International Young Scientists Conference
on Molecular and Cell Biology

February 25th-26th, 2021, Warsaw

ABSTRACT BOOK

Content:

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Andrzejowska, Aleksandra: Gaseous phase hydration and sorption isotherm of the Antarctic lichenized fungus *Umbilicaria antarctica* Frey & I.M. Lamb thallus

Aleksandra Andrzejowska, Paulina Kijak[1], Angelica Casanova-Katny[2], Kazimierz Strzałka[5,6] Maria Olech[3,4] and Hubert Harańczyk[1]

1. M. Smoluchowski Institute of Physics, Jagiellonian University, Cracow, Poland,
2. Faculty of Natural Resources, Catholic University of Temuco, Chile,
3. Institute of Botany, Jagiellonian University, Cracow, Poland,
4. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland,
5. Malopolska Centre of Biotechnology, Jagiellonian University, Cracow, Poland,
6. Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Cracow, Poland

Contact for the abstract: Aleksandra Andrzejowska; aleksandra.pacura@student.uj.edu.pl

Antarctic lichenized fungi may survive extremely low temperatures and desiccation. This research is focused on molecular mechanisms of these abilities. The Antarctic lichen *Umbilicaria Antarctica* collected from the sites on rocks of Isla Robert, Southern Shetlands, maritime Antarctics, on July 7th, 2018, at Chilean 54. ECA (54 Expedition Cientifica Antartica). The rate and the sequence of saturation of three bound water fractions was tested. Gaseous phase hydration and dehydration courses and sorption isotherm of *U. antarctica* thalli were measured. The hydration courses revealed bound water fractions: (i) a very tightly bound water A_0^h = 0,01(1) still present after dehydration over the silica gel, (ii) a tightly bound water fraction A_1^h = 0,08(1) with the hydration time t_1^h = 2,4(3) h, and (iii) a loosely bound water fraction with the hydration time t_2^h = 27(3) h, and for higher relative humidities p/p_0 > 52% a loosely bound water fraction. For $p/p_0 \geq$ 88% the total level of bound water significantly increases up to *ca*. 0,6 which may be interpreted as a recovery of life activity in *U. antarctica*. The dehydration kinetics is well described by a single-exponential function with the dehydration time t^d = 10(1) h. The sorption isotherm showed the multilayer sorption fitted well by a sigmoidal function. We fitted two models of the sorption process, namely a classic Brunauer-Emmett-Teller (BET) model, and a newer Dent model (Guggenheim-Anderson-de Boer = GAB). The GAB fits yield the value of primary binding sites contribution equal to $\Delta M/m_0$ = 0,05(1), as expressed in units of dry mass, m_0. The fraction of unoccupied binding sites at p/p_0 = 100% equals $1/b_1$ = 0,01%, which may suggest elevated hydrophilicity level of the surface of *U. antarctica* thallus.

Keywords: Antarctica, extremophiles, lichens, lichenized fungi, *Umbilicaria,* dehydration, hydration, sorption isotherm, hydration kinetics.

This research was performed thanks to INACH (Instituto Antártico Chileno, Ministerio de Relaciones Exteriores, Chile): Sistema de Proyectos INACH, RT_27_16.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Banaś, Anna Marta: Two *Campylobacter jejuni* oxidoreductases CjDsbA1 and CjDsbA2 interact with CjDsbB and display high oxidase activity *in vitro*

Anna Marta Banaś[1], Katarzyna Bocian-Ostrzycka[1], Maciej Plichta[2], Elżbieta Katarzyna Jagusztyn-Krynicka[1]

1. Department of Bacterial Genetics, Institute of Microbiology, University of Warsaw, Warsaw, Poland
2. Department of Biopysics, Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland;

Contact for the abstract: Anna Banaś anna.banas@biol.uw.edu.pl

Dsb protein family is involved in disulfide bond generation in bacteria. The well studied *Escherichia coli* Dsb system acts in two pathways – oxidation (EcDsbA responsible for disulfide generation, EcDsbB – DsbA's redox partner) and izomerization/reduction (EcDsbC rearranges incorrect disulfide bonds, EcDsbD - DsbC's redox partner). Homologues of EcDsbA in *Campylobacter jejuni* are CjDsbA1 and CjDsbA2. Phylogenetic analyses revealed that both proteins belong to small cluster named DsbA1-2, that is closely related to the DsbA cluster harboring classical DsbA proteins. Structural modeling showed that CjDsbA1/CjDsbA2 are more closely related to EcDsbL (untypical DsbA protein) than to EcDsbA. The aim of presented work was to characterize the biochemical properties of CjDsbA1/CjDsbA2 and to verify their interactions with CjDsbB (homologue of EcDsbB). *cjdsbA2, cjdsbB, astA* (encodes DsbA2 substrate) genes are organized in an operon, *cjdsbA1* is a separate transcriptional unit. Performed analysis (evaluation of the redox potentials and ability to oxidize reduced RNaseA demonstrated that both proteins are strong oxidazes and similarly to EcDsbL, are inefficient in insulin reduction assay (in contrast to EcDsbA)). As *C. jejuni* encodes only one DsbB protein we evaluated whether it interacts with both CjDsbA1 and CjDsbA2. MST technique enabled us to verify that both proteins interact with CjDsbB (with different strength). It is for the first time when we observed that DsbB encoded in the same operon as DsbL/DsbA2 is also a redox partner for the DsbA located outside of this operon. Our results demonstrated that biochemical properties of CjDsbA1/CjDsbA2 resemble much more those presented by EcDsbL than those of EcDsbA. The presented data are in accordance with phylogenetic analyses which suggested that *C. jejuni* DsbA1-2/DsbB/AstA system was horizontally transfer to a common ancestor of the gamma-Proteobacteria and that the divergence of *Cjdsba1 /CjdsbA2* occurred after the horizontal transfer.

Keywords: thiol oxidoreductases, disulfide bonds, protein interactions, *Campylobacter jejuni.*

The National Science Centre supported this work (grant no.2015/17/B/NZ1/00230)

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Baran, Brygida: Identification of kinases that phosphorylate Gli proteins

Brygida Baran[1], Sylwia Niedziółka[1], Paweł Niewiadomski[1]

1. Centre of New Technologies University of Warsaw

Contact for the abstract: Brygida Baran; b.baran@cent.uw.edu.pl

Posttranslational modifications (PTMs) are key regulatory events for the majority of signaling pathways. In mammalian cells phosphorylation is the most common regulatory PTM and is involved in virtually all major signaling cascades. Transcription factors are often phosphorylated on multiple residues, which regulates their trafficking, stability, or transcriptional activity, depending on the kinase and the site involved. It has been shown that, in addition to the PKA-mediated inhibitory phosphorylation, Gli molecules undergo another phosphorylation event, which is only evident in activated nuclear Gli proteins. Gli proteins are transcriptional effectors of the Hedgehog signaling pathway. In mammals, they are represented by three proteins: Gli1, Gli2 and Gli3. Gli1 acts principally as a transcriptional activator, whereas Gli2 and Gli3 display both activator and repressor functions. They play key roles in the development of many organs and tissues, and are deregulated in birth defects and cancer. It has been proposed that a separate phosphorylation event, independent of PKA, might be required for Gli nuclear translocation, but the site(s) nor the kinase have not been identified. Our research fill this critical gap in our understanding of the regulation of Gli proteins by taking an survey of Gli posttranslational modifications that are triggered by activation. We identified two novel kinases interacting with Gli3 protein. Our research focused on the examination of physical interactions between these kinases and all Gli variants. We also investigated how loss of function of these kinases affect Gli PTMs and thus their activity. This study aims to provide a better understanding of the mechanism of the HH pathway and may lead to the finding of new therapeutic solutions for diseases related to the activity of Gli proteins.

