tumor regrowth. The aim of our study was to investigate the potential of inhibition of murine melanoma growth by combining two agents: antivascular - DMXAA and the inhibitor of HIF-1α - digoxin and explaining the mechanism of action of this combination. After DMXAA treatment tumor size was reduced only for limited time. After 7 days regrowth of tumors was observed and number of vessels was increased especially in the peripheral areas of the tumor. DMXAA also induced the influx of inflammatory cells such as macrophages, CD8+ cytotoxic lymphocytes, NK cells and CD4+ lymphocytes. Digoxin administration inhibited the growth of tumors. Administeration of both agents in the proper sequence significantly inhibited the regrowth of tumors. Combination therapy reduced the number of newly formed vessels. In the tumor bearing mice treated with the combination of DMXAA and digoxin, the number of M1 macrophages, CD8+ cytotoxic lymphocytes, NK cells and, to a lesser extent, CD4+ cells was increased. The combination of antivascular agents with HIF-1α inhibitors appears to be an effective therapeutic option.

The study was supported by Grant UMO-2015/17/N/NZ4/02738 from the Ministry of Science and Higher Education (Poland).

[B-39] HSPA2 KNOCKDOWN AFFECTS REDOX HOMEOSTASIS BUT HAS NO EFFECT ON GROWTH, MOTILITY, ADHESION AND CHEMORESISTANCE OF HUMAN NON-SMALL CELL LUNG CANCER CELLS

Damian Sojka[1], Damian Matyśniak[1], Agnieszka Gogler-Pigłowska[1], Katarzyna Klarzyńska[1], Natalia Vydra[1], Małgorzata Łysek-Gładysińska[2], Zdzisław Krawczyk[1], Dorota Ścieglińska[1]

[1]Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Institute - Oncology Center, Gliwice Branch, Poland; [2]Department of Cell Biology and Electron Microscopy, The Jan Kochanowski University in Kielce, Kielce, Poland

The HSPA2 gene is a poorly characterized member of the human heat shock protein HSPA (HSP70) family encoding chaperone proteins. HSPA2 is abundantly expressed in the testis and plays a crucial role in regulating spermatogenesis. Beside, HSPA2 is present in several types of somatic tissues, including epidermis and bronchial epithelium. We have recently found that HSPA2 participates in the process of epithelial keratinocyte differentiation. HSPA2 is frequently overexpressed in wide variety of cancers. We showed previously that a high HSPA2 expression in tumors correlates negatively with survival of non-small cell lung carcinoma (NSCLC) patients. Recent literature data suggests that HSPA2 can have significant impact on the malignant phenotype of various cancer cells.

The aim of this study was to examine the role of HSPA2 in NSCLC cells. We used lentiviral vector-mediated gene transfer and RNA Interference technology to stably knockdown HSPA2 expression in normal bronchial epithelial cells, several types of NSCLC cell lines, and cells derived from breast and cervical tumors (to compare our results with data published by others). Specific and significant reduction of the HSPA2 level in bronchial epithelial cells had no effect on proliferation and migration, but coincided with reduced ability to form colonies and lower adhesion to fibronectin. On the contrary, cancer cells deficient in HSPA2 expression showed no alterations in proliferations, clonogenic potential, adhesion and migration. However, only in cancer cells HSPA2 deficit manifested by ROS disbalance accompanied by decrease in mitochondrial membrane potential, reduction in number of active mitochondria and alterations in mitochondrial ultrastructure. Surprisingly, these changes did not contributed to higher sensitivity of HSPA2-deficient cells to classic chemotherapeutic agents. Finally, we excluded the possibility that lentiviral vectors used by us for gene transfer would introduce unwanted bias into our study and obscure potential effect of HSP2 depletion on cancer cells. For that purpose, we used lentiviral vectors to knockdown the expression of the HSPA1 gene, a well-studied cancer-related member of the HSP70 family. As expected, HSPA1 silencing led to senescent phenotype of cancer cells. This results ultimately confirmed suitability of lentiviral vectors for studying effects of HSP depletion in cancer cells.

In conclusion, on the contrary to results published by others, our findings convincingly show that HSPA2 plays minor (if any) role in promoting growth, migration and adhesion of various types of cancer cells. HSPA2 can be important for the maintenance of redox homeostasis in cancer cells, however functional significance of this phenomenon is at present unclear. Instead, we found that HSPA2 can regulate clonogenic potential and adhesion of normal bronchial epithelial cells. This implicates, that similarly to epidermis, HSPA2 can be involved in the process of bronchial epithelial cell differentiation.

This work has been supported by National Science Centre, research grant NCN no. DEC-2013/09/B/NZ5/01815

[A-10] SUBSTRATES TRAPPING BY PROTEIN SURFACE – IN SILICO STUDY

Agnieszka Stańczak[1,2], Artur Góra[1]

[1]Tunneling Group, Biotechnology Centre, Silesian University of Technology, ul. Krzywoustego 8, 44-100 Gliwice, Poland, [2]Department of Chemistry, Silesian University of Technology, ks. Marcina Strzody 9, 44-100 Gliwice, Poland

LinB [1] (PDB ID: 1MJ5) is a haloalkane dehalogenase of the α/β hydrolase family of enzymes. It is an enzyme responsible for transforming haloalkanes into inorganic halide ions and alcohols. It consists of 296 amino acid residues form into two domains. The active site is formed by residues located inside a cavity between these two domains. This cavity is mostly hydrophobic and hence suitable for haloalkane binding. A halide binding site is formed in a pocket in the active site, and adjacent is the catalytic triad, Asp108, His272, and Glu132.

Prior to reaction, substrates need to be transferred from the surrounding solution to the protein surface and then to the active site cavity. Often substrates have a long road within different- hydrophobic and hydrophilic- regions of a protein until they reach active site. The individual steps of a reaction may occur in different environments. Transporting substrates from one region to another is not always trivial [2]. To help experimentalists in understanding delivering substrates and process of catalysis, in silico study were done.

The Amber14 [3] package was used to study spontaneous substrate delivery. Classical 100 ns long molecular dynamics simulation were performed in ten repetition for each ligand. The preliminary results of the analysis of simulations with two different ligands: bromocyclohexane and 1,2 dibromoethane show that we are able to capture the substrates entry phenomena using classical MD simulations. Moreover, we are observing that particular compartments of protein surface are able to trap and hold substrate prior to their entry to active site. Detailed analysis provides information about the retentions of ligands in different part of protein surface. Based on our study, the surface-ligand contact map has been constructed which might shade the light on importance of ligand transportation phenomena for selectivity and activity of LinB enzyme.

[1].Oakley AJ. et al. Biochemistry.2004, 43, 870-878.
[2]. Góra A et al. Chem Rev 2013, 113, 5871-5923.
[3].Case DA et al. AMBER 14. University of California, San Francisco, 2014.

This work is supported by National Science Centre Poland (www.ncn.gov.pl) grant HARMONIA DEC-2015/18/M/NZ1/00427.

[A-28] A WEB APPLICATION FOR REAL-TIME PCR DATA ANALYSIS

Sebastian Student[1], Jakub Porc[1]

[1]*Silesian University of Technology, 44-100 Gliwice, Akademicka 16, Poland*

As progress is being made in the molecular biology and genetics areas, a great number of researchers aim to find an application of this knowledge in the industry or try to explain the mechanisms of genetic disorders and diseases. Techniques used in these research, such as real-time PCR, provide data of great size that cannot be analyzed without using computational methods. These can be very challenging and time consuming for researchers without proper experience and skills, so there is a demand for free and simple to use tools that would provide comprehensive and clear results.
A web application called "Simple qPCR" was created in order to perform an automated analysis of real-time PCR fluorescence data. It was created using the R environment and Shiny package to build interactive web app. After providing the raw Ct values data directly from Real-Time PCR instrument, the program calculates the fold difference of gene expression. We use modified CT method to take multiple reference genes and gene specific amplification efficiencies into account. We can quickly analyze huge experiment schemes with more 96- and 384-well plates by uploading the configuration file with the information about the placement of the samples on each multiwall plate. Using the graphical interface we can choose the reference genes and the control samples.
The result table with normalized relative gene expression qPCR data is shown directly in the online application, which can be downloaded in the Excel file format. We can visualizes the data using the boxplots figure for each provided sample. The tool has a clear user interface containing examples of the files needed to start the analysis and a comprehensive user guide. The user can use this application without any program installation directly form the internet There is no need to install the program, it is available on the internet, making it easily accessible and simple to use by any researcher.

The research was supported by grant BKM-508/RAU1/2017 t.32 from the Silesian University of Technology.

[B-20] ECTOSOMES RELEASED BY METASTATIC CUTENUOUS MELANOMA CELLS PROMOTE PROLIFERATION AND MIGRATION OF LESS INVASIVE CELL LINE

Magdalena Surman[1], Sabina Szwed[1], Anna Dróżdż[2], Ewa Stępień[2], Małgorzata Przybyło[1]

[1]Department of Glycoconjugate Biochemistry, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, 30-387 Kraków, ul Gronostajowa 9, Poland; [2]Department of Medical Physics, M. Smoluchowski Institute of Physics, Jagiellonian University in Krakow, 30-348 Kraków, ul. Łojasiewicza 11, Poland

Ectosomes are small heterogeneous membrane vesicles generated by budding from the plasma membrane in a variety of cell types, including tumor cells. They are shed into the extracellular space and considered as an alternative form of intracellular communication in which information is transmitted from the originating cell to recipient cells without direct cell-to-cell contact. Through horizontal transfer of a variety of biologically active molecules (proteins, lipids and/or nucleic acids), tumor-derived ectosomes may play functional roles in oncogenic transformation, tumor progression, invasion, metastasis, angiogenesis promotion, escape from immune surveillance, and drug resistance.

In the present study ectosomes released in vitro by metastatic cutaneous melanoma (CM) WM266-4 cells were isolated from conditioned culture media by sequential centrifugation. Subsequently, their cancer-promoting effect on less invasive, primary CM WM115 cell line was analyzed in terms of altered proliferation (Alamar Blue assay) and migratory properties (wound healing assay).

The results showed that 18-hour incubation with two different doses (7.5 μL or 15 μL) of WM266-4 ectosome sample caused an increased migration of WM115 cells in wound healing assay. The velocity of wound closure was approximately three times higher for ectosome-treated cells in comparison to control, however this effect was not dose dependant. Moreover, higher fluorescence intensity of reduced Alamar Blue reagent was measured after incubation with ectosome sample (7,5 μl or 15 μl) and this effect was dose dependent.

Taken together, our results suggest that ectosomes released by metastatic CM cells might directly stimulate proliferation and migratory properties of less invasive cells, thereby facilitating disease progression.

This work was supported by the grant from the Jagiellonian University in Krakow (K/DSC/004659).

[A-13] THE APPLICATION OF DEEP CONVOLUTIONAL NEURAL NETWORKS IN THE AUTOMATED DIAGNOSIS OF EARLY ALZHEIMER'S DISEASE ON MAGNETIC RESONANCE IMAGES

Aleksandra Suwalska, Franciszek Binczyk

Silesian University of Technology, Institute of Automatic Control, Data Mining Group, 44-100 Gliwice, ul. Akademicka 16, Poland

The objective of the work was to design and implement the deep convolutional neural network as a tool for the automated early detection of Alzheimer disease, that means at mild cognitive impairment stage. Early detection can be achieved by the use of medical images from magnetic resonance imaging.

The neural networks were first proposed in 1947 and for many years were not popular due to the complicated training process. The introduction of multicore CPU and graphic CPU's allows for not only much faster training but also an increase of hidden layers' dimensionality (depth of the network). The convolutional networks (CNN) are an example of deep networks that are constructed of a cascade of layers containing convolutional filters. In such a cascade the size of the filter varies and allows to detect different image features. Alzheimer's disease (AD) is a degenerative and progressive neurological disease of the brain. Mild cognitive impairment (MCI) is considered as an early stage of AD.

The proposed model of CNN consists of: 4 convolutional layers of 32, 64, 128 and 128 filters (7x7, 5x5, 5x5 and 3x3 pixel size), 3 2x2-pixel max pooling layers and 3 fully-connected layers. The activation functions were ReLU and Softmax.