Keywords: Hedgehog, cancer, Gli, kinases, cell signaling.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Bilska, Aleksandra: Noncanonical poly(A) polymerase TENT5C regulates B cell humoral response and differentiation

Aleksandra Bilska[1], Monika Kusio-Kobiałka[1], Paweł S. Krawczyk[2], Olga Gewartowska[2], Dominika Nowis[3,4], Jakub Golab[5,6], Andrzej Dziembowski[2,1], Seweryn Mroczek[1]

1. Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Pawinskiego 5a, 02-106 Warsaw, Poland
2. Laboratory of RNA Biology, International Institute of Molecular and Cell Biology, Trojdena 4, 02-109 Warsaw, Poland
3. Genomic Medicine, Medical University of Warsaw, Banacha 1a, 02-097 Warsaw, Poland
4. Laboratory of Experimental Medicine, Center of New Technologies, University of Warsaw, Banacha 2c, 02-097 Warsaw, Poland
5. Department of Immunology, Medical University of Warsaw, Banacha 1a, 02-097, Warsaw, Poland
6. Centre of Preclinical Research, Medical University of Warsaw, Banacha 1a, 02-097 Warsaw, Poland

Contact for the abstract: Aleksandra Bilska, abilska@biol.uw.edu.pl

Polyadenylation is an important mRNA processing step affecting it's stability and translational efficiency. Except nuclear polyadenylation, poly(A) tails can be extended in a cytoplasm by noncanonical poly(A) polymerases (ncPAPs). TENT5C is a ncPAP, which acts as oncosupressor in multiple myeloma, a cancer of terminally differentiated B cells, peculiarly modifying mRNAs encoding ER-targeted proteins. We have carried out a global analysis of poly(A) tail length distribution in activated B cells from WT and *Tent5C* KO mice using Nanopore direct RNA sequencing approach. This analysis revealed mRNAs encoding immunoglobulins as primary TENT5C substrates, as we observed specific shortening of their poly(A) tails in *Tent5C* KO cells with no change in global polyadenylation status. This results in reduced mRNAs half-lives and consequently decreased steady-state level of these transcripts in *Tent5C* KO B cells. Therefore, Tent5C-depleted B cells produce and secrete less immunoglobulins *in vitro* and, in agreement with this, mice lacking TENT5C have diminished concentration of immunoglobulins in blood serum and show impaired humoral response after immunization with T-independent antigen. Additionally, *Tent5C* KO B cells are characterized by accelerated proliferation rate and faster differentiation to $CD138^{high}$ plasma cells. Despite this, a lack of TENT5C results in a decreased ER compartment volume, reduced dynamics of its expansion during B cell activation, and downregulation of unfolded protein response. To conclude, we showed that TENT5C plays a vital role in B cells physiology as the first ncPAP engaged in polyadenylation of immunoglobulin mRNAs and regulation of humoral immunity.

Keywords: TENT5C, noncanonical polyadenylation, B cells, immunoglobulins.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Biruk, D.V.: The analysis of the processes of hippocampus's endogenous stem cells migration when being electrically stimulated

D. V. Biruk[1], M. O. Dosina[2], S. G. Pashkevich[3]

1. The Belorussian State University, The Physics Faculty, The Department of Biophysics
2. The State Scientific Institution 'The Institute of Physiology, NAS of Belarus'
3. The State Scientific Institution 'The Institute of Physiology, NAS of Belarus'

Contact for the abstract: Dan Biruk; dan.biryuk@yandex.by

Interneuron communications effectiveness in the model of a surviving rats' hippocampal slice is a three-dimensional and natural standard for functional interaction of neural networks in long-term cultivation. The effects of electrical stimulation depend on the stimulating and recording electrodes' localization, as well as the compositional features of the perfused artificial cerebrospinal fluid, ambient temperature, oxygenation, and the functional state of a particular hippocampal slice. The migration processes of endogenous stem cells occur in the adult animals' brain. It was suggested: factors contributing to signal transmission in the nervous tissue include the natural endogenous stem cells' movement located in the dentate fascia-section of the hippocampus. **The aim was** to study the regulated activation of endogenous stem cell migration processes during the short- and long-term electrical stimulation in the rat hippocampal slices. The hippocampal slices (n=5, N=27) 400-microns thick were perfused with artificial cerebrospinal fluid under conditions of saturation of 95%-O2 and 5%-CO2 for 40min, at a temperature of 28.9°C. Electrical stimulation and registration of evoked responses was carried out in the hippocampus's CA1-region using tungsten electrodes. Single stimuli were applied with a duration of 200μs and an interval between stimuli of 20s, or 100Hz for 1s – for modeling long-term potentiation. When obtained for microscopic examination of the CA1-region of the hippocampus (after 30min), the slices 8-microns thick were incubated with a fluorescent dye with FITC-labeled anti-CD90 antibodies ('+'-marker on stem cells), examined using a fluorescence microscope (Zeiss AxioVert-200M) at the $\lambda_{excitation}$=490nm and $\lambda_{emission}$=502nm. The fluorescent cells' number in the hippocampus's CA1-region (1mm^2): 1) intact sections (n=5, N=5) – not visualized; 2) placement of electrodes (n=4, N=7) – (3.8±1.3) cells; 3) single stimulation (n=4, N=7) – (11.3±2.6) cells; 4) long-term potentiation (n=5, N=8) – (36.3±8.6) cells. Establishing the endogenous stem cell migration processes' rate is necessary to justify ways to increase the neurogenesis's effectiveness.

Keywords: hippocampus, stem cells, rats, hippocampal slices, the dentate fascia-section of the hippocampus, the hippocampus's CA1-region, evoked potentials.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Bogusz, Karolina: Rapamycin-induced p150^{glued} and β2-adaptin interaction requires dynactin integrity and low CLIP-170 level

Karolina Ryczek[1], Aleksandra Tempes[1], Anna Malik[1,2], Agnieszka Brzozowska[1], Jacek Jaworski[1]

1. International Institute of Molecular and Cell Biology, Warsaw, Poland
2. Nencki Institute of Experimental Biology, Warsaw, Poland

Contact for the abstract: Karolina Ryczek; kryczek@iimcb.gov.pl

The intracellular transport along microtubules (MTs) is essential for proper growth and cell development. It requires molecular motors (dynein-dynactin or kinesins), adaptors and several additional proteins like MT plus-end tracking proteins, including cytoplasmic linker protein 170 (CLIP-170). The processivity of dynein, which transports cargo towards minus ends of MT, is ensured by dynactin. Dynactin consists of 23 subunits, where p150^{glued} protein has a vital role in interaction with adaptors and MTs. On the other hand, adaptors are required for specific recognition of transported cargo, for instance, the recently described adaptor - AP2 complex, known for its role in clathrin-mediated endocytosis, enables autophagosome transportation in neurons [Kononenko et al., 2017]. Preliminary data obtained by our group indicated the modulatory effect of mammalian Target of Rapamycin (mTOR) on the interaction between p150^{glued} and β2-adaptin (AP2 subunit), but the exact mechanism remains unknown. Therefore, the aim of this work was to determine the role of dynein-dynactin integrity and CLIP-170 on an increase of p150^{glued}-AP2 interaction induced by rapamycin. To study p150^{glued}-β2-adaptin interaction, the experiments were conducted with the use of Proximity Ligation Assay (PLA) on RAT-2 cell lines with genetic modifications affecting dynein-dynactin integrity and CLIP-170 levels. PLA results confirmed that inhibition of mTOR with rapamycin increases p150^{glued}-β2-adaptin interaction, which was prevented by overexpression of the p50 subunit, used to disassembly of dynactin complex. On the other hand, the shRNA-driven silencing of CLIP-170, a known p150^{glued}-interacting protein, and confirmed mTOR target caused an increase in p150^{glued}-β2-adaptin interaction comparable with rapamycin treatment. The transfection of RAT-2 cells with control scrambled RNA had no such effect. In conclusion, our results showed that mTOR influences p150^{glued}-β2-adaptin interaction and the entire dynactin complex is needed for that. Moreover, the presence of CLIP-170 seems to adversely regulate this interaction suggesting a possible mechanism of mTOR contribution.

Keywords: dynein-dynactin, p150^{glued}-AP2 interaction, CLIP-170, mTOR.