In the study MRI images from the Alzheimer's Disease Neuroimaging Initiative (ADNI) were applied. The database collects data such as MRI and PET images, genetics, cognitive tests, CSF and blood biomarkers. Our dataset includes 2,014 historical scans. Each slice of MP-RAGE sequences with brain tissue was normalized, rescaled and given as an input for the CNN.

The results of the classification with CNN application allow to differentiate between patients with MCI, patients with AD and healthy controls with 92% accuracy. The sensitivity the early stage of Alzheimer's disease (MCI) detection is 95%. It was proven that deep neural networks could be successfully used as a method for the automated image analysis resulting in diagnosis of early AD. Currently a 3D model of convolutional neural network is being developed, which will be used on the whole MRI image. This approach may result in better performance as compared to 2D convolutions on slices. The researchers also believe that the application of the deconvolution process could help identify a robust imaging biomarker.

This work was financed by project granted by Narodowe Centrum Nauki: UMO-2015/19/B/ST6/01736. Additionally, FB was financed by BKM- 508 /RAU1/2017; 02/010/BKM17/0083 topic 42.

[B-52] C-RAF KNOCKDOWN IMPROVES CHEMOSENSITIVITY OF HELA CELLS TO THE XANTHONE TREAMENT

Daniel Sypniewski[1], Magdalena Czajka-Uhryn[1], Michał Szapski[1], Natalia Szkaradek[2], Ewa Nowak[1], Tomasz Loch[1], Anna M. Waszkielewicz[2], Agnieszka Gunia-Krzyżak[2], Henryk Marona[2], Ilona Bednarek[1]

[1]Department of Biotechnology and Genetic Engineering, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland; [2]Department of Bioorganic Chemistry, Chair of Organic Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland

The Raf/Ras/MEK/ERK pathway plays crucial role in regulating various cellular processes such as cell growth, proliferation, survival and apoptosis. Raf (Rapidly accelerated fibrosarcoma) kinases are members of serine/threonine protein kinase family of signal transmitters and include three isoforms: C-Raf (also termed Raf-1 or C-Raf-1), A-Raf and B-Raf. C-Raf overexpression is a phenomenon occurring in many types of cancer where it is considered to be a crucial factor in tumorigenesis and invasion of neoplastic cells. Drugs targeting the MEK/ERK pathway at the level of Raf may be particularly useful in the cancer therapy because Raf is the key activator of the ERK pathway.

The aim of the study was to verify whether C-Raf knockdown in HeLa cell cultures would improve efficiency of anticancer therapy with xanthone derivatives. Five aminoalkanol xanthone derivatives, synthesized at the Department of Bioorganic Chemistry (CMUJ, Kraków, Poland), were included in the study due to their previously designated significant antitumor activity. Natural xanthones, gambogic acid and α-mangostin, were used as reference compounds. Antisense oligonucleotide (ASO) and endoribonuclease-prepared siRNA (esiRNA) were used to knock down C-Raf expression.

Expression of C-Raf was efficiently silenced in cell cultures transfected with either esiRNA or ASO. In cultures subjected to C-Raf silencing IC50 values for or all xanthone derivatives significantly decreased (by the average of 52.03±5.37%) in comparison to non-transfected cultures or cultures transfected with control (scrambled) siRNA. C-Raf silencing also led to significant apoptosis enhancement, as well as a decrease in proliferation rate, determined by EdU incorporation assay (ThermoFischer Scientific) and by clonogenic assay. Finally, significant decrease in BD Matrigel invasion was observed. Additionally, a selective modulator of C-Raf, GW5074 (Sigma-Aldrich) was used to study potential synergism between xanthones and C-Raf inhibition. The values of Combination Index were in the range between 0.685 and 0.874 indicating synergism between all the studied xanthones and GW5074.

Taken together, these results suggest that C-Raf silencing can be a strategy to improve efficiency of the xanthones-based anticancer therapy. Furthermore, the use of pharmacological inhibitor GW5074 leads to synergistic effects in the combined xanthones treatment.

The study was supported by Medical University of Silesia funding (KNW-1-043/N/6/B and KNW-1-090/N/7/B).

[B-53] RUNX1 AND RUNX3 GENES IN ACUTE MYELOID LEUKEMIA DEVELOPMENT

Dagmara Szmajda, Adrian Krygier, Marta Żebrowska, Agnieszka Jeleń, Ewa Balcerczak

Department of Pharmaceutical Biochemistry and Molecular Diagnostics, Laboratory of Molecular Diagnostics and Pharmacogenomics, Medical University of Lodz, Muszynskiego 1, 90-151 Lodz, Poland

Acute myeloid leukemia is a malignancy of white blood cells characterized by clonal proliferation of myleoid progenitor cells in bone marrow and peripheral blood. The etiology of acute myeloid leukemia is not yet fully identified, however it is known that some genetic factors can contribute to its manifestation. RUNX1 and RUNX3 genes can be mentioned here, both playing role in the transcription process.

The aim of the study was to evaluatef RUNX1 and RUNX3 genes expression level in patients diagnosed with AML. The investigated group consisted of 43 patients, 22 women and 21 men. Peripheral blood remaining after routine tests was used for research. Relative RUNX1 and RUNX3 genes expression level was assessed using real-time PCR method.

The relative expression level of RUNX1 and RUNX3 gene varies among selected cases. The obtained results were related to some clinicopathological features. Expression levels of RUNX1 gene tended to be higher and more variable among women (P=0.044). Death occurrence was more frequent among patients with higher RUNX3 expression level (P=0.036). These findings suggest that gender can affect the expression level of RUNX1 gene and probably influence the process of AML development among women and men. It can be also concluded that RUNX3 can serve as a new potential prognostic factor. Patients with a higher expression level have generally poorer outcomes. However, the obtained results must be confirmed on a larger group of patients.

This study was supported by Statutory Funds of the Department of Pharmaceutical Biochemistry and Molecular Diagnostics, Medical University of Lodz 503/3-015-02/503-31-001 and Funds of the Faculty of Pharmacy, Medical University of Lodz 502-03/3-015-02/502-34-089 and 502-03/3-015-02/502-34-088.

[B-21] PROTEOMIC ANALYSIS OF CUTENUOUS MELANOMA ECTOSOMES

Sabina Szwed[1], Magdalena Surman[1], Dorota Hoja-Łukowicz[1], Sylwia Kędracka-Krok[2], Urszula Jankowska[3], Ewa Stępień[4], Małgorzata Przybyło[1]

[1]Department of Glycoconjugate Biochemistry, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, 30-387 Kraków, ul Gronostajowa 9, Poland; [2]Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, 30-387 Kraków, ul Gronostajowa 7, Poland; [3]Laboratory of Proteomics and Mass Spectrometry, Malopolska Centre of Biotechnology, Jagiellonian University in Krakow, 30-387 Kraków, ul Gronostajowa 7A, Poland; [4]Department of Medical Physics, M. Smoluchowski Institute of Physics, Jagiellonian University in Krakow, 30-348 Kraków, ul. Łojasiewicza 11, Poland

Ectosomes are rather heterogeneous population of extracellular vesicles with diameter ranging from 0.1 to 1 μm that is released to intercellular space by almost all cell types, including tumor cells. The biological and clinical significance of ectosome secretion in cancer has been a subject of sustained research. It is well established that tumor-derived ectosomes play functional roles in oncogenic transformation, tumor progression, invasion, metastasis, angiogenesis promotion, escape from immune surveillance, and drug resistance, thereby facilitating disease progression.

The present study was the first to characterize ectosomal proteome from two primary cutaneous melanoma (CM) cell lines – WM115 and WM793. Ectosomes were isolated from conditioned culture media by sequential centrifugation and analyzed with the use of nanoLC-MS/MS. First, the ectosome protein samples were prepared using paramagnetic bead technology, reduced and subjected to trypsin digestion. Then, peptides were analyzed using an UltiMate 3000 RSLCnano System coupled with Q-Exactive mass spectrometer with DPV-550 Digital PicoView nanospray source. The RAW files were later processed by the Proteome Discoverer platform and searched against the SwissProt database. Subsequent Gene Ontology (GO) annotations were made with the use of DAVID 6.8 database.

As a result, total numbers of 1349 and 1207 proteins were identified in WM115 and WM793 ectosomes, respectively. Both ectosome samples had the common set of 807 proteins that could severely impact melanoma invasive and metastatic potential. GO analysis showed the most abundant groups of ectosomal proteins are implicated in tumor cell motility, ECM remodeling, angiogenesis as well as in drug and immune response.

Data provided by this study might be of great interest to the melanoma scientific community and further the knowledge on the role of ectosomes in CM progression. It might also become valuable for the identification of diagnostic and prognostic biomarkers for CM.

This work was supported by the grant from the Polish National Science Centre (2013/11/B/NZ4/04315).

[B-41] HSP70 INHIBITION AS THERAPEUTIC STRATEGY FOR NON-SMALL CELL LUNG CARCINOMA, IN VITRO STUDY

Dorota Ścieglińska[1], Damian Sojka[1], Agnieszka Gogler-Pigłowska[1], Katarzyna Klarzyńska[1], Natalia Vydra[1], Krystyna Klyszcz[1], Sylwia Hasterok[2], Zdzisław Krawczyk[1]

[1]Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Poland; [2]Faculty of Biology and Environmental Protection, University of Silesia in Katowice, Poland

Heat shock proteins (HSPs) are molecular chaperones subdivided into several families based on their molecular weight. The HSPA (HSP70) family, one of the most conserved family in evolution, in human consist of twelve proteins. The HSPA proteins are involved in protein quality control and can be considered as a potent buffering system for cellular stress, either from extrinsic (environmental, physiological) or intrinsic (oncogenic) stimuli. HSPA have been implicated in numerous diseases. HSPAs are usually overexpress in various human tumors, including non-small cell lung carcinoma (NSCLC), and a high expression of these proteins is typically a marker for poor prognosis. HSPAs are considered important for survival of cancer cells. Due to their cytoprotective roles, these proteins may help protect cancer cells against chemotherapy-induced cell death.

In this study we assessed the response of human NSCLC cell lines to targeting HSPAs activity, either by specific RNAi-mediated knockdown of HSPAs isoform expression or by application of small molecule pan-HSPA inhibitor. We also tested whether such strategies could sensitize cancer cells to cisplatin or bortezomib, anticancer drugs used in NSCLC therapy. We found that neither specific silencing of HSPA1 (the major stress-inducible and cytoprotective protein) nor HSPA2 (differentiation-related chaperone) expression, showed significant impact on NSCLC proliferation or viability. It is wort noting that both HSPA1 and HSPA2 were found highly overexpressed in NSCLC cells. Instead, we observed a massive reduction in cellular viability when NSCLC cells were exposed to pan-HSPA inhibitor VER155008. We found that VER155008 effectively induced apoptotic cell death via reducing the level of Bag-1, a multifunctional and antiapoptotic protein. Unfortunately, combination treatment with cisplatin were unable to decrease cellular viability beyond that achieved by VER155008 or cisplatin alone. Nevertheless, we observed increased toxicity when VER155008 was combined with bortezomib, a clinically useful proteasome inhibitor. Similar effect we observed when proteasome function in VER155008-treated cells was blocked using MG132.

We found that simultaneous inhibition of multiple HSPA family members by pan-HSPA inhibitor showed effective anticancer activity. Our study also showed that HSPA and proteasome inhibitors combination have additive toxic effect in NSCLC cells. Our findings indicate that HSPAs can represent an interesting target to establish a novel approach for the treatment of NSCLC.

This work has been supported by National Science Centre, research grant NCN no. DEC-2013/09/B/NZ5/01815

[B-4] PHARMACOGENETIC MODELS OF ADVERSE REACTIONS TO FAC CHEMOTHERAPY IN BREAST CANCER PATIENTS

Karolina Tęcza, Jolanta Pamuła-Piłat, Joanna Łanuszewska, Dorota Butkiewicz, Lucyna Ponge, Iwona Domińczyk, Ewa Grzybowska

Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Institute-Oncology Center, Gliwice Branch

One of the major problems in breast cancer treatment are the differences in patients' response to the same medication. Adverse reactions to chemotherapy make a significant clinical problem due to decreased quality of life, prolongation of treatment and reinforcement of negative emotions associated with therapy.