This work has been supported by Polish National Science Centre OPUS grant 2016/21/B/NZ3/03639

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Čėsna, Vytautas: *Pinus sylvestris* secondary compounds impact on mass outbreaks of *Lymantria monacha*

Vytautas Čėsna[1], Ieva Lučinskaitė[1], Diana Marčiulynienė[1], Adas Marčiulynas[1], Vaida Sirgedaitė-Šėžienė[1]

1. Institute of Forestry, Lithuanian Research Center for Agriculture and Forestry, Liepų str. 1, LT-53101 Girionys, Kaunas District, Lithuania

Contact for the abstract: Vytautas Čėsna v.cesna1994@gmail.com

Insect mass outbreaks are the main factors that cause forest diseases, deaths and change ecosystem structure in all over the world. The purpose of the research is to evaluate activity and coherence of *Pinus sylvestris* L. accumulated secondary compounds (phenols, flavonoids) total content and its antioxidant activity from needles with *Lymantria monacha* L. outbreaks in temperate zone. The insect's grubs gnaw Scots pine needles and cause pines defoliation. For protection against the insect foresters use biological preparation "Foray 76B" made of bacteria *Bacillus thuringiensis*. The *Lymantria monacha* insect mass outbreaks were fixed in the summer of 2019 in Kuršių Nerija, Lithuania. The needles from Scots pines were collected 3 days after spraying (when the outbreaks were widely spread) and 1 year after spraying (when the outbreaks were controlled). It was analyzed Scots pines needles secondary compounds total content (phenols (TPC) and flavonoids (TFC)) and antioxidant activity dependence on the Scots pines and the data was separated into 3 parts: 1) Control – Scots pines had damaged by the mass outbreaks and spraying was not used, 2) Not Sprayed – Scots pines had not damaged by the mass outbreaks and spraying was not used, 3) Sprayed – Scots pines had damaged by the mass outbreaks and spraying was used. The results showed that TPC, TFC and antioxidant activity have the same tendency – when the outbreaks start, Scots pines increase their TPC, TFC and antioxidant activity, moreover, the biggest contents of TPC (4.00±0.25 mg/g^{-1}) and TFC (9.24±0.48 mg/g^{-1}), also antioxidant activity (640.25±34.04 µmol/g) were established in Scots pines which were not affected by the *Lymantria monacha* outbreaks. The key point of the research showed that Scots pine TPC, TFC and antioxidant activity have a huge value for the trees internal protection against pests.

Keywords: *Pinus sylvestris*, microorganisms, black arches, phenols, flavonoids, antioxidant activity.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Chalkley, Alannah: Molecular dissection of the mechanisms of nanoparticle penetrance into 3D cancer cell models

Alannah Chalkley[1,2], Jeremy C. Simpson[1,2]

1. School of Biology and Environmental Science, University College Dublin (UCD), Dublin, Ireland.
2. Conway Institute of Biomolecular and Biomedical Research, UCD, Dublin, Ireland.

Contact for the abstract: Alannah Chalkley; alannah.chalkley@ucdconnect.ie

One of the biggest problems facing mankind today is the cost and complexity of developing new drugs to treat various of human diseases. The interdisciplinary field of nanomedicine is fast becoming essential for specific and targeted drug delivery, offering new solutions for the development of more efficacious therapeutics. Nanoparticles (NPs) can be engineered to contain information to target a drug to a specific site in the body. However, there is little mechanistic knowledge about how NPs interact with cells, the subcellular pathways they take, if they can pass from cell-to-cell, or the genes that are important for these events. In this study we are applying the use of 3D spheroids, constructed from human cells, as a model to study NP membrane trafficking pathways in and between cells in the context of drug delivery. The chosen spheroids are analysed using high-content screening microscopy, which enables high-resolution quantitative imaging of trafficking events in individual cells within each spheroid. The spheroids are constructed from cell lines that represent various tissues, including the gut and lung, enabling direct comparisons of NP penetrance and trafficking pathways between different tissue models. Overall, this project aims to provide the first molecular understanding of the mechanisms by which synthetic NPs, as potential drug delivery vehicles, penetrate into solid tumours, using in vitro grown cellular spheroids as a model system.

Keywords: Nanoparticles, Spheroids, Membrane Trafficking, Drug Delivery.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Chumachenka, Maria: GC-MS-based plasma lipidomics for the discovery of potential biomarkers from a rat model for chronic and acute allergic contact dermatitis

Chumachenka Maryia[1], Korik Elena[1], Alekseev Nikolay[1], Semak Igor [1]

1. Belarusian State University, Faculty of Biology, Department of Biochemistry

Contact for the abstract: Maryia Chumachenka chumachenkomaria19@gmail.com

Lipidomics is a lipid-targeted metabolomics approach aiming at comprehensive analysis of lipids in biological systems. Recently, lipid profiling has captured increased attention due to the well-recognized roles of lipids in numerous diseases. Contact allergies are complex diseases, and one of the important challenges for public health and immunology. An estimated 15–20% of the general population suffers from contact allergy. The purpose of this study was to determine the differential lipidomic profiles of blood plasma fatty acids in animals with experimental allergic contact dermatitis. Allergic contact dermatitis (ACD) was induced in rat by repeated application of 2,4 - dinitrochlorobenzene. The animals were divided into 3 experimental groups (n=7): control group, group with acute phase ACD, group with chronic phase ACD. The separation and quantitation of fatty acids methyl esters achieved by gas chromatography mass spectrometry (GC-MS) technique. The chromatographic procedure provides separation between saturated and unsaturated fatty acids of different chain lengths (C4-C22) as well as between most positional isomers. Fatty acids are extracted in the presence of internal standards for high quantitation accuracy. Mass spectrometer conditions are optimized for broad detection capacity and sensitivity capable of measuring trace amounts of fatty acids in complex biological samples. To identify the most informative compounds, discriminant analysis method was used in Statistica 12.0. Discriminant analysis revealed a clear separation between all experimental groups. The main contribution to the discrimination of the group with acute phase of ACD was made by linoleic, arachidic, cis-11-eicosenoic, cis-8,11,14-eicosatrienoic, nervonic acids. The change in the levels of α-linolenic, cis-11,14-eicosadienoic, arachidonic, docosahexaenoic was the most significant in group with chronic phase of ACD.

Keywords: Lipidomics, allergic contact dermatitis, fatty acid.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Ciesielski, Oskar: Pharmacological inhibition of peptidylarginine deiminases driving histone citrullination and its impact on endothelial cells proliferation

Oskar Ciesielski[1, 2], Aneta Balcerczyk[1]

1. University of Lodz, Department of Molecular Biophysics, Pomorska 141/143, 90-236 Lodz, Poland
2. University of Lodz, the Bio-Med-Chem Doctoral School of the University of Lodz and Lodz Institutes of the Polish Academy of Sciences

Contact for the abstract: Oskar Ciesielski oskar.ciesielski2@unilodz.eu

Citrullination is an enzymatic posttranslational modification that has been found to play important role in multiple disorders, mainly in autoimmune diseases, however, its role in the regulation of metabolism is still poorly identified. Protein citrullination is catalyzed by a family of enzymes – peptidylarginine deiminases (PADs), with dominant role of two isoforms: PAD2 and PAD4 described as capable of modifing histone tail arginine to citrulline. The purpose of this study was to select the most efficient commercially available citrullination inhibitor and to investigate their effects on selected metabolic parameters of endothelial cells (Ecs) such as viability, cell cycle progression and histone H3 citrullination (H3cit) status. Two human ECs models were used: primary vein (HUVEC) and microvascular endothelial cells (HMEC-1). Three irreversible inhibitors were used: BB-Cl-amidine, Cl-amidine and F-amidine. Cellular viability was measured *via* resazurin reduction assay. The effects on proliferation and cell cycle progression were analysed by the means of flow cytometry and qPCR. Inhibitor concentrations below IC_{50} were then used to examine the effects on histone H3 citrullination status by Western-blotting. Out of the three inhibitors tested, surprisingly only BB-Cl-amidine exerted a cytotoxic effect on ECs (IC_{50}.= 1.75 ± 0.05). The selected concentrations of all inhibitors were respectively: BB-CL-amidine: 0.25 µM; 0.5 µM; 1 µM, Cl-amidine: 2 µM; 5 µM; 10 µM and F-amidine: 2 µM; 5 µM and 10 µM, but none of them had significant effect on cell cycle progression. However, the inhibitors at the concentrations indicated above, decreased the H3cit (BB-Cl-amidine proved to be the most efficient, followed closely by Cl-amidine). No significant differences were observed between the primary and immortalized ECs models. The performed experiments revealed that BB-Cl-amidine is the most efficient histone citrullination inhibitor in endothelial cells, and that pharmacological inhibition of PADs has no impact on cell cycle progression, however, impact their proliferation.