In this study we evaluated the genetic and clinical risk factors of FAC chemotherapy-related toxicities in the group of 324 breast cancer patients. Selected genes and their polymorphisms were involved in FAC drugs transport (ABCB1, ABCC2, ABCG2, SLC22A16), metabolism (ALDH3A1, CBR1, CYP1B1, CYP2C19, DPYD, GSTM1, GSTP1, GSTT1, MTHFR, TYMS), DNA damage recognition, repair and cell cycle control (ATM, ERCC1, ERCC2, TP53, XRCC1).

The multifactorial risk models that combine genetic risk modifiers and clinical characteristics were constructed for 12 toxic symptoms. The majority of toxicities was dependent on the modifications in components of more than one pathway of FAC drugs. Also, the impact level of clinical factors was comparable to the genetic ones. Furthermore, for the carriers of multiple high risk factors the chance of developing given symptom was significantly elevated. Our results emphasize the complex nature of adverse effects during FAC breast cancer therapy, including the interplay among the polygenic inheritance of genetic and clinical risk factors.

The predictive models that engage multiple factors could be potentially useful in personalized approach to cancer treatment. The tool that enable the separation of patients group in terms of expected toxicity and therefore allows the tailoring of treatment to the characteristics of given patients, could significantly improve its tolerance, patients' quality of life and also outcome.

This work was financially supported by National Science Centre grant no. 2012/07/N/NZ5/00026

[B-5] CORRELATION BETWEEN RADIOSENSITIVITY AND BACKGROUND RADIATION-INDUCED COPY NUMBER VARIATIONS

Joanna Tobiasz[1], Najla Al-Harbi[2], Sara Bin Judia[2], Salma Majid[2], Ghazi Alsbeih[2], Joanna Polanska[1]

[1]Institute of Automatic Control, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100 Gliwice, Akademicka 16, Poland; [2]Faisal Specialist Hospital & Research Centre, Riyadh 11211, Kingdom of Saudi Arabia

Copy Number Variations (CNVs) are construed as DNA segments longer than 1 kb which vary in copy-number between genomes. They include deletions and amplifications, which arise by several DNA repair mechanisms, also as a response to radiation. CNVs influence gene expression levels in numerous ways. Hence, they are associated with various diseases, including mental, neurological and autoimmune disorders as well as cancers. The aim of this study is to investigate the relationship between cell lines' radiosensitivity and detected CNVs as well as to select genes affected by those significant copy-number changes.

Fibroblasts collected from 135 non-irradiated patients served as the material for this study. After the clonogenic assay the parameter of surviving fraction at 2 Gy (SF2) with the threshold value equal to 0.325 was applied to characterize the radiosensitivity. Subsequently, 52 radiosensitive and 83 regular response non-irradiated cell lines were used for CNV detection with Affymetrix CytoScan HD microarrays. As a result, values of Fold Change (FC) defined as the binary logarithm of ratio of signal intensity from a sample and the reference set were obtained for every cell line and every measured marker. Both groups were compared with the use of various statistical methods: Shapiro-Wilk test, Mann-Whitney U test and Storey's procedure for multiple testing correction as well as the estimation of the size of differences between the groups with non-parametric Cohen's d modification. Differentiating CNVs with at least medium effect size based on the Cohen's d value were tested for Spearman's correlation between their FC and SF2. Functional analysis of genes affected by significantly correlated CNVs was performed on the basis of the Gene Ontology database.

Over 10% (17,198 markers) of all differentiating features have at least medium effect, with 46 of them having an effect classified as large. 11,546 of these CNVs are not located inside any gene. Remaining are located within 5,480 introns, 284 exons, 144 3'-UTRs, and 16 5' UTRs. Correlation between FC and SF2 was proved to be nonsignificant for only less than 1% of exons, 5'- and 3'-UTRs. Although the correlation was not corroborated for over 3% of introns and intergenic fragments, these proportions are not high enough to state that they do not occur by chance. Significant correlation was confirmed for almost 97% of all at least medium effect differentiating markers (including all with large effect), which amounts to 16,617 features. Those markers are located inside 2,545 genes. Biological processes involved in functioning of nervous system are numerously represented (GO:0097150, GO:0035235, GO:0050896, GO:0099537, GO:0007399) in the set of GO results for selected genes, which is reflected in many publications and studies concerning individual radiosensitivity and neurological dysfunctions.

The performed study proved that there is a significant correlation between radiosensitivity and some autosomal Copy Number Variations in non-irradiated cells. These results indicate that selected copy-number alterations involved genes which products are associated with various processes crucial for the proper functioning of organisms.

This work was financially supported by SUT Grant Number BK 204/RAU1/2017/9, NSTIP KACST BIO1429-20 and 15-MED4114-20.

[B-40] HEAT SHOCK TRANSCRIPTION FACTOR 1 (HSF1) AS A POTENTIAL REGULATORY ELEMENT OF ESTROGEN SIGNALING

Agnieszka Toma-Jonik[1], Patryk Janus[1], Tomasz Stokowy[2], Ryszard Smolarczyk[1], Natalia Vydra[1], Wieslawa Widlak[1]

[1]Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, 44-101 Gliwice, Wybrzeze Armii Krajowej 15, Poland; [2]Department of Clinical Science, University of Bergen, Norway

Estrogens are known as the important regulators of the female reproductive functions. The principle estrogenic hormone, 17β-estradiol (E2), is synthesized by testosterone aromatization in the ovary and in other tissues. Its action is mediated by intracellular hormone-specific estrogen receptors (ERs). Estrogen signaling is implicated in the progression of the human breast cancers, the majority of which start as estrogen-dependent.
We found that E2 treatment leads to activation of HSF1 in human, estrogen receptor (ER)-positive breast adenocarcinoma MCF7 cells, but not in ERa-negative breast cancer cells. HSF1 is known to play a key role in tumor biology supporting the malignant transformation as well as tumor progression. Thus, we aimed to study whether HSF1 can support the genomic action of the estrogen receptor. We down-regulated HSF1 expression in MCF7 cells using specific lentiviral shRNA. HSF1 down-regulation has no effect on proliferation of MCF7 cells, although can partially reverse the disorganized growth of MCF7 cells in 3D culture. To find out the changes in the whole transcriptome after E2 or heat shock treatment we performed the RNA-Seq. The Gene to GO BP (Gene Ontology Biological Process) analysis revealed that silencing of HSF1 resulted not only in the downregulation of genes involved in response to stress and protein folding but also in deregulation of genes involved in signal transduction. Looking for differences in response to estrogen treatment we found that among activated genes, those involved in cell adhesion were over-represented in cells with silenced HSF1, but not in control ones. These findings suggest that in the presence of estrogen HSF1 could change the ability of cells to contact with extracellular matrix and other cells influencing their motility as well as their metastatic potential.

This work was supported by grants no 2014/13/B/NZ7/02341 and 2015/17/B/NZ3/03760 from the Polish National Science Centre.

[C-19] NANOMATERIALS - NEW THREAT TO THE ENVIRONMENT AND HEALTH

Mariusz Tomaszewski, Grzegorz Cema, Aleksandra Ziembińska-Buczyńska

Silesian University of Technology, Faculty of Energy and Environmental Engineering, Environmental Biotechnology Department, Akademicka 2, 44-100 Gliwice, Poland

Nanotechnology has gained a great deal of public interest due to the needs and applications of nanomaterials in many fields. Nanoparticles are used in electronics components, automation, solar energy, chemistry and biomedical applications. Moreover, they become a part of our daily life in sports equipment, clothing or cosmetics products. Nanomaterials can be released into the environment at every stage of the product life cycle. Increased exposure to these materials are likely to increase the possibilities of their adverse health effects. The potential entry routes into the human body are inhalation, ingestion and dermal penetration. However, there are still limited information about their emissions to the environment, toxicity and impact on human health. Nanoparticles toxicity is related to their size, shape, specific chemical and physical properties, but interactions at the nanoscale with biological systems are often unrecognized. More attention is paid to understand how the composition of nanoparticles interact with environment and living organisms, but there are still no standards and definitions for their toxicity determination. Moreover, there is a lack of the acts regulating the conditions of the manufacturing, placing on the market or use of nanomaterials. As a result, a new field of research has emerged - nanotoxicology.

This work presents a brief summary of recent knowledge about nanoparticles emissions, their behavior and toxicity in the environment, including current and future challenges for the safe nanotechnology.

Work was supported by The Silesian University of Technology (08/080/BKM16/0038)

[C-20] A NOVEL 1,8-NAPHTHALIMIDES INHIBITS CANCER CELLS GROWTH BY TARGETING DNA AND TOPOISOMERASE II

Mateusz D. Tomczyk[1], Anna Byczek-Wyrostek[1], Przemysław Kasprzycki[1], Klaudia Strama[1], Anna Maroń[2], Krzysztof Z. Walczak[1]

[1]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, Krzywoustego 4, 44-100 Gliwice, Poland; [2]Department of Crystallography, Institute of Chemistry, University of Silesia, Szkolna 9, 40-006 Katowice, Poland

DNA and DNA-associated processes are classes of the most important targets for the anticancer agents from the very beginning. Cancer cells are generally more susceptible to perturbation in DNA structure and functions because of higher replication and transcription demands. In fact, DNA has been successfully targeted by Mechlorethamine to treat cancers even before the discovery of DNA double helix structure. Apart from the agents that directly affected DNA, the agents targeting DNA-processing enzymes such as topoisomerases (Topo) are also extremely investigated and proceeded into clinical treatment [1-3].

1,8-Naphthalimides are well-known DNA-targeting agents that have been extremely explored as anticancer agents, and some of them, such as Amonafide, have reached clinical trials, however, all were withdrawn because of unfavorable toxicity and limited therapeutic efficacy [4]. Thus novel derivatives with improved efficacy and toxicity have received great research interests. Herein we report synthesis of a set of □-hydroxylalkylamine substituted 1,8-naphthalimides, which have shown good cytotoxicities against HCT-116 and A549 cell lines, mostly with IC_{50} in the low micromolar range comparable to that of Amonafide.

The mode of action of these new 1,8-naphthalimides in the cancer cells probably is similar to Amonafide. The molecular interactions mechanism of interaction with DNA and intracellular targets were investigated by molecular modeling simulations and spectroscopic methods. The preliminary tests of their antitumor activities indicated that they could inhibit Topo II catalytic activity in cell-free system as indicated by kDNA assay kit. We also chose some of the most active compounds and confirmed they DNA intercalation activity. Finally, the inhibition of cell growth by representative compounds were associated with induction of apoptosis.

[1] Martínez R et al. Curr. Med. Chem. 2005, 12, 127-151.
[2] Nitiss JL Nat. Rev. Cancer. 2009, 9, 338-350.
[3] Majumder P et al. Subcell. Biochem. 2007, 41, 145-189.
[4] Brana MF et al.Curr. Med. Chem. Anticancer Agents 2001, 1, 237-255.

[B-6] HAX1 ROLE IN PROMOTING BREAST CANCER METASTASIS CAN BE LINKED TO ITS IMPACT ON COLLECTIVE MIGRATION, ADHESION AND CONTRACTILITY

Alicja Trebinska-Stryjewska[1], Anna Balcerak[1], Maciej Wakula[1], Lukasz Szafron[2], Alina Rembiszewska[3], Mateusz Chmielarczyk[1], Urszula Smietanka[1], Renata Sienkiewicz-Kozlowska[4], Joanna Owczarek[3], Anna Felisiak-Golabek[5], Tymon Rubel[6], Sylwia Tabor[7], Ewelina Macech-Klicka[1], Ryszard Konopiński[1], Ewa A. Grzybowska[1]

[1]Department of Molecular and Translational Oncology, [2]Department of Immunology, [3]Department of Pathology and Laboratory Diagnostics, [4]Department of Breast Cancer and Reconstructive Surgery, [7]Department of Lung Cancer and Chest Tumours, The Maria Sklodowska-Curie Institute - Oncology Center, Warsaw, Poland; [5]Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland, USA; 6Institute of Radioelectronic and Multimedia Technology, Warsaw University of Technology, Warsaw, Poland

HAX1 protein is involved in regulating apoptosis, cell motility and calcium homeostasis. Its overexpression was reported in several tumors, including breast cancer. This study demonstrated that cytoplasmic HAX1 protein levels are significantly higher in primary tumors of breast cancer patients who experience progression during the disease course. Cytoplasmic HAX1 level above a threshold specified in ROC analysis was found to be an independent, negative prognostic factor for cancer progression. Our analyzes in breast cancer cell lines showed that HAX1 affects collective, but not single cell migration, thus indicating the importance of cell-cell contacts for the HAX1-mediated effect. Further analyzes demonstrated that HAX1 knockdown affects cell-cell junctions, ECM adhesion and actomyosin contractility. Together, these data indicate that the role of HAX1 in promoting metastasis may include stabilizing cell-cell junctions and maintaining the integrity of the epithelial cell layer, possibly influencing the formation of highly metastatic tumor cell clusters.

This work was supported by the Polish National Science Center grants no. 2011/01/B/NZ1/03674 and 2014/14/M/NZ1/00437.

[B-33] DIMER STABILITY IS DEPENDENT ON BOTH INTRA- AND EXTRACELLULAR PART OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

Cezary Tręda[1,2], Dagmara Grot[1,2], Aneta Włodarczyk[1,2], Ewelina Stoczyńska-Fidelus[1,2], Piotr Rieske[1,2]

[1]Department of Research and Development, Celther Polska Ltd., Milionowa 23 St., 93-193 Lodz, Poland; [2]Department of Tumor Biology, Medical University of Lodz, Zeligowskiego 7/9 St. 90-752 Lodz, Poland

Epidermal Growth Factor Receptor (EGFR) amplifications and rearrangements (e.g. EGFRvIII, characterized by the deletion of 2-7 exons that encode the extracellular domain of EGFR) are very common in most tumors types. Therefore, EGFR is model target for developing targeted therapies that not only distinguish between wild-type and mutant receptor, but also different specific mutations become a target for ultra-targeted therapies.

We created various mutants of EGFRvIII as well as wild-type EGFR that were introduced to AD293 cell line by lentiviral vector. AD293 cell line is characterized by a negligible amount of EGFR. Further, with use of various inhibitors, we analyzed wild-type EGFR and EGFRvIII dimer formation using semi-native western blot technique.

Our goal was to evaluate how the disturbance of selected intra- and extracellular part of EGFR influences stability and amount of dimer formed by EGFR. Interestingly, we found that inhibition of intracellular kinase domain significantly influences dimer stabilization. We also found that wild-type extracellular part hinders possibility of dimer formation and truncated version enhances the dimer formation and stability. Furthermore, in contrast to many earlier studies, our results indicated that EGFRvIII easily homodimerize.

To conclude, we show data of different effects of either various popular TKIs or EGFR mutations, and we suggest the possibility to translate these data not only to just dimer formation ability and its stability, but also biological effect of this phenomena. This would readily support the necessity of creation of new types of anti-EGFR molecules.

This study was supported by the Polish Agency for Enterprise Development (grant number POIG.01.04.00-10-037/11-00).

[B-55] INNOVATIVE LIBRARY PREPARATION TECHNOLOGIES: RNA-SEQ AND CHIP-SEQ AT THE SERVICE OF EPIGENOMICS

Anne-Clémence Veillard, Gilles Brocart, Miklós Laczik, Emilia Danilowicz-Luebert, Céline Sabatel

DIAGENODE SA, LIEGE SCIENCE PARK / rue du Bois Saint-Jean 3 / 4102 Seraing / Belgium

Epigenetics is crucial for the regulation of gene expression and has broad relevance in biological processes like development, disease and response to the environment. Epigenomics, the study of the epigenetic state of the genome, is therefore a key layer of biological systems. For more than 10 years Diagenode has been offering innovative tools to study epigenetic marks such as histones post-translational modifications and DNA methylation. Moreover Diagenode facilitates the use of Next-Generation Sequencing by developing progressive library preparation protocols dedicated to epigenetics analysis. Current library preparation protocols are long assays, with numerous steps and reduced efficiency on low input samples. To overcome this Diagenode is now presenting two new protocols for epigenomic studies. Chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) has become the gold standard for whole-genome mapping of protein-DNA interactions.

Since non-coding RNAs play key roles in the regulation of epigenetic marks -- Diagenode provides now a new solution to prepare libraries for RNA sequencing. We developed a technique called “Capture and Amplification by Tailing and Switching (CATS)” that is able to generate high diversity libraries from ultra-low RNA inputs. We present here results how the technology performs in our hands.

Diagenode presents a new ChIP-seq solution based on tagmentation and which permits to integrate the library preparation into the ChIP experiment. Therefore ChIPmentation allows straightforward and fast ChIP-seq experiments.

Results show that both, CATS RNA-seq and ChIPmentation are standardized, fast and reliable technologies that support exciting discoveries in the fascinating field of epigenomics.

[B-35] MECHANISMS OF BK CHANNEL ACTIVATION BY PHYSICOCHEMICAL STIMULI (Ca2+ CONCENTRATION, MEMBRANE POTENTIAL, TEMPERATURE, PRESSURE) – SIMULATION STUDIES

Agata Wawrzkiewicz-Jałowiecka, Zbigniew J. Grzywna

Silesian University of Technology, Faculty of Chemistry, Department of Physical Chemistry and Technology of Polymers

BK channels are transmembrane proteins which enable for highly effective and selective transport of potassium ions through the cell membrane. Because of their distinctively high conductance and, by extension, crucial role in many physiological processes, the mechanisms of their activation and gating awaken still high interest within scientific community. Nevertheless, not all aspects of the BK channel's functioning have been unraveled yet. In literature exist many models of their functioning at different external conditions, but they are often introduced without direct references to the channel's structure.

In this work we propose models of BK channel activation describing how physical and chemical factors (Ca2+ concentration, membrane potential, temperature, pressure) may synergistically operate on its activation gate. During formulation of models we incorporate available structural information about different types of ion channels, that share many similarities with the BK channels. As a validation of models we compare the results of their realizations with the experimental data obtained by the use of patch clamp method.

The main inferences from these studies suggest relatively large voltage-sensor translation within cell membrane in direction of extracellular side during membrane depolarization, pore widening exerted by the Ca2+ binding, electromechanical interaction of Ca2+ bonded to RCK1 with voltage sensor, and considerable effects of temperature and pressure on membrane properties, that affect channel gating

[C-13] GOLD NANOPARTICLES/ PEDOT: PSS LAYERED COMPOSITES AS BIOLOGICALLY ACTIVE COATINGS FOR NEURAL DEVICES

Daria Więcławska[1], Małgorzata Skorupa[1], Katarzyna Krukiewicz[1,2], Manus Biggs[2]

[1]Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology, Gliwice, Poland, [2]Centre for Research in Medical Devices, National University of Ireland, Galway, Ireland

Neural devices, such as neural probes or electrode arrays, are advantageous tools used to study the function of the nervous system and related degenerative disorders such as Parkinson's or Alzheimer's diseases. These devices also pose a potential way of treatment, by stimulation as well as recording of the neural signals. The inherently desirable features of materials applied in neural electrodes are high conductivity, low impedance and great biocompatibility. The proposed solution to meet these requirements is to employ metal-polymer composites.

In this study multilayer coatings have been investigated, consisting of gold nanoparticles and a biocompatible conductive polymer - poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS). The electrochemical characterization was done by means of cyclic voltammetry and electrochemical impedance measurements. Through comparison of the results of composites with different amount of layers, the composite with superior electrochemical qualities has been established. By SEM characterization of the film surface it has been proven that the gold nanoparticles form fractal-like structures, which are favourable for cell growth. In vitro cytotoxicity has been investigated by culturing of SH-SY5Y cell line on the surface of nanocomposites and performing the live/dead assay.

This research is supported in part by a research grant from Science Foundation Ireland (SFI) and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073. MB is an SFI, Starting Investigator SIRG COFUND fellow, grant no. 11/SIRG/B2135. KK is grateful to the National Science Centre in Poland for financing the research in the framework of SONATA program (2016/23/D/ST5/01306).

[A-22] GENETIC ALGORITHM IN TRAINING SET SELECTION FOR TUMOR SEGMENTATION PROBLEM

Wojciech Wilgierz[1,2], Dariusz Kuchta[1,2], Grzegorz Mrukwa[1,2], Mykola Chekan[3], Piotr Widłak[3], Monika Pietrowska[3], Joanna Polańska[1]

[1]Data Mining Division, Silesian University of Technology; [2]Spectre Team, Data Mining Division, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100, Gliwice, Poland [3]Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Institute Oncology Center, Gliwice Branch, 44-100, Gliwice, Poland

Introduction: Mass Spectrometry Imaging (MSI) is an emerging technique in an untargeted tissue molecular imaging. Analysis of data gathered using MALDI-MSI method is problematic due to high volume of collected datasets (in both spatial and molecular domains) and enormous information redundancy. Duplication of the information might be misleading in extraction of tissue specific molecular profiles. In this work we address these issue, through tumor segmentation, proposing a method that may be useful for capturing cancer molecular pattern.
Materials: Two head and neck cancer tissue preparations were captured in range of 800-4000 Da (109,568 mass channels) with ultrafleXtreme mass spectrometer, giving 7,671 and 11,668 spectra respectively. The preparations underwent examination by an experienced pathologist and tumor area has been manually segmented as a reference.
Methods: SVM classifier was chosen to identify cancer tissue signature. Since great number of the spectra provides redundant information, genetic algorithm was applied to select the most representative ones. To validate the obtained signature, one preparation was used during the training/testing step, while the second served as validation one. Random split of preparation 1 into training & testing sets was performed with the ratio 70% vs. 30%. Two simultaneous numerical experiments were performed: standard, where all spectra from training set were used in the learning process, and modified where genetic algorithm (GA) was used for selection of the most representative spectra from standard training set. The following values of GA parameters were chosen: 0.3 preservation rate, roulette wheel parent selection, single-point crossover, 0.1 mutation rate with 0.01 probability for each bit to change. Population size was set to 10 and different number of generations was considered. SVM was trained up to 10,000 iterations and 10-6 convergence threshold, no regularization was done. The experiment was repeated 30 times. Classifier accuracy was assessed by Accuracy, FDR, and Dice similarity index between pathologist defined and classifier detected tumor area.
Results: GA significantly reduced the size of training set from original 7,671 spectra to by average 263 spectra only (ranged from 134 to 381). The accuracy of standard SVM classifier on testing set was 88.75%, while for GA based training was 96.14%. The accuracy drops down when the spectra from independent validation set were classified: in case of standard approach FDR was 50.00%, for GA based: 33.18%. If Dice similarity index is considered, its value equals to 15.89% in case of standard approach, and significantly increases to 82% for GA based technique. The results validation set were 15.51% and 37.31% respectively.
Conclusions: Genetic algorithm can serve as a tool for smart reduction of training dataset in case of MALDI-MSI classification problems.

This study has been financially supported by NCN grant BITIMS no 2015/19/B/ST6/01736 (JP, GM), and PO WER NzN! 2.0 SPECTRE project (0303400-00-P009/16). Computations were carried out using the GeCONiI infrastructure (grant POIG.02.03.01 24 099).

[C-11] IL-2 SIGNAL PEPTIDE IS NOT SUFFICIENT TO EFFICIENTLY PURIFY RECOMBINANT K-RAS PROTEIN FROM CULTURE MEDIUM

Aneta Wlodarczyk, Dagmara Grot, Marta Popeda, Karolina Janik, Katarzyna Wasiak, Marcin Ciechanowski, Piotr Rieske

Research and Development Unit, Personather Ltd., 93-193 Lodz, ul. Milionowa 23, Poland

Recombinant proteins are widely used not only as therapeutic molecules themselves, but also as tools for functional analyses in the process of new drug development. We made an attempt to obtain KRAS protein of enough quantity to be further used in GTPase activity assay. For this purpose sequence of our target gene was fused with 6xHis and FLAG tags at the C-terminal and IL-2 signal peptide at the N-terminal end. The former tags were added in order to enable direct protein purification using nickel columns and its uncomplicated detection, while the latter peptide was aimed to trigger secretion of protein into the culture medium for easier purification of correctly folded protein, not subjected to protease enzymes. Following transduction of CHO cell line and further selection with appropriate antibiotic, expression of transgene was assessed at both mRNA (by means of real-time PCR) and protein level (WB analysis).