Keywords: endothelium, histone citrullination, peptidylarginine deiminases.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Cios, Aleksandra: Influence of extremely low frequency EMF on ccRCC

Aleksandra Cios[1], Martyna Ciepielak[1], Łukasz Szymański[1], Wanda Stankiewicz[1]

1. Department of Microwave Safety, Military Institute of Hygiene and Epidemiology, Kozielska 4, 01-163 Warsaw

Contact for the abstract: Cios Aleksandra aleksandracios@gmail.com

Electromagnetic field (EMF) is generated by natural and artificial sources of radiation, differing in wavelength, frequency or photon energy. The electromagnetic spectrum extends from static electric and magnetic fields to electric and magnetic fields of low frequency, indirect and radio electromagnetic fields, microwaves, optical radiation and ionizing radiation. Extremely low frequency electromagnetic field (ELF-EMF) is a field with a frequency of 0-300 Hz generated mainly by power lines. The relationship between EMF and its impact on the human body has become one of the main topics of research on health problems, especially cancer, caused by the electromagnetic field. It has been shown that exposure to ELF-EMF can affect many cellular activities, including gene expression. So far, no studies have been conducted to investigate the effect of EMF exposure on clear cell renal cell carcinoma cell lines. We studied the effects of ELF-EMFs (50 Hz, 4,5 mT) on 4 cell lines: HEK293, 786-O, 769-P and Caki1 by monitoring cellular proliferation and necrosis, cell morphology, their migration and invasion capacity as well as cell cycle and ROS production. Based on the results we showed that ELF-EMF decreases viability and proliferation of tumor cell lines, inhibits their migratory, invasive and metastatic capacity, causes G1 cancer cell arrest and higher production of early apoptotic cells and reactive oxygen species. This preliminary study revealed that ELF-EMF might serve as a potential tool for manipulating viability of renal cancer cells.

Keywords: electromagnetic field, cancer cells, clear cell renal cell carcinoma, viability, cell cycle.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Czarnota, Anna: Characterization of anti-hepatitis C neutralizing antibody response after immunization with chimeric hepatitis B/hepatitis C virus like particles

Anna Czarnota[1], Patryk Zalewski[1], Krystyna Bieńkowska-Szewczyk[1], Katarzyna Grzyb[1]

1. Laboratory of Virus Molecular Biology, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and Medical University of Gdańsk

Contact for the abstract: Anna Czarnota; anna.czarnota@phdstud.ug.edu.pl

Hepatitis C virus (HCV) infection is a major health problem worldwide, affecting an estimated 70 million people worldwide. An HCV vaccine, however, still remains an elusive goal. In contrast, hepatitis B virus (HBV) infection can be prevented with commercially available recombinant vaccine based on small surface antigen of hepatitis B (sHBsAg), which has the ability to form highly immunogenic virus-like particles (VLPs) and represents an attractive antigen carrier for the delivery of foreign sequences. In our studies, we examined the immunogenic properties of a bivalent HBV/HCV vaccine candidates based on the novel chimeric particles in which highly conserved epitopes of HCV E2 glycoprotein were inserted into the hydrophilic loop of sHBsAg. The expression of chimeric particles was performed in the *Leishmania tarentolae* expression system, which has the potential to produce high-yields of proteins with the mammalian-like N-glycosylation pattern. Chimeric proteins were next purified using ultracentrifugation and the particles assembly was confirmed using direct transmission electron microscopy. After immunization of mice we confirmed that the mouse sera were able to recognize not only the synthetic peptides covering HCV E2 epitopes, but also yeast-derived sHBsAg. Moreover, we assessed the cross-reactivity and neutralizing antibody response against different HCV genotypes. In our study we proved that sHBsAg-based VLPs are able to successfully present several HCV-derived regions and to elicit strong and specific anti-HCV antibody response. Although more evaluation of those constructs is still needed, this approach may prove useful in the development of a rationally designed prophylactic vaccine against HCV.

Keywords: Hepatitis C virus, virus-like particles, sHBsAg, vaccines.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Daszczuk, Patrycja: Identification of cis-regulatory elements of Smad transcription factors and their role in cyclic regulation of hair follicle Stem Cells

Patrycja Daszczuk[1], Łukasz M. Boryń[1], Xiaoyang Wang[2], Agnieszka Kikulska[1], Paula Mazurek[1], Guangfang Wang[2], Eve Kandyba[3], Maksim Plikus[2], Krzysztof Kobielak[1]

1. University of Warsaw, Centre of New Technologies, Warsaw, Poland,
2. University of California Irvine, Stem Cell Research Center, Irvine, CA, USA,
3. University of California, San Francisco

Contact for the abstract: Krzysztof Kobielak, k.kobielak@cent.uw.edu.pl

Hair follicle (HF) is a dynamic miniorgan which undergoes cyclic phases of growth, degradation and quiescence. In every HF, in a place referred as a bulge, resides small population of hair follicle Stem Cells (hfSCs) capable of epidermis regeneration, sebaceous glands and whole hair follicle. Recently, we discovered molecular mechanism of the canonical Bone Morphogenetic Protein (BMP) signaling governs the homeostasis of hfSCs. Smad1, 5 and 9 are very similar to each other signal transducers and transcriptional modulators activated by phosphorylation by BMP type 1 receptor kinase type IA (Bmpr1a) in HF. However recently we discovered non-overlapping role of Smad 1/5/9 in the maintaining and regulation of hfSCs emphasized the distinct function of Smad proteins at the morphogenesis stage and in postnatal homeostasis. Smad 1/5 play crucial role in differentiation of hair shaft during morphogenesis while Smad9 is responsible for epidermis development. In adults, Smad 1/5 maintain hfSCs in quiescence when Smad9 plays role in hair differentiation. Seeing that phosphorylated Smad (pSmad) proteins play alternative role in hfSCs regulation, we focused further on identifying the cis-regulatory elements and the target genes of Smads in hfSCs isolated from the skin. Using our inducible constitutively active Bmpr1a transgenic murine system and conditional knockouts, we were able to perform ChIP-seq analysis of phosphorylated form of Smad proteins. Comparison between differentially expressed genes in hfSCs after inhibition of BMP signaling to whole genome pSmads interactome received from our Chip-seq predicts the direct connection between the pSmad binding sites in regulatory regions and the change of specific gene expression. This gives significant insight into the transcriptional regulation and maintenance of hfSCs quiescence.

Keywords: hair follicle Stem Cells, Smad, homeostasis, cis-regulatory elements.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Fluks, Monika: Optical coherence microscopy as a label-free tool for imaging subcellular structure and assessing quality of mammalian oocytes and embryos

Monika Fluks[1], Aleksandra Sobkowiak[1], Szymon Tamborski[2], Maciej Szkulmowski[2], Anna Ajduk[1]

1. Reproductive Biology Group, Department of Embryology, Faculty of Biology, University of Warsaw, Poland.
2. Optical Biomedical Imaging Group, Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University in Torun, Poland.