Protein was initially purified using HisTrapTM FF columns from various culture media, either those dedicated for high-yield protein production such as ExpiCHO and F17 Freestyle or standard culture medium DMEM HG. Unfortunately, efficiency of such purification approach turned out to be very low, as high amount of KRAS protein was still detected in CHO-S cells lysate following medium collection and protein was not detected using SDS-PAGE electrophoresis. Therefore, purification from cell lysates was performed, using lysis buffers of different compositions and the lysis buffer without addition of agents that may chelate nickel ions from the column (such as EDTA or EGTA) turned out to be the most efficient option.

These data suggest that IL-2 signal peptide is not sufficient to trigger secretion of KRAS protein to culture medium. It may be due to the fact that RAS proteins undergo the process of farnesylation and geranylgeranylation, post-translational modifications anchoring protein to cell membrane, or because protein was folded in such a way that sterically hindered its secretion.

This study was supported by the Operational Programme Smart Growth grant no. POIR.01.02.00-00-0034/15-00

[B-34] EGFRvIII EXPRESSION MAY TRIGGER APOPTOSIS IN NEURAL STEM CELLS

Aneta Włodarczyk, Kamila Rosiak, Maciej Smolarz, Piotr Rieske

Department of Tumor Biology, Medical University of Lodz, 90-752 Lodz, ul. Zeligowskiego 7/9, Poland

Nowadays, cancer is one of the most common causes of death worldwide. Despite years of extensive research and insightful knowledge in the area of oncology, the scientists still fail to develop entirely effective antineoplastic therapy. Intriguingly, such a failure may result from simplified understanding of the role of particular genes involved in the process of neoplasia - protumorigenic oncogenes and antineoplastic suppressors. There are some premises, however, to think that that the role of these genes is not as obvious as it is considered to be, as it may depend on the molecular context of the cell. We made an attempt to analyse whether oncogenes are able to induce apoptosis in normal cells, possibly via their interaction with other oncogenes or tumor suppressors.

As an analytical model induced neural stem cells (iNSc) were chosen due to their possible role in glioblastoma pathogenesis. We established iNS cells with inducible expression of mutated variant of epithelial growth factor receptor - EGFRvIII, as confirmed on both mRNA (using real-time PCR) and protein level (WB and ICC analyses). The impact of variable level of EGFRvIII expression on cell proliferation was evaluated using real-time observations. Additionally, WB analysis with anti-PARP antibody was applied for apoptosis assessment. Cells characterized by high EGFRvIII expression were proliferating at significantly lower rates and tended to undergo apoptosis, while low EGFRvIII expression exhibit pro-proliferative effect. As the phenomenon of synthetic lethality is currently gaining recognition, we additionally transduced iNSc-EGFRvIII with another oncogene - KRASG12V. Obtained results indicated the proapoptotic effect of EGFRvIII and KRASG12V co-expression, even when expression of the former oncogene was low.

Results of the study indicate that oncogenes may trigger programmed cell death when overexpressed in normal cells. Further studies are clearly required to determine the molecular context of the cells that enables these genes to act in such non-obvious way. Results of such analyses may lead to better comprehension of the role of particular genes involved in the process of carcinogognesis as well as constitute a breakthrough in the development of new anticancer therapies.

This study was supported by Medical University of Lodz grant No. 503/0-166-01/ 503-01-003

[A-23] ROUGH LOCAL RESHAPING FOR MULTIMODAL IMAGES CO-REGISTRATION USING ELLIPSIS' SHAPE APPROXIMATION

Michał Wolny[1,2], Grzegorz Mrukwa[1,2], Joanna Polańska[1]

[1]Data Mining Division, Silesian University of Technology, [2]Spectre Team, Data Mining Division, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100, Gliwice, Poland

Introduction: Mass spectrometry imaging (MSI) is a recent approach in biomedical research and it is accounted as a powerful tool in the study of various types of diseases. MSI allows to nicely combine molecular and morphological information, since molecular images are spatially resolved and well-correlated with the respective histological images. Integrative analysis of such multimodal data would support knowledge discovery in MSI experiment, therefore co registration of images is an issue of increasing interest and importance for present bioinformatics. The study focuses on the fusion of images obtained in MALDI ToF/ToF MSI experiment and respective histological H&E stained images. Resolving the co-registration problem would be of significant help in the studies on inter- and intra-tumor heterogeneity.
Material: The 5 FFPE tissue samples were sectioned, preprocessed and subjected to MS imaging with the use of MALDI-TOF/TOF ultrafleXtreme (Bruker) spectrometer with a raster width of 100 μm. Optical images were registered at the resolution of 1200 dpi. Spatial information on spectra collected in MSI experiment was used to construct the binary tissue map. The series of classical image processing algorithms were applied to detect sample edge. The similar operations were performed on H&E optical image.
Methods: The developed image fusion algorithm consists of three main steps. At the beginning, the rough estimates of global sample rotation, shift and scaling are obtained by single ellipse based shape modeling. The second step requires detailed modeling of each sample image and identification of corresponding ellipses on both models by k-NN algorithm. It allows for estimation of local shift and rotation transformation. Average values are used for images coarse co-registration, while the third step calls for local nonlinear image warping to maximize the value of Dice index.
Results: The algorithm was tested on the set of five pairs of tissue samples. To model tissue shape by ellipses approximation, 5-10 ellipses were used. Average distance between matched ellipses was 43,23 px. The final results shown that the developed algorithm has a potential to serve as an automated tool for H&E and MSI images co-registration.

This study has been financially supported by NCN grant BITIMS no 2015/19/B/ST6/01736 (JP, GM), and PO WER NzN! 2.0 SPECTRE project, (0303400-00-P009/16). Computations were carried out using the GeCONiI infrastructure (grant POIG.02.03.01 24 099).

[B-43] NFKB GENE EXPRESSION IN GASTRIC CANCER IN POLISH POPULATION - PRELIMINARY RESEARCH

Marta Żebrowska, Sebastian Szulczewski, Magdalena Wiernicka, Adrian Krygier, Dagmara Szmajda, Agnieszka Jeleń, Marek Mirowski, Ewa Balcerczak

Laboratory of Molecular Diagnostics and Pharmacogenomics, Department of Pharmaceutical Biochemistry and Molecular Diagnostics, Muszynskiego 1 90-151 Lodz, Poland

One of the most common malignant diseases in Poland is gastric cancer. The pathogenesis of gastric cancer development is not entirely clear. Next to the environmental risk factors, such as Helicobacter pylori infection or dietary habits, the host genetic factors as predispositions to gastric cancer development are discussed.

The transcriptional kb factor, encoded by the NFkB gene, binding to the promoter of the immunoglobulin light chain gene in mature B cells, may play an important role in immune and inflammatory processes. Since the NFkB protein product is a transcription factor it can control the functioning of many other genes involved in important cellular processes. Studies, form last years, show that expression of this gene may also be involved in apoptosis and proliferation processes. Probably NFkB by stimulating the expression of anti-apoptotic genes leads to the inhibition of apoptosis. Thus can promote the development of cancer diseases.

The aim of this preliminary study was to assess NFkB gene mRNA expression level in the group of patients with gastric cancer.

In 22 tissue samples collected from patients with gastric cancer the relative level of NFkB expression was measured by real-time PCR. The ΔΔ Ct method was used to calculate relative changes in gene expression.

Relative expression level of the NFkB gene to GAPDH expression (a housekeepnig gene) was variable among all cases.

[B-56] UV radiation influences glutathionylation potential in colorectal cancer cells

Małgorzata Adamiec[1], Magdalena Skonieczna[1,2]

[1]Institute of Automatic Control, Silesian University of Technology Akademicka 16, 44-100 Gliwice, Poland; [2]Biotechnology Centre, Silesian University of Technology, Krzywoustego 8, 44-100 Gliwice, Poland
Corresponding author: magdalena.skonieczna@polsl.pl

Reactive oxygen species (ROS) are chemically reactive molecules that contain oxygen and which are important players in the regulation of cellular signaling and energy production processes. The main producers of ROS in living cells are mitochondria (complex I and III of the cytochrome chain, where superoxide anions may appear as byproducts (rev. in [1]). There exist in cells endogenous ROS-scavenging systems, based on the activity of glutathione peroxidase (GPX) and peroxiredoxin (Prx) and also thioredoxin (Trx) and glutathione (GSH) redox potential [2]. However, to reduce oxidized glutathione and thioredoxin other enzymes, namely glutathione reductase (GSR) and thioredoxin reductase (TrxR), are required. These ROS-scavenging systems also reduce oxidized proteins, which control redox-dependent signaling pathways [2].

Aim of the studies: one of the most important anti-oxidants is glutathione, a molecule which undergoes S-glutathionylation process. It is a reversible reaction of glutathione as well as cysteine residues in proteins, leading to the formation of disulphides. One of the most important effects of glutathionylation is protection against irreversible oxidative changes in biomolecules (DNA, proteins, lipids, etc.). In this work the effects of UV radiation, on the glutathionylation potential in colorectal cancer cells, were studied.

Materials and methods: the HCT 116 cells were exposed to UV radiation of various wavelengths (UVA, UVB and UVC) and powers (10 kJ/m^2, 5 kJ/m^2 and 100 J/m^2, respectively). The profiles of glutathionylation in cells were estimated at a different time points after UV exposure (1, 3, 6, 8, 12 and 24 h), followed by glutathione reductase expression evaluation (Real-Time PCR). Detection of the total, oxidized or reduced glutathione was performed by flow cytometry, fluorometric assays, Western blotting and fluorescence microscopy imaging. Oxidative stress (hydrogen peroxide intracellular wave) was estimated by cytometry and correlated to the glutathionylation process.

Results and conclusions: 24 h after UVC radiation GSR expression in HCT 116 cells was inhibited, what encouraging an oxidative stress. The results demonstrate that post-UV radiation GSR expression in cells decreased, so transcript level changes are opposite to the those of GSH (increase). The lowest level of reduced glutathione was observed in the first hours after irradiation. Reduction of the oxidized (dimer) form of glutathione to the reduced (monomer) form is the most effective after GSR expression induction. The cycle of glutathione or thioredoxin oxidation/reduction is dose- and time-dependent, and this protects the cell from oxidative stress.

[1] T. Finkel, 2011, Cell Biol, 194 7-15
[2] Drug Discovery Today, 1, 2016, REVIEWS

The work was supported by grant (M.S.) No. 02/010/RGH17/0092, founded by Rector of the Silesian University of Technology in Gliwice, Poland.

[C-36] MESENCHYMAL STEM CELLS TRANSFER ONCOLYTIC MYXOMA VIRUS INTO MELANOMA CELLS

Joanna Jazowiecka-Rakus[1], Aleksander Sochanik[1], Aleksandra Rusin[1], Wojciech Fidyk[2], Agnieszka Ciomber[2], Agata Hadryś[3], Joanna Ciepła[3], Grant McFadden[4]

[1]Center for Translational Research and Molecular Biology of Cancer; [2]Department of Bone Marrow Transplantation and Oncohematology; Maria Skłodowska-Curie Institute - Oncology Center, Branch Gliwice, Poland; [3]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland; [4]Center for Immunotherapy, Vaccines and Virotherapy, Biodesign Institute, Arizona State Univ., Tempe, AZ (USA)

Intravenous administration of naked oncolytic virus triggers immune response and mobilizes the phagocytic system (MPS) leading to sequestration of the therapeutic construct. Effective delivery of oncolytic viruses into tumors requires shielding of viruses. Among promising carrier candidates are mesenchymal stem cells (MSC). They can be preloaded with virus *ex vivo* and delivered iv. to the recipient. Upon completion of transit to the tumor site these cells can release the oncolytic cargo. MSC cells show low expression of MHC I and II and they can regulate immune response by inhibiting activity of dendritic cells, natural killer cells as well as CD8+ and CD4+ cells. We attempted oncolytic therapy with myxoma virus (MYXV); this representative of poxviruses is not pathogenic to humans or other vertebrates (except rabbits). MYXV has great therapeutic potential as it shows tropism to various types of cancer cells, selectively infecting and replicating within them, which leads to cancer cells rupture and elimination (oncolysis) of tumor mass, without affecting adjacent tissues. We examined MSC carrier cells for their ability to transfer MYXV cargo to melanoma cells in culture. **Methods**: MSC cells were obtained from healthy donor bone marrow. Following MSCs isolation, their phenotype was confirmed using Human MSC Analysis Kit and flow cytometry. vMyx-GFP or vMyx GFP/TrFP viral constructs were propagated in RK13 rabbit cells and used to infect both murine (B16-F10) and human (451Lu, WM35, WM793B) melanoma cells, as well as MSC cells. B16-F10 and WM793B cells stably expressing mRFP were used (retrovirus-based pLNCX2 system derived from MLV). To determine if input and/or progeny virus are transferred from infected MSC cells to melanoma cells, cytarabine (cytosine arabinoside, Ara-C), a known inhibitor of viral DNA replication and late gene expression of MYXV, was used. MYXV infection was monitored by fluorescence microscopy, virus titering and flow cytometry; cell viability was measured by Alamar Blue assay. **Results:** The phenotype of isolated MSC cells has shown them suitable for further experiments. Our data confirmed that the murine melanoma and three human melanoma cell lines, as well as MSC cells, are permissive to MYXV infection. We examined the effect of MYXV infection on the viability of MSCs, B16-F10 and RK13 cells. We observed ~98% MSC cell survival at MOI=10. Murine and human melanoma cell lines, as well as RK13 cells were susceptible to MYXV-mediated killing. MYXV-infected MSCs successfully cross-infect murine and human melanoma cells in *in vitro* co-culture. When MSC cells were exposed to vMyx GFP/TrFP followed by the addition of Ara-C, the late gene expression of MYXV (TrFP+) was inhibited, which resulted in very low levels of viral late gene expression in both melanoma cells and MSC cells. Moreover, GFP expression in melanoma cells (infected by MYXV donated by MSC cells) was reduced, but not eliminated, by the Ara-C treatment. This means that both progeny virus (inhibited by Ara-C) and input virus (unaffected by Ara-C) can infect targeted melanoma cells. Together, these results support the notion that both input and progeny virus derived from MSC cells are both delivered into melanoma cells, which results in their productive infection. **Conclusion:** The results suggest that MSC cells ("Trojan horse") might be indeed suitable for improving MYXV-mediated oncolytic therapy of melanoma tumors.

This study was supported by grant No. CRP/POL16-02_EC from ICGEB, Italy to JJ-R.

[C-37] DERIVATIVES OF GRAPHENE OXIDE AS POTENTIAL DRUG CARRIERS

Aleksander Sochanik[1], Violetta Kozik[2], Andrzej Bąk[2], Marcin Rojkiewicz[2], Piotr Kuś[2], Waldemar Priebe[3], Joanna Jazowiecka-Rakus[1]

[1]Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Institute - Oncology Center, Gliwice Branch, 44-100 Gliwice, Poland
[2]Institute of Chemistry, University of Silesia, Szkolna 9, 40-006 Katowice, Poland;
[3]Department of Experimental Therapeutics, University of Texas MD Anderson Cancer Center, Houston, TX (USA).

This study has been focused on synthesis and initial assessment of biologically active novel derivatives of graphene oxide (GO) as potential anticancer drug nanocarriers.

Chemical functionalization of graphene oxide conferring novel physical and chemical properties involves either covalent alterations of GO, leading to surface changes via formation of chemical bonding whereas alterations of non-covalent kind involve van der Waals forces, hydrogen bonding and π–π stacking interactions. Covalent modifications appear to be superior as they yield compounds with defined properties whereas carriers prepared by non-covalent methods are less stable.

Therefore, novel GO-based nanocarriers were synthesized using covalent approach involving nucleophilic substitution of graphene oxide nanoparticles with iminodiacetic acid (IDA) or glycine (Gly). As the first step, iminodiacetic acid or glycine were transformed into iminodiacetic acid or glycine methyl ester hydrochlorides, respectively, for C-terminus protection. The obtained products were then activated in situ, and used to form amide bonds between graphene oxide and iminodiacetic acid, or glycine, respectively.

Ease of functionalization and ability to transport biologically active substances made it possible to investigate usefulness of both GO-IDA and GO-Gly as nanocarriers of an advanced antimelanoma chemotherapeutic. As numerous studies have suggested, chemotherapeutics useful for melanoma treatment require customization; we thus tested in here GO-based nanocarriers loaded with WP760, one of the first melanoma-specific chemotherapeutic candidates It is a bis-anthracycline with previously demonstrated antimelanoma activity *in vitro*. It exploits daunomycinone and adriamycinone as intercalating moieties, two minor groove-binding sugars related to daunosamine and 4-amino-2,3,6-trideoxy-alpha-L-lyxo-hexopyranose, all joined by tailored linkers (‘click’ chemistry). Doxorubicin was used as a reference compound.

[C-38] MESENCHYMAL STROMAL CELLS (MSC) AS CARRIERS OF IL-12 CDNA IN TREATMENT OF MICE BEARING MELANOMA B16-F10

Natalia Kułach[1,2], Tomasz Cichoń[1], Magdalena Jarosz-Biej[1], Marek Rusin[1], Ewelina Pilny[1,3], Stanisław Szala[1] , Ryszard Smolarczyk[1]

[1]Center for Translational Research and Molecular Biology of Cancer Maria Sklodowska-Curie Institute - Oncology Center, Gliwice Branch, Wybrzeże Armii Krajowej Street 15, 44-101 Gliwice, Poland [2]Department of Animal Physiology and Ecotoxicology, University of Silesia, Bankowa 9 St., Katowice, Poland [3]Department of Organic Chemistry, Biochemistry and Biotechnology, Silesian University of Technology, Gliwice

In recent years, mesenchymal stromal cells (MSC) have become the subject of intensive research efforts. MSC are multipotent, nonhematopoietic cells, present in many tissues of the body. *In vivo* MSC are an important functional element of hematopoietic stem cells niche and an integral part of blood vessel walls. MSC cells are a reservoir of cytokines and growth factors for hematopoietic cells. MSC secrete proangiogenic and cell proliferation stimulating factors. MSC have regenerative properties, accelerate healing and participate in bone, cartilage and myocardium regeneration. MSC have the ability to migrate to the damaged site and exhibit taxis towards tumor cells. Owing to these specific migratory abilities, mesenchymal cells have been exploited as carriers of anticancer therapeutic agents. IL-12 is a powerful immunostimulant which activates CD4+, CD8+ and NK cells as well as triggers release of IFN-γ, all of which induce a strong immune response directed against cancer cells. IL-12 cytokine also acts as an antiangiogenic factor. The aim of the project is melanoma bearing mice treatment using IL-12-modified MSC. Mesenchymal stromal cells (MSC) with Sca-1^+ $CD105^+$ $CD90^+$ $CD29^+$ $CD44^+$ $CD106^-$ $CD45^-$ phenotype were isolated and characterized. The migration ability to tumor cells was examined. The capacity of MSC to differentiate into osteogenic cells and adipocytes was checked. An adenoviral vector was constructed with genes encoding both murine IL-12 subunits. The vector with IL-12 was introduced into the cells. The level of IL-12 secreted by transduced cells was determined by ELISA. The results show that the cells isolated from murine bone marrow: have MSC phenotype, differentiate towards osteocytes and adipocytes, exhibit taxis to B16-F10 cells, transduced with modified adenovirus vector produce IL-12 and brought to B16-F10 tumor significantly retard its growth.

The study was supported by Grant UMO-2015/17/N/NZ4/02738 from the Ministry of Science and Higher Education (Poland).

[C-39] APPLICATION OF MODERN POLYMER MATERIALS SUPPORTING INCREMENTAL TECHNOLOGIES IN REGENERATIVE MEDICINE

Andrzej S. Swinarew[1], Klaudia Kubik[1], Jarosław Paluch[3], Anna Kwaśniewska[2], Anna Bielicka[2], Marta Łężniak[1], Tomasz Flak[1], Karolina Gajos[1]

[1]Institute of Materials Science, Faculty of Computer Science and Material Science, University of Silesia in Katowice, 75 Pułku Piechoty 1A, 41-500 Chorzów, Polan; [2]Department of Radiology, Diagnostic Imaging Laboratory School of Medicine in Katowice, Medical University of Silesia in Katowice, Francuska 20/24, 40-027 Katowice, Poland; [3]Department and Clinic of Laryngology, School of Medicine in Katowice, Medical University of Silesia in Katowice, Francuska 20/24, 40-027 Katowice, Poland

Implantation of composite constructs is a one of the possibilities which allowed to avoid complications after resection in the course of benign tumor, traumatic and degenerative lesions. Implants can be made of different materials. Porous materials are a new scaffolding solution for the organ and suitable cells such as collagen, chondrocytes, osteoblasts grown up. This work focuses on the material used as a filament in 3D printing and its application to alloplastics and transplantology.

The aim of the work was to create a biocompatible composite construct dimensionally personalized to the patient, which in terms of anatomical and functional can replace the function of the organ after implantation.

The porous material obtained from 3D printing was covered with a suitable cell culture for cell proliferation. This solution became a new path to reduce the risk of recurrence of the implant by the patient.

Pre-made model of the acetabulum was tested for friction coefficient as well as wear tribological accordance with the standards in combination with a ceramic femoral head. In this tribological combination the material exhibited significantly less wear and lower friction coefficient than the corresponding ceramic or polypropylene acetabulum.

The obtained results may indicate that the mentioned material could be a base for replacing previously used metallic materials in the future. In addition, the ability to print from material allows for accurate personalization of the implant without the need for greater resection of the patient's bone fragment during implantation.