Contact for the abstract: Monika Fluks; m.fluks@student.uw.edu.pl

Confocal laser microscopy and other fluorescence imaging methods applied in biology allow for detailed structural and dynamic studies of a single cell but require fluorescent markers to visualize cellular architecture and may cause short- and long-term photo-damage. Traditional light microscopy, although relatively non-invasive, does not provide detailed structural information. Optical coherence microscopy (OCM) is a promising alternative, as it does not require sample pre-processing or labelling and is capable of providing three-dimensional (3D) images of intracellular structures and their development in time in a non-invasive way. Here we show that images obtained by OCM provide information useful for oocyte and embryo quality assessment. We showed that OCM allows for assessment of chromatin conformation in immature (germinal vesicle, GV) oocytes (surrounded by cumulus cells or denuded) and for the selection of oocytes with so-called SN (surrounded nucleoli) chromatin conformation that have higher developmental potential. We also showed that OCM provides data that allows for a precise nuclei (i.e. cell) count in compacted mouse embryos and that the number of nuclei (cells) at the compacted morula stage correlates with the embryo's ability to form high-quality blastocysts. Neither chromatin conformation in GV oocytes, nor nuclei number in morulas can be assessed non-invasively by traditional light microscopy. The OCM scanning protocols were verified to be safe for oocytes and preimplantation embryos, as evidenced by indisturbed maturation process and similar Ca^{2+} response to a fertilizing spermatozoon in scanned and control oocytes, as well as comparable development of scanned and non-scanned morulas to the blastocyst stage. Our results suggest that OCM may be a valuable addition to imaging toolkit used in assisted reproduction procedures.

Keywords: assisted reproductive technologies, optical microscopy, oocytes, embryos.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Gargas, Justyna: Primary glial cultures as tools for investigating *in vitro* cell interactions after neonatal hypoxic-ischemic insult

Justyna Gargas, Justyna Janowska, Karolina Ziabska, Malgorzata Ziemka-Nalecz, Joanna Sypecka

NeuroRepair Department, Mossakowski Medical Research Institute Polish Academy of Sciences

Contact for the abstract: Justyna Gargas; jgargas@imdik.pan.pl

Mutual interactions between cells forming the nervous tissue might contribute to development of neurological disorders. *In vitro* culture and co-culture systems are particularly useful tools to model selected neurological disorders and to study various biological mechanisms triggered by exo- and endogenous signals. However, to eliminate the influence of external stimuli which might potentially modulate the investigated processes, cells should be cultured in restricted conditions, yet maximally resembling a physiological tissue microenvironment. Taking the above into consideration, we established purified glial (oligodendroglial, astroglial and microglial) monocultures to study and to evaluate previously selected biological processes, like cell survival, proliferation, polarization and differentiation. The glial cultures were established from the brain hemispheres of neonatal Wistar rats. After being mechanically homogenized, the primary mixed glial population was cultured for 12 days and then a monofraction of each glial type was either mechanically or enzymatically separated. To mimic *in vitro* the neonatal hypoxic-ischemic (HI) insult, the cultures were deprived of oxygen-glucose deprivation for 50 minutes. After that, the cells were cultured for 24h or 72h in a serum-free medium and physiologically normoxic conditions (5% O_2). Subsequently, the cells were subjected to detailed examination to evaluate the response of the neonatal glial cells to the applied pathological conditions (hypoxia and limitation of trophic support). Analysis of cell survival and proliferation, as well as their morphology revealed that HI exerts diversified effects in regard to glial cell type which should be taken into account during evaluation of mutual cell interations in pathogenesis of fatal consequences of HI episode. Applied model and the obtained results allowed us to model *in vitro* neonatal HI insult and to design subsequent projects for potential drug testing with aim of searching for effective treatment options to prevent the development of neurological disorders.

Keywords: glial cells, hypoxic-ischemic episode, perinatal asphyxia.

Supported by National Science Centre (Poland) 2014/15/B/NZ4/01875 and ESF, POWR.03.02.00-00-I028/17-00.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Gorchakova, Olga: MUC 1 / MUC 13 in the development of hereditary colon tumors

Harchakova Volha[1], Kuznetsov Oleg[2], Valko Nikita[3]

Contact for the abstract: Harchakova Volha, daniil_go@inbox.ru

MUC-1 / MUC-13 - glycoproteins, tumor-associated antigens that modulate the metabolism of a tumor cell, thereby supporting its growth and development. In our study, we assessed the significance of MUC1 and MUC13 as biological markers for the development of the tumor process of the large intestine and studied the aspects of their possible use in morphological and serological studies. Work performed in the Grodno region of Belarus, 2018-2019. Selected 113 samples of tissue and serum of patients (almost healthy individuals and individuals with histologically verified CRC). The age of the studied group was 60.07 ± 11.5 years, the control group 53.46 ± 8.02 years. Diagnostic stage: enzyme-linked immunosorbent assay with monoclonal antibodies to MUC1 / MUC13 receptors; molecular genetic analysis of mutations in the MSH2 gene by PCR with an electrophoretic detection scheme. Statistical processing - Statistica 10.0. As a result of the research, it was established that the concentration of antibodies to the MUC1 receptor in the serum of practically healthy individuals was 0.22 ± 0.05 ng / ml (n = 23), MUC13 - 0.56 ± 0.20 ng / ml (n = 23) ; in the serum of patients with histologically verified CRC MUC1 - 0.26 ± 0.08 ng / ml (n = 18), MUC 13 - 0.66 ± 0.22 ng / ml (n = 18); in samples of healthy colon tissue (without a diagnosed tumor process) MUC1 - 0.19 ± 0.02 ng / ml (n = 51), MUC13 - 0.72 ± 0.36 ng / ml (n = 51); in samples of tumor tissue - MUC1 - 0.82 ± 0.12 ng / ml (n = 20), MUC 13 - 0.89 ± 0.36 ng / ml (n = 20). The concentration of MUC1 / MUC13 antibodies is reliable in samples of “healthy” intestinal tissue and tissue samples from patients with verified tumors (p = 0.0001 / p = 0.03), in samples of tumor tissue and blood serum from patients with confirmed tumors (p = 0, 0001 / p = 0.002). The concentration of MUC1 / MUC13 in the serum of patients in the control group and the serum of patients with an established CRC was significantly different (p = 0.001 / p = 0.05). For the study group in tissue samples, a molecular genetic study was performed - mutations in the MSH2 gene (1-9 Exon) were detected. Assessment of the significance of the level of expression of MUC-1/13 in patients with a high clinical risk of developing intestinal neoplasms suggests the possibility of their use as early biological markers of the presence of mutations in the MSH2 gene and possible tumor development caused by increased proliferation of intestinal cells.

Keywords: colorectal cancer, molecular genetic testing, biological markers, mucin 1 (13).

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Gorczyca, Gabriela: Endocrine active compounds modulate calcium homeostasis in porcine cumulus-oocyte complexes during in vitro ageing

Gabriela Gorczyca[1], Kamil Wartalski[2], Małgorzata Duda[1]

1. Department of Endocrinology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University in Krakow, Poland
2. Department of Histology, Jagiellonian University Medical College, Kopernika 7, 31-034 Krakow, Poland

Contact for the abstract: Gabriela Gorczyca; gabriela.gorczyca@doctoral.uj.edu.pl

The oocyte grows and matures in a complex environment of the ovarian follicle within the cumulus-oocyte complex (COCs) where it occupies central location and is surrounded by a few layers of granulosa cells, named cumulus cells (CCs). Within this structure cell-to-cell contacts and communication allow the oocyte to acquire its developmental competence. During regular oocyte maturation after LH surge an increase in [Ca^{2+}] concentration first in cumulus cells and then in the oocyte has been observed. Therefore, alterations of proper extracellular [Ca^{2+}] content and disorders concerning its uptake may be at least partially responsible for oocyte ageing resulting in their poor-quality and finally infertility of the female. Because one of the factors causing the premature ovarian failure is the ever-increasing level of environmental pollution with endocrine active compounds (EACs) disrupting normal function of the endocrine system the aim of the presented work was to investigate how exposure of porcine COCs to selected EACs during in vitro ageing affects calcium concentration and CCs viability. The COCs were isolated from healthy, medium-size porcine follicles (4-6 mm in diameter), encapsulated in alginate beads (3D in vitro culture) and then cultured in the presence of vinclozolin (Vnz; a fungicide), nandrolone (Ndn; an anabolic steroid), and cyclosporin A (CsA; an immunosuppressant). After termination of culture (96h), analysis of calcium concentration in the media and CCs viability (Live-Dead detection kit, TUNEL) was performed. Results of this study demonstrated an abnormal, decreased concentration of [Ca^{2+}] in the culture media after COCs exposure to Vnz and CsA. Decreased CCs viability was observed in all experimental groups when compared to the control. Results obtained with TUNEL method together with abnormal [Ca^{2+}] oscillations confirm CsA-induced apoptosis. Viability analysis results also suggest that both vinclozolin and nandrolone may activate different types of cell death which requires further research.

Keywords: cumulus-oocyte complexes, calcium, ageing, EACs.

This work was supported by grant 2018/29/N/NZ9/00983 from National Science Centre, Poland

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Gorjão, Neuton: RNA polymerase I and III common subunit influences its own expression

Neuton Gorjão[1], Abu Musa Md Talimur Reza [1] Monika Wiśniewska [1], and Damian Graczyk[1]

1. Affilation - Department of Genetics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Pawińskiego 5a, 02-106 Warsaw

Contact for the abstract: Neuton Gorjão, n.gorjao@ibb.waw.pl

Colorectal cancer (CRC) is the second-most and third-most common cancer in women and men, respectively. Most patients with metastatic colorectal cancer fail to respond or develop resistance to the conventional treatments. Therefore, the identification of molecular mechanisms underlying colorectal cancer progress is critical to improve patient outcome and has enormous clinical value. POLR1D is a small subunit that is common to RNA polymerase I and III, which synthesize rRNA and tRNA, respectively. The products of these polymerases are crucial for protein synthesis. POLR1D is frequently upregulated in colorectal cancer and its high expression is positively correlated with tumour size and poor survival of CRC patients. In contrast, POLR1D knock-down inhibits CRC cells proliferation and tumour-growth in mouse xenograft model. Here, we provide evidence that that the ectopic overexpression of POLR1D in normal colon cells and several colorectal cancer cell lines decreases the expression of endogenous POLR1D. Furthermore, this phenomenon seems to be conserved in evolution, since we observe this effect also in budding yeast. Thus, there is a mechanism that tightly controls the levels of POLR1D protein. Moreover, this mechanism is likely to be defective in CRC cells, since they show elevated POLR1D protein levels, thus, potentially contributing to the tumorigenic properties of CRC cells. Interestingly, our recent work demonstrated that POLR1D is not regulated at transcription or degraded at the proteasome or by autophagy. Another possible mechanism will be presented and discussed. Our long-term goal is to decipher the mechanism of POLR1D self-regulation and its role in CRC. We hypothesize that free POLR1D protein triggers cellular response that acts to restrain the expression of itself and to balance the assembly of polymerases I and III.

Keywords: Colorectal cancer, RNA polymerases I and III, POLR1D, Protein self-regulation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Górka, Sylwia: Localization and change in the amount of active transcription of the RNA polymerase II form in mesophilic *Arabidopsis thaliana* leaf cells during hypoxia and reoxygenation

Sylwia Górka[1], Małgorzata Ciesińska, Janusz Niedojadło

1. Departament of Cellular and Molecular Biology, Nicolaus Copernicus University, Toruń

Contact for the abstract: Sylwia Górka; sylwiagorkaa@gmail.com

Hypoxia, or hypoxia, in flooded organs plant waters is a common and global problem in the world. Stress conditions lead to a decrease in crop yields due to difficult access to oxygen, resulting from weaker diffusion and solubility of oxygen in water. Prolonged flooding of some plants causes a disturbance of metabolism and the transition from aerobic to anaerobic respiration, which causes "energetic action" which limits the growth and development of the entire plant. As a result of low oxygen and ATP levels in the cells, there are changes in the transcriptome as well as a decrease in the level of translations. In *A.thaliana*, this effect is reversible upon recovery to physiological conditions after the stressor has been removed. Modulation of changes at the level of gene expression is important in adapting plants to abiotic stress. RPB1 is an integrated RNA II polymerase subunit, activated using the CTD domain is necessary for survival of activity during hypoxia. The aim of the measurement was to determine the effect of hypoxia on the change of *RPB1* gene expression and RNA II polymerase activity in *A.thaliana* leaf mesophilic cells. Immunolocation results for the serine 2 CTD domain change in size and distribution of transcriptionally active RNA II polymerase. At 1 and 6 hours hypoxia summarize the increase in transcription levels. Within several hypoxia, a significant decrease in serine 2 levels was observed relative to control conditions. Removal of stress allowing the level of transcription to return to the level observed under physiological conditions. Use of qPCR methods revealing that a reduced amount of RPB1 mRNA correlated with a decrease in the amount of active RNA II polymerase during hypoxia. Research considers that the *RPB1* gene is important in plant survival strategies during continuous flooding and after returning to aerobic conditions.

Keywords: hypoxia, RPB1, polymerase RNA II.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Górska, Agata: Gravitational stress and multi drug resistance phenomena in human ovarian cancer cells exposed to altered gravity

Dawid Przystupski[1,2], Agata Górska[1], Piotr Wawryka[1,2], Jack van Loon[3,4], Julita Kulbacka[1]

1. Department of Molecular and Cellular Biology, Wroclaw Medical University, Poland
2. Faculty of Medicine, Wroclaw Medical University, Wroclaw, Poland
3. DESC (Dutch Experiment Support Center), Dept. Oral and Maxillofacial Surgery / Oral Pathology, VU University Medical Center &Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, The Netherlands,
4. Life & Physical Science, Instrumentation and Life Support Laboratory (TEC-MMG), ESA/ESTEC, Noordwijk, The Netherlands

Contact for the abstract: Agata Górska; a.d.gorska@gmail.com

Some types of cancer - such as breast, colon and ovarian cancer - display multidrug resistance (MDR), which leads to failures in applied treatment. The search for the therapies overcoming cellular MDR mechanisms or acting effectively despite their occurrence has been the subject of countless studies. Interestingly, the answer to successful cancer treatment may be found due to gravity-related experiments. Gravity variations have been shown to remarkably influence the growth and biological processes of malignant cancer cells related to cell death and cell cycle arrest, drug resistance, angiogenesis, cytokine secretion. Furthermore, it has been found that gravity variations modulate the cytoskeleton structure of cancer cells. Thus, altered gravity experiments have become a promising approach to improve our understanding of cancer biology in order to detect interesting target proteins for future cancer treatment. Henceforward we hypothesized that gravitational stimuli may modify biophysical and chemical properties of cell membrane and cytoskeleton leading to modulation of MDR pathways on genetic and proteomic levels. The investigation and clarification of these phenomena may constitute an initial step toward enhancing our understanding of the link between cellular resistance to cytostatics and the response to various gravitational stimuli. This idea underlies the HyperCells project, which was aimed to investigate how hypergravity affects the drug sensitivity of human ovarian cancer cells. Our approach assuming implementation of Large Diameter Centrifuge (LDC) has been selected to perform in hypergravity conditions by researchers from the European Space Agency, as a part of the Spin Your Thesis! 2019 program. Here we present a comprehensive approach for analysis of protein-protein interactions network to establish, how the exposure to altered gravity alters the sensitivity of malignant cells to chemotherapeutic drugs. In preliminary research profound *in silico* analysis has been performed, underlying the further analysis of expression levels of chosen proteins.