Participant affiliation and e-mail addresses

2nd name	1st name	Participation[1]	Affiliation	City	e-mail address
Abramowicz	Agata	L	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	agata.abramowicz@io.gliwice.pl
Adamczyk	Agnieszka	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Kraków*	z5adamcz@cyfronet.pl
Adamiec	Małgorzata		*Silesian University of Technology*	*Gliwice*	malgosiaa93@gmail.com
Altmann	Sandra	P	*University of Silesia*	*Katowice*	sandra_dg@interia.pl
Balcerczak	Ewa	P	*Medical Univeristy of Lodz*	*Łódź*	ewa.balcerczak@umed.lodz.pl
Banach	Anna	P	*Silesian University of Technology*	*Gliwice*	anna.banach@polsl.pl
Bandoła	Dominika	P	*Silesian University of Technology*	*Gliwice*	dominika.bandola@polsl.pl
Baumann	Marc	L	*University of Helsinki*	*Helsinki*	marc.baumann@helsinki.fi
Bednarczyk	Martyna	P	*Medical University of Silesia*	*Katowice*	martyna.bednarczyk@outlook.com
Bednarczyk	Katarzyna		*Silesian University of Technology*	*Gliwice*	katarzyna.bednarczyk@polsl.pl
Biecek	Przemysław	L	*Warsaw University of Technology*	*Warszawa*	przemyslaw.biecek@gmail.com
Bielas	Rafał	P	*Silesian University of Technology*	*Gliwice*	rafal.bielas@polsl.pl
Bil	Patryk	P	*Silesian University of Technology*	*Gliwice*	patryk.bil@polsl.pl
Binczyk	Franciszek	P	*Silesian University of Technology*	*Gliwice*	Franciszek.E.Binczyk@polsl.pl
Butkiewicz	Dorota	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	Dorota.Butkiewicz@io.gliwice.pl
Byczek-Wyrostek	Anna	P	*Silesian University of Technology*	*Gliwice*	anna.byczek@polsl.pl
Bzówka	Maria	P	*Silesian University of Technology*	*Gliwice*	maria.bzowka@gmail.com
Cecotka	Agnieszka	P	*Silesian University of Technology*	*Gliwice*	RR1@polsl.pl
Chadalski	Marek	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	marek.chadalski@io.gliwice.pl
Chorąży	Mieczysław		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	chorazy@io.gliwice.pl
Ciechanowski	Marcin		*Medical University of Lodz*	*Łódź*	ammarcin.ciechanowski@gmail.com
Cieślar-Pobuda	Artur	C	*Silesian University of Technology*	*Gliwice*	artur.cieslar-pobuda@polsl.pl
Collas	Philippe	L	*Universtity of Oslo*	*Oslo*	philippe.collas@medisin.uio.no
Cortez	Alexander	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	alehandro17@wp.pl
Cykowiak	Marta	P	*Poznan University of Medical Sciences*	*Poznań*	marta.cykowiak@gmail.com
Cypryk	Wojciech		*Centre of Molecular and Macromolecular Studies PAS*	*Łódź*	wcypryk@cbmm.lodz.pl
Czernek	Liliana	P	*Centre of Molecular and Macromolecular Studies PAS*	*Łódź*	lczernek@cbmm.lodz.pl
Czerwińska	Dominika	P	*Silesian University of Technology*	*Gliwice*	Dominika.Czerwinska@polsl.pl
Danilowicz-Luebert	Emilia	P	*Diagenode SA*	*Seraing (Ougrée)*	edal@diagenode.com
Dawidowska	Małgorzata	L	*Institute of Human Genetics, PAS*	*Poznań*	malgorzata.dawidowska@igcz.poznan.pl
Duechler	Marcus	L	*Centre of Molecular and Macromolecular Studies PAS*	*Łódź*	mduchler@cbmm.lodz.pl
Elas	Martyna		*Jagiellonian University*	*Kraków*	martyna.elas@uj.edu.pl
Fendler	Wojciech	L	*Medical University of Lodz*	*Łódź*	fendler.wojciech@gmail.com
Fidyk	Wojciech	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	wojciech.fidyk@io.gliwice.pl
Filipczyk	Adam	L	*Oslo University Hospital*	*Oslo*	dr.adam.filipczyk@gmail.com
Fussiova	Lenka		*Adaptive Biotechnologies*	*Prague*	lenka.fussiova@gmail.com
Gaipl	Udo	L	*Universitaetsklinikum Erlangen*	*Erlangen*	Udo.Gaipl@uk-erlangen.de
Gajda	Karolina		*Silesian University of Technology*	*Gliwice*	k.gajda86@wp.pl
Gajowik	Aneta	P	*National Institute of Public Health - National Institute of Hygiene*	*Warszawa*	agajowik@pzh.gov.pl

Gamoń	Filip	P	*Silesian University of Technology*	*Gliwice*	filip.gamon@gmail.com
Gawecki	Robert	P	*University of Silesia*	*Katowice*	robert.gawecki@us.edu.pl
Gdowicz-Kłosok	Agnieszka	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	agagdowicz@gmail.com
Główka	Marek		*The Institute of Heavy Organic Synthesis "Blachownia"*	*Kędzierzyn-Koźle*	marekglowka_1993@tlen.pl
Gnus	Małgorzata	P	*Silesian University of Technology*	*Gliwice*	malgorzata.gnus@polsl.pl
Goczał	Agata	P	*University of Lodz*	*Łódź*	agata.goczal@wp.pl
Gogler-Pigłowska	Agnieszka	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	agogler@o2.pl
Gola	Joanna	L	*Medical University of Silesia*	*Katowice*	jgola@sum.edu.pl
Golec	Marlena	P	*University of Silesia*	*Katowice*	marlena.golec90@gmail.com
Górka	Judyta		*Jagiellonian Univerisity*	*Kraków*	judyta.gorka@doctoral.uj.edu.pl
Gracka	Maria	P	*Silesian University of Technology*	*Gliwice*	maria.gracka@polsl.pl
Grot	Dagmara	P	*Medical University of Lodz*	*Łódź*	d.jadwiszczak@gmail.com
Grzenik	Anna		*Silesian University of Technology*	*Gliwice*	annagrzenik95@gmail.com
Grzybowska	Ewa		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	ewagrzybowska@yahoo.com
Hejmo	Tomasz		*Medical University of Silesia*	*Katowice*	tomekhejmo@gmail.com
Hudy	Dorota	P	*Silesian University of Technology*	*Gliwice*	dorota.hudy@polsl.pl
Idkowiak	Jakub	P	*Institute of Bioorganic Chemistry PAS*	*Poznań*	jidkowiak@ibch.poznan.pl
Izanloo	Azra	P	*Imam Reza International University*	*Mashhad*	a.izanloo64@gmail.com
Jaksik	Roman	P	*Silesian University of Technology*	*Gliwice*	roman.jaksik@polsl.pl
Janik	Karolina	P	*Medical University of Lodz*	*Łódź*	karolina.janik@stud.umed.lodz.pl
Janus	Patryk		*Silesian University of Technology*	*Gliwice*	pjanus@io.gliwice.pl
Jarosz-Biej	Magdalena		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	Magdalena.Jarosz-Biej@io.gliwice.pl
Jazowiecka-Rakus	Joanna	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	joanna.jazowiecka@io.gliwice.pl
Józkowicz	Alicja	L	*Jagiellonian University*	*Kraków*	alicja.jozkowicz@uj.edu.pl
Jura	Jolanta	L	*Jagiellonian University*	*Kraków*	jolanta.jura@uj.edu.pl
Jureczko	Marcelina	P	*Silesian University of Technology*	*Gliwice*	Marcelina.Jureczko@polsl.pl
Kała	Sylwia	P	*Silesian University of Technology*	*Gliwice*	sylwia.kala@gmail.com
Kałużyński	Piotr		*Silesian University of Technology*	*Gliwice*	piotr.kaluzynski@polsl.pl
Kardyńska	Małgorzata	P	*Silesian University of Technology*	*Gliwice*	malgorzata.kardynska@polsl.pl
Karpiel	Ilona	P	*University of Silesia*	*Katowice*	ilona.karpiel@smcebi.edu.pl
Kluszczyńska	Katarzyna		*Centre of Molecular and Macromolecular Studies PAS*	*Łódź*	kaska.kluszczynska@gmail.com
Kocot	Szymon	P	*Silesian University of Technology*	*Gliwice*	szymko1995@gmail.com
Kogut	Daria		*Silesian University of Technology*	*Gliwice*	daria.kogut0@gmail.com
Kozik	Violetta		*University of Silesia*	*Katowice*	violetta.kozik@us.edu.pl
Krawczyk	Monika	P	*Silesian University of Technology*	*Gliwice*	monika.krawczyk@polsl.pl
Krol	Lukasz		*Silesian University of Technology*	*Gliwice*	lukasz.krol@polsl.pl
Kruszewski	Marcin	L	*Institute of Nuclear Chemistry and Technology*	*Warszawa*	m.kruszewski@ichtj.waw.pl
Krześniak	Małgorzata	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	Malgorzata.Krzesniak@io.gliwice.pl
Krzywoń	Aleksandra	P	*Silesian University of Technology*	*Gliwice*	aleksandra.krzywon@polsl.pl
Kuczak	Michal	P	*University of Silesia*	*Katowice*	mkuczak@us.edu.pl
Kujawa	Katarzyna		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	katarzyna.kujawa@io.gliwice.pl
Kułach	Natalia		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	wojtyla.natalia@gmail.com
Kupczak	Maria	P	*Silesian University of Technology*	*Gliwice*	mery.105@wp.pl

Kurasz	Karolina	P	*Silesian University of Technology*	*Gliwice*	karolina.kurasz@polsl.pl
Kurpas	Monika	P	*Silesian University of Technology*	*Gliwice*	monika.kurpas@gmail.com
Kuśmierczyk	Paweł		*CELTHER POLSKA SP Z O O*	*Konstantynów Łódzki*	paw.kusmiercz@gmail.com
Lalowski	Maciej	L	*University of Helsinki*	*Helsinki*	maciej.lalowski@helsinki.fi
Lisowska	Katarzyna	C, L	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	klisowska@io.gliwice.pl
Ludwig	Sonja	L	*University of Duisburg-Essen*	*Essen*	Sonja.Ludwig@uk-essen.de
Lumniczky	Katalin	L	*OSKKI/NRIRR*	*Budapest*	lumnicz@hp.osski.hu
Łanuszewska	Joanna		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	joanna.lanuszewska@io.gliwice.pl
Łysek-Gładysińska	Małgorzata	P	*Jan Kochanowski University*	*Kielce*	mglad@ujk.edu.pl
Malarz	Katarzyna		*University of Silesia*	*Katowice*	kmalarz@us.edu.pl
Marczyk	Michal	P	*Silesian University of Technology*	*Gliwice*	Michal.Marczyk@polsl.pl
Marona	Paulina	P	*Jagiellonian University*	*Kraków*	paulina.marona@doctoral.uj.edu.pl
Marzec-Burszewska	Joanna		*Polgen Sp. z o.o.*	*Łódź*	joanna_marzec@polgen.com.pl
Matysniak	Damian	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Warszawa*	d.matysniak@nencki.gov.pl
Mazurek	Agnieszka	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	Agnieszka.Mazurek@io.gliwice.pl
Melka	Bartlomiej	P	*Silesian University of Technology*	*Gliwice*	bartlomiej.melka@polsl.pl
Mika	Justyna	P	*Silesian University of Technology*	*Gliwice*	Justyna.Mika@polsl.pl
Mirowski	Marek	P	*Medical Univeristy of Lodz*	*Łódź*	marek.mirowski@umed.lodz.pl
Mitusińska	Karolina	P	*Silesian University of Technology*	*Gliwice*	karolina.mitusinska@polsl.pl
Mohareb	Fady	L	*Cranfield University*	*Cranfield*	f.mohareb@cranfield.ac.uk
Mothersill	Carmel	L	*McMaster University*	*Hamilton*	mothers@mcmaster.ca
Mrozek-Wilczkiewicz	Anna		*University of Silesia*	*Chorzów*	anna.mrozek-wilczkiewicz@us.edu.pl
Muc-Wierzgoń	Małgorzata		*Medical University of Silesia*	*Katowice*	mwierzgon@sum.edu.pl
Mularski	Jacek	P	*Silesian University of Technology*	*Gliwice*	jacek.mularski@gmail.com
Musioł	Robert		*University of Silesia*	*Katowice*	robert.musiol@us.edu.pl
Narożna	Maria	P	*Poznan University of Medical Sciences*	*Poznań*	maria.narozna@onet.eu
Niebudek	Katarzyna	P	*Medical University of Lodz*	*Łódź*	katarzyna-niebudek@wp.pl
Niemiec	Joanna	L	*Maria Sklodowska-Curie Institute - Oncology Center, Cracow Branch*	*Kraków*	z5niemie@cyfronet.pl
Nowak	Marcin	P	*Silesian University of Technology*	*Gliwice*	marcin9.nowak3@gmail.com
Nowak	Ewa	P	*Medical University of Silesia*	*Katowice*	ewa.nowak@sum.edu.pl
Ochab	Magdalena	P	*Silesian University of Technology*	*Gliwice*	magdalena.ochab@polsl.pl
Odrobińska	Justyna	P	*Silesian University of Technology*	*Gliwice*	Justyna.Odrobinska@polsl.pl
Olbryt	Magdalena		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	magdalena.olbryt@io.gliwice.pl
Pacholczyk	Marcin		*Silesian University of Technology*	*Gliwice*	marcin.pacholczyk@polsl.pl
Pamuła-Piłat	Jolanta	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	jpamula@io.gliwice.pl
Papierska	Katarzyna	P	*Poznan University of Medical Sciences*	*Poznań*	kpapierska@ump.edu.pl
Papież	Anna	P	*Silesian University of Technology*	*Gliwice*	anna.papiez@polsl.pl
Pastuch-Gawołek	Gabriela	P	*Silesian University of Technology*	*Gliwice*	gabriela.pastuch@polsl.pl
Paszek	Anna	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	annanaumowicz@o2.pl
Paszek	Paweł	L	*University of Manchester*	*Manchester*	pawel.paszek@manchester.ac.uk
Patra	Hirak	L	*University of Cambridge*	*Cambridge*	hp401@cam.ac.uk
Pawlowska	Roza	P	*Celther Diagnostics Sp. z o.o.*	*Konstantynów Łódzki*	roza.pawlowska@celther.com
Pęciak	Joanna	P	*Medical University of Lodz*	*Łódź*	joanna.peciak@stud.umed.lodz.pl