Keywords: multi drug resistance, gravity response, altered gravity, hypergravity, Large Diameter Centrifuge

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Grabowska, Ewa: Does structure matter? An impact of thiamine analogues on kinetic properties of pyruvate dehydrogenase complex

Ewa Grabowska[1], Magdalena Czerniecka[1], Urszula Czyżewska[1], Aneta Zambrzycka[1], Zenon Łotowski[2], Adam Tylicki[1]

1. Faculty of Biology, University of Białystok, Ciołkowskiego 1J, 15-245 Białystok, Poland
2. Faculty of Chemistry, University of Białystok, Ciołkowskiego 1K, 15-245 Białystok, Poland

Contact for the abstract: Ewa Grabowska e.grabowska@uwb.edu.pl

Thiamine is an essential vitamin for carbohydrate metabolism. One of the main reaction involving thiamine pyrophosphate (TPP) is catalyzed by the pyruvate dehydrogenase complex (PDHC). Structural analogues of thiamine (eg. oxythiamine – OT) can disturb this reaction and can be considerate as an cytostatics or antibiotics. At the beginning of the 20th century, a thiamine derivative called deazathiamine (DAT) was synthesized. In the study of enzymes isolated from microorganisms it shows strong inhibitory potential against some TPP-dependent reactions. Knowing the effect of thiamine derivatives on TPP-dependent enzymes and the structure of DAT, this compound could also have an inhibitory effect on mammalian PDHC. Therefore, the purpose of our research was to compare the effect of DAT and OT on PDHC isolated from the pig's heart. The reaction mixture contained saturating concentrations of pyruvate, NAD^+, CoA and Mg^{2+} in phosphate buffer (50 µM; pH 7,5), TPP in the range of 0,02-5 µM and 0,01 µM OT or DAT pyrophosphates (OTPP/DATPP). Kinetic parameters of PDHC were determined using the Lineweaver-Burk and Hanes-Woolf models (Km, Vmax) as well as the Hill model (nH, $S_{0,5}$). Results obtained from the Hill model indicate the hyperbolic kinetic of PDHC responses to TPP. Km values for TPP in the presence of DATPP and OTPP were 5- and 1,5-fold higher respectively than Ki values for TPP without antivitamins. Obtained data confirm the competitive inhibition of the PDHC by both thiamine derivatives. The Ki for DATPP (0,003 µM) was about 4-fold lower than Ki value for OTPP (0,025 µM). The results indicate that DATPP is a much more potent PDHC inhibitor compared to OTPP and may have cytostatic potential. Obtained results require confirmation in studies on cell *in vitro* cultures. If DATPP inhibits the growth of cancer cells *in vitro*, it may be considered as a potential cytostatic agent.

Keywords: oxythiamine, deazathiamine, inhibition constant.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Grochowski, Maciej: Characterization of uridyltransferases of fission yeast

Maciej Grochowski, Lidia Lipińska, Jürg Bähler, Michał Małecki

Genome wide sequencing of RNA 3'-ends revealed the complexity of mRNA post transcriptional regulation by tail modifcations, which is emerging as an important layer of gene regulation. It can rapidly alter the fate of both noncoding and coding RNAs. Except from well described adenylation, RNA tails uridylation, guanylation and cytidylation were detected. While the function of the latter two is unknown uridylation is responsible for mRNA halflives shortening in humans, and tethering of uridyl transferase to an mRNA leads to a modest decrease of mRNA abundance. During mouse oocyte maturation, uridylation is instrumental for controlling transcript levels and to enable further development. However, in human somatic cells, as in yeast and plants, depletion of enzymes responsible for uridylation results in weak, if any, phenotypes. Therefore, the global impact of uridylation on the transcriptome is still an open question. It is likely that uridylation, like cytoplasmic adenylation, is important in specific situations where the impact of transcription on RNA level is compromised. Fission yeast have well-described RNA uridylation that is catalysed by two uridyltransferases Cid1 and Cid16. We aimed to characterize these enzymes and effect of their activity on RNA fate. Here we show preliminary data on our studies. We analyzed transcriptional changes caused by deletion of these genes and examined their synthetic interactions with other genes in SGA experiment. We confirmed hits by crossing deletion strains and complex examining phenotype of double mutants.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Gralewska, Patrycja: DNA damaging and cytotoxic effect of ATR and CHK1 inhibitors combined with olaparib on HR proficient HGSOC cell line SKOV3

Patrycja Gralewska[1], Arkadiusz Gajek[1], Agnieszka Marczak[1], Aneta Rogalska[1]

1. Department of Medical Biophysics, Faculty of Biology and Environmental Protection, University of Lodz

Contact for the abstract: Patrycja Gralewska; patrycja.gralewska@unilodz.eu

The initial, standard-of-care, adjuvant chemotherapy in epithelial ovarian cancer is usually a platinum drug, such as cisplatin or carboplatin, combined with a taxane. However, despite surgical removal of the tumour and initially, high response rates to first-line chemotherapy, around 80% of women will develop cancer recurrence. Replication stress response (RSR) is characteristic for tumours development, including ovarian cancer. Olaparib (AZD2281, PARP inhibitor), is an FDA approved drug for the treatment of $BRCA^{mut}$ high-grade serous ovarian cancers (HGSOCs), however, resistance to olaparib often occurs. RSR activates DNA repair checkpoint proteins, such as ataxia telangiectasia and Rad-3 related protein (ATR) and checkpoint kinase 1 (CHK1). The objective of the study was to evaluate the effect of the new compounds – ATR inhibitor (AZD6738), CHK1 inhibitor (MK8776) and their combination with olaparib on the homologous recombination (HR) proficient, ovarian cancer cell line – SKOV3. The mode of cell death was assessed colorimetrically, densitometrically by immunoblot analyses of the level of cytochrome c and through measuring DNA fragmentation. Compounds concentrations for the experiments were selected based on MTT assay, which established the optimal molar doses of compounds for the biological effect. The concentrations ratio of 1:1 has been applied in alkaline and neutral version of the comet assay and western blot method. Expression of cytochrome c was measured after 24 h treatment using GAPDH as a loading control. The DNA damage was measured after increasing incubation times (up to 48 h). Combined treatment led to the intensified release of cytochrome c and accumulation of SSB and DSB in comparison to PARPi monotherapy. The studies suggest that simultaneous administration of PARPi with ATRi or CHK1i may have a high genotoxic effect on the SKOV3 ovarian cancer cell line and may lead to apoptotic changes, on the path of synthetic lethality.

Keywords: ATR inhibitor, CHK1 inhibitor, ovarian cancer, PARP inhibitor.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Gralińska, Ela: Association Plots visualize condition-specific genes from high-dimensional data

Ela Gralińska[1], Martin Vingron[1]

1. Department of Computational Molecular Biology, Max Planck Institute for Molecular Genetics, Berlin, Germany

Contact for the abstract: Ela Gralińska; gralinska@molgen.mpg.de

Today's large transcriptome data sets pose a significant challenge to the existing analysis and visualization methods. Although methods such as principal component analysis (PCA) have been successfully employed for many years, the 2D- or 3D- embedding of a high-dimensional data set may be of little help in the interpretation of the data and in the search for marker genes. Therefore, we introduce the Association Plot – a planar representation of gene-condition relationships in a high-dimensional data – for finding condition-specific genes in complex data. We demonstrate our method on GTEx RNA-seq data and PBMC single-cell RNA-seq data, with Association Plots depicting clearly the genes which characterize tissues or cell clusters.

Keywords: Association Plot, condition-specific genes, marker genes, correspondence analysis.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Gregorová, Pavlína: Broad-range RNA modification analysis of complex biological samples using rapid C18-UPLC-MS

Pavlína Gregorová[1], Nina H. Sipari[2], L. Peter Sarin[1]

1. RNAcious Laboratory, Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Finland
2. Viikki Metabolomics Unit, University of Helsinki, Helsinki, Finland

Contact for the abstract: Pavlína Gregorová; pavlina.gregorova@helsinki.fi

Post-transcriptional RNA modifications play an important role in cellular metabolism with homoeostatic disturbances manifesting as a wide repertoire of phenotypes, reduced stress tolerance and translational perturbation, developmental defects, and diseases, such as type II diabetes, leukemia, and carcinomas. Hence, there has been an intense effort to develop various methods for investigating RNA modifications and their roles in various organisms, including sequencing-based approaches and, more frequently, liquid chromatography–mass spectrometry (LC-MS)-based methods. Although LC-MS offers numerous advantages, such as being highly sensitive and quantitative over a broad detection range, some stationary phase chemistries struggle to resolve positional isomers. Furthermore, the demand for detailed analyses of complex biological samples often necessitates long separation times, hampering sample-to-sample turnover and making multisample analyses time consuming. To overcome this limitation, we have developed an ultra-performance LC-MS (UPLC-MS) method that uses an octadecyl carbon chain (C18)-bonded silica matrix for the efficient separation of 50 modified ribonucleosides, including positional isomers, in a single 9-min sample-to-sample run. To validate the performance and versatility of our method, we analyzed tRNA modification patterns of representative microorganisms from each domain of life, namely Archaea (*Methanosarcina acetivorans*), Bacteria (*Pseudomonas syringae*), and Eukarya (*Saccharomyces cerevisiae*). Additionally, our method is flexible and readily applicable for detection and relative quantification using stable isotope labelling and targeted approaches like multiple reaction monitoring (MRM). In conclusion, this method represents a fast and robust tool for broad-range exploration and quantification of ribonucleosides, facilitating future homoeostasis studies of RNA modification in complex biological samples.