Pieprzyca	Anna	P	*Silesian University of Technology*	*Gliwice*	anna.m.pieprzyca@gmail.com
Pietrowska	Monika	C	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	monika.pietrowska@io.gliwice.pl
Pietrzak	Jacek		*Medical University of Lodz*	*Łódź*	jacek.pietrzak@stud.umed.lodz.pl
Pigłowski	Wojciech	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	wojciech.piglowski@io.gliwice.pl
Pilny	Ewelina	P	*Silesian University of Technology*	*Gliwice*	ewelina.pilny@polsl.pl
Plefka	Ernst	L	*BIOCRATES Life Sciences AG*	*Innsbruck*	
Poczta	Anastazja	P	*University of Lodz*	*Dobrzyca*	anapoczta5@gmail.com
Polańska	Joanna	C	*Silesian University of Technology*	*Gliwice*	Joanna.Polanska@polsl.pl
Poleszczuk	Jan	P	*Nalecz Institute of Biocybernetics and Biomedical Engineering PAS*	*Warszawa*	jpoleszczuk@ibib.waw.pl
Popeda	Marta	P	*Personather Ltd.*	*Konstantynów Łódzki*	marta.popeda@gmail.com
Poterała	Aleksandra	P	*Silesian University of Technology*	*Gliwice*	aleksandra.poterala@gmail.com
Psiuk-Maksymowicz	Krzysztof	P	*Silesian University of Technology*	*Gliwice*	krzysztof.psiuk-maksymowicz@polsl.pl
Raczyńska	Agata	P	*Silesian University of Technology*	*Gliwice*	agata.raczynska3@gmail.com
Raczyńska-Szajgin	Magdalena		*University of Silesia*	*Katowice*	magdalena.raczynska-szajgin@us.edu.pl
Rieske	Piotr	L	*Medical University of Lodz*	*Łódź*	piotr.rieske@umed.lodz.pl
Rogoliński	Jacek		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	Jacek.Rogolinski@io.gliwice.pl
Rolnik	Bożena		*Silesian University of Technology*	*Gliwice*	bozena.rolnik@polsl.pl
Rusin	Marek	L	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	Marek.Rusin@io.gliwice.pl
Ruszel	Karol	P	*Uniwersytet Medyczny w Lublinie*	*Lublin*	karol.ruszel@wp.pl
Rybak	Aleksandra	P	*Silesian University of Technology*	*Gliwice*	aleksandra.rybak@polsl.pl
Rzeszowska	Joanna	C	*Silesian University of Technology*	*Gliwice*	Joanna.Rzeszowska@polsl.pl
Rzyman	Witold	L	*Medical University of Gdańsk*	*Gdańsk*	wrzyman@gumed.edu.pl
Sierra	Angeles	L	*IDIBAPS & UVic*	*Barcelona*	asierrajim@gmail.com
Sikora	Bartosz	P	*Medical University of Silesia*	*Katowice*	bartoszsikora90@gmail.com
Simka	Klaudia	P	*Medical University of Silesia*	*Katowice*	simka.klaudia@gmail.com
Składowski	Krzysztof	L	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	Krzysztof.Skladowski@io.gliwice.pl
Skonieczna	Magdalena		*Silesian University of Technology*	*Gliwice*	magdalena.skonieczna@polsl.pl
Skubis	Aleksandra	P	*Medical University of Silesia*	*Katowice*	aleksandra.skubis@gmail.com
Smolarczyk	Ryszard	P	*Maria Sklodowska-Curie Institute - Oncology Center, Gliwice Branch*	*Gliwice*	ryszard.smolarczyk@io.gliwice.pl
Smolarz	Mateusz		*Maria Sklodowska-Curie Institute - Oncology Center, Gliwice Branch*	*Gliwice*	mateusz.smolarz@io.gliwice.pl
Sochanik	Aleksander	P	*Maria Sklodowska-Curie Institute - Oncology Center, Gliwice Branch*	*Gliwice*	aleksander.sochanik@io.gliwice.pl
Sojka	Damian	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	damian.sojka@io.gliwice.pl
Stańczak	Agnieszka	P	*Silesian University of Technology*	*Gliwice*	agasami@wp.pl
Stoczyńska-Fidelus	Ewelina		*Medical University of Lodz*	*Łódź*	ewelinastoczynska@gmail.com
Stokowy	Marcin		*Silesian University of Technology*	*Gliwice*	szoko1@autograf.pl
Student	Sebastian		*Silesian Universit of Technology*	*Gliwice*	sebastian.student@polsl.pl
Surman	Magdalena	P	*Jagiellonian University*	*Kraków*	magdalena.surman@doctoral.uj.edu.pl
Suwalska	Aleksandra	P	*Silesian University of Technology*	*Gliwice*	aleksandrasuwalska95@wp.pl
Swinarew	Andrzej		*University of Silesia*	*Katowice*	andrzej.swinarew@us.edu.pl
Szmajda	Dagmara	P	*Medical University of Lodz*	*Łódź*	dagmara_szmajda@interia.pl
Szurko	Agnieszka		*University of Silesia*	*Katowice*	agaspl@o2.pl
Szwed	Sabina	P	*Jagiellonian University*	*Kraków*	sabina.szwed@doctoral.uj.edu.pl
Ślęzak-Prochazka	Izabella		*Silesian University of Technology*	*Gliwice*	izabella.slezak-prochazka@polsl.pl

Tęcza	Karolina	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	karolina.tecza@io.gliwice.pl
Tobiasz	Joanna	P	*Silesian University of Technology*	*Gliwice*	joanna.tobiasz@polsl.pl
Toma-Jonik	Agnieszka	P	*Maria Skłodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	agnieszka.toma-jonik@io.gliwice.pl
Tomaszewski	Mariusz	P	*Silesian University of Technology*	*Gliwice*	mariusz.tomaszewski@polsl.pl
Tomczyk	Mateusz	P	*Silesian University of Technology*	*Gliwice*	mateusz.d.tomczyk@polsl.pl
Trębińska-Stryjewska	Alicja	P	*Maria Sklodowska-Curie Institute - Oncology Center, Warsaw Branch*	*Warszawa*	alicja.trebinska@coi.pl
Tręda	Cezary	P	*Medical University of Lodz*	*Łódź*	cezary.treda@umed.lodz.pl
Tudrej	Patrycja		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	patrycja.tudrej@io.gliwice.pl
Vydra	Natalia	P	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	Natalia.Vydra@io.gliwice.pl
Wardega	Piotr	L	*NanoTemper Technologies*	*Kraków*	
Wasiak	Katarzyna		*PERSONATHER Sp. z o.o.*	*Konstantynów Łódzki*	kat.wasiak93@gmail.com
Wawrzkiewicz-Jałowiecka	Agata	P	*Silesian University of Technology*	*Gliwice*	agata.wawrzkiewicz@polsl.pl
Whiteside	Theresa	L	*University of Pittsburgh*	*Pittsburgh*	whitesidetl@upmc.edu
Widłak	Piotr	C	*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	piotr.widlak@io.gliwice.pl
Widłak	Wiesława		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	wieslawa.widlak@io.gliwice.pl
Więcławska	Daria	P	*Silesian University of Technology*	*Gliwice*	RR1@polsl.pl
Witkowski	Jakub	P	*Adamed*	*Pieńków*	jakub.witkowski@adamed.com.pl
Włodarczyk	Aneta	P	*Medical University of Lodz*	*Łódź*	anetawlodarczyk@onet.pl
Włosowicz	Agata		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	agata.wlosowicz@io.gliwice.pl
Zając	Gracjana		*Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch*	*Gliwice*	gracjana.zajac@io.gliwice.pl
Ziółkowski	Piotr	L	*Wrocław Medical University*	*Wrocław*	ziołkows@interia.pl

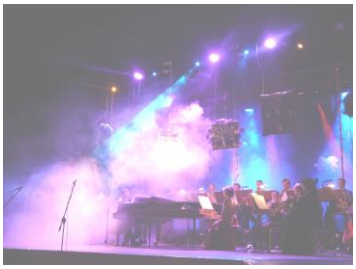
[1]C-chairperson; L-lecture; P-poster

ORKIESTRA
im. Arcyksiężnej
Marii Krystyny Habsburg

Archduchess Maria Christina Habsburg Memorial Orchestra has been performing on stage since 2010. Its musicians have been giving concerts throughout Poland, more than two hundred so far.

The founder and artistic director of the Orchestra is Sylwester Targosz-Szalonek, who has brought together many talented musicians from all over southern Poland. Orchestra musicians are accompanied by several excellent soloists, all forming together a pleasantly-sounding harmonious company. Family atmosphere during rehearsals makes playing together a real pleasure for these musicians, so audiences can later be truly delighted during stage concerts while experiencing the sheer joy of music.

In their repertoire the Orchestra musicians feature symphonic works, operas, operettas and musicals. Since its beginnings, the Orchestra released two own DVDs.


Currently, the Orchestra musicians are working on "Pavarotti and Friends"program.


Sylwester Targosz-Szalonek a tenor, actor, instrumentalist, conductor and television personality, is the distinguished graduate of Karol Szymanowski Academy of Music in Katowice (Maestra Henryka Januszewska-Stańczyk class). He has completed numerous vocal master courses, e.g. those led by Professor Helena Lazarski (Austria), Daniel Weeks (USA), Kalmyk Kalutowa (Bulgaria) or Paul Esswooda (United Kingdom). He graduated from the vocal master class of Professor Cesare Colona in Berlin. His artistic activity encompasses wide-range repertoire and stylistics (chamber recitals, performances and operas, operettas, oratorios and cantata concerts).

Studenckie Koło Naukowe Biotechnologów

przy Centrum Biotechnologii Politechniki Śląskiej

KIM JESTEŚMY?

Jesteśmy międzywydziałowym kołem naukowym – SKNB zrzesza wspaniałych studentów z wydziału Inżynierii Środowiska i Energetyki, wydziału Automatyki, Elektroniki i Informatyki oraz wydziału Chemicznego, których łączy wspólna pasja - **BIOTECHNOLOGIA.**

CZYM SIĘ ZAJMUJEMY?

Nasze Koło posiada szeroką ofertę dla osób żądnych przygód i nowych doświadczeń. U nas masz możliwość uczestniczyć w: projektach naukowych, konferencjach naukowych, spotkaniach integracyjnych, eventach o charakterze promocyjnym, redagowaniu kwartalnika SKNB „BioLetyn". Organizujemy: Śląskie Dni Biotechnologii BAKCYL, pokazy i warsztaty, Funlab, BioBus.

Chcesz do nas dołączyć? Interesuje Cię nasza działalność?

MASZ PYTANIE? NAPISZ DO NAS!

sknbiotechnologow@gmail.com

BioLetyn

Kwartalnik Studenckiego Koła Naukowego Biotechnologów

ISSN 2392-2982

BioLetyn

KWARTALNIK STUDENCKIEGO KOŁA NAUKOWEGO BIOTECHNOLOGÓW PRZY CENTRUM BIOTECHNOLOGII POLITECHNIKI ŚLĄSKIEJ

Kwartalnik BioLetyn tworzony jest przez studentów Politechniki Śląskiej, dedykowany wszystkim osobom zainteresowanym tematyką biotechnologiczną. Zachęcamy do dołączenia do zespołu redakcyjnego, gdzie można nabyć nowych umiejętności, między innymi pracy w grupie, organizacji czasu, ustalania priorytetów oraz rozwinąć swoje zdolności językowe przy pisaniu artykułów.

bioletyn@gmail.com

Gliwickie Spotkania Naukowe 2017 - zadanie finansowane w ramach umowy 634/P-DUN/2017
ze środków Ministerstwa Nauki i Szkonictwa Wyższego przeznaczonych na działalność upowszechniającą naukę

Silesia.
Positive energy

Konferencja "Gliwickie Spotkania Naukowe 2017" jest współorganizowana przez Województwo Śląskie

Projekt dofinansowany z budżetu Miasta Gliwice