Keywords: modified ribonucleosides, high-throughput, UPLC, mass spectrometry.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Grochowina, Igor: Chaperone complexes involved in Fe-S cluster transfer

Igor Grochowina[1], Marcin Jeleń[1], Katarzyna Dąbrowska[2], Łukasz Nierzwicki[3], Aneta Grabińska[1], Bartłomiej Tomiczek[1], Michał Dadlez[2], Jacek Czub[3], Rafał Dutkiewicz[1], Jarosław Marszałek[1]

1. Laboratory of Evolutionary Biochemistry, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Poland
2. Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics of Polish Academy of Sciences, Warsaw, Poland
3. Department of Physical Chemistry, Gdansk University of Technology, Gdańsk, Poland

Contact for the abstract: Igor Grochowina; igor.grochowina@phdstud.ug.edu.pl

Mitochondrial biogenesis of iron-sulfur clusters via ISC pathway requires subsequent interactions of multiple proteins with the conserved scaffold protein Isu1, on which clusters are built before their transfer to recipient proteins. Following the cluster assembly process, the transfer of Fe-S cluster to a target protein is mediated by molecular chaperones. Initially, holo-Isu1 is bound by a J-protein Hsc20, which targets the double complex to Hsp70(Ssq1), leading to formation of a transient triple complex followed by stimulation of Hsp70 ATP-ase and release of Fe-S cluster. Residues critical for formation of the double and triple complexes were determined using substitution variants of Hsp70(Ssq1), Hsc20 and Isu1, but the structural mechanism of these interactions remains unclear. To characterize the productive interactions between Isu1 and its chaperones, we used homology modeling and molecular dynamics simulations, constructing a structural model of the triple complex between Hsp70(Ssq1), Hsc20 and Isu1. We were also able to purify stable Hsc20-Isu1 and Hsp70(Ssq1)-Hsc20-Isu1 complexes in amounts sufficient for biochemical analyses. Then, we applied hydrogen exchange mass spectrometry to purified complexes and individual proteins to investigate the effect of complex formation on proteins' hydrogen bond network and to verify our model. Obtained differential deuteration patterns indicate that Isu1 binds the C-terminal IBD domain of Hsc20, which is also able to interact with the nucleotide domain of Hsp70(Ssq1) in the triple complex. Hsp70(Ssq1)–Hsc20 binding occurs via interaction between nucleotide domain of Hsp70(Ssq1) and J-domain of Hsc20, and the PVK motif on Isu1 is involved in binding of the Hsp70(Ssq1) substrate domain. The presence of chaperones results in stabilization of Isu1 hydrogen bond network, however the substantial heterogeneity of Isu1 suggests that further analyses are required to fully understand the Hsp70(Ssq1)-Isu1 interaction.

Keywords: Hsp70, hydrogen-exchange mass spectrometry, molecular dynamics, Fe-S clusters.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Grubliauskaitė, Monika: Quality control methods for thawed ovarian tissue after cryopreservation

Monika Grubliauskaitė[1,2], Živilė Gudlevičienė[1], Gabrielis Kundrotas[1]

1. Department of Biobank, National Cancer Institute, Lithuania
2. Life Sciences Centre, Vilnius University

Contact for the abstract: Monika Grubliauskaitė; monika.grubliauskaite@gmc.vu.lt

Due to advancements in cancer diagnostics and treatment, female survival rates have increased in the past decades, especially in young patients. However, aggressive treatments – usually systemic chemotherapy and radiotherapy, reduces possibility of successful reproduction dramatically. One of the most promising yet still considered to be experimental option for female fertility preservation is ovarian tissue (OT) cryopreservation before oncologic treatment. However, for successful retransplantation, quality and viability of thawed tissue must be evaluated. The aim of this study was to assess structural quality, contamination with malignant cells and miRNA expression of frozen-thawed ovarian tissue. The study was approved by the Vilnius Regional Biomedical Research Ethics Committee. During 2015–2018 OT samples were cryopreserved in National Cancer Institute (Lithuania) from young women (<40 years) who signed Informed consent. Samples were frozen by slow freezing technique and stored at -80°C. Total RNA extraction and miRNA expression analysis were performed as quality control. Additional thawed ovarian tissue fragments (n=5) were xenotransplanted into female NOD SCID mice for 5 weeks. Histological hematoxylin/eosin and immunohistochemical (IHC) Ki-67 staining of ovarian tissue grafts and only thawed OT samples were applied. Results have shown good quality of extracted RNA from thawed OT – A260/A280 was 1.96–2.04, RIN 6.6–7.6 and 74 of 84 (88 %) investigated miRNAs were expressed. The earliest expression of *hsa-miR-1280* was detected. Xenotransplantation and IHC (Ki-67 staining of <5%) analysis have not shown any signs of malignant cells in both – grafts and thawed tissue samples. Successful engraftment of 80% was achieved, structurally grafts and thawed OT fragments were intact meaning tissue samples are viable after thawing. Molecular methods in combination with morphological and immunohistochemical analysis could be used as reliable and quick quality assurance tests avoiding standard and time consuming xenotransplantation method.

Keywords: Ovarian tissue, fertility, cryopreservation, xenotransplantation, cancer, miRNA.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Haque, Effi: Novel NRF2 Mutations Lead to Aberrant Transcriptional Regulation in HCC

Effi Haque[1], Magdalena Śmiech[1], Hiroaki Taniguchi[1]

1. Institute of Genetics and Animal Biotechnology, Laboratory for Genome Editing and Transcriptional Regulation, Polish Academy of Sciences, 05-552 Jastrzębiec, Poland

Contact for the abstract: Effi Haque e.haque@igbzpan.pl

Next-generation sequencing of the cancer genome identified that oxidative stress-related transcription factor NRF2 (NFE2L2) is mutated in different cancers including hepatocellular carcinoma (HCC). In this study, we demonstrated NRF2 DLG mutations (NRF2 D29A and L30F), found in Japanese liver cancer patients, upregulate the transcriptional activity of NRF2 in Hepa1-6 cells. Moreover, the transcriptional activity of NRF2 mutations was not suppressed by KEAP1, presumably because NRF2 MTs disturb proper NRF2-KEAP1 binding and block KEAP1-mediated degradation of NRF2. Additionally, both MTs upregulate the transcriptional activity of NRF2 on *MMP9* promoter in Hepa1-6 and Huh7 cells, suggesting that MTs derived gain-of-function of NRF2 may be important for liver tumor progression. We also found ectopic overexpression of oncogenic BRAF WT and V600E increased the transcriptional activity of NRF2 WT on both 3xARE reporter and *MMP9* promoter. Interestingly, NRF2 D29A and L30F MTs with oncogenic BRAF V600E MT synergistically upregulated the transcription activity of NRF2 on 3xARE reporter and *MMP9* promoter in Hepa1-6 and Huh7 cells. In summary, our findings suggest that MTs in NRF2 have pathogenic effect, and NRF2 MTs together with oncogenic BRAF V600E MT synergistically cause more aggressive tumor phenotype.

Keywords: NRF2, KEAP1, BRAF, MMP9

Grant (This work was supported by the Polish National Science Center OPUS 13 (2017/25/B/NZ5/02762) for HT

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Jancewicz, Iga: The role of SWI/SNF chromatin remodelling complex ATPase subunit BRM, in triple negative breast cancer

Iga Jancewicz[1], Natalia Rusetska[2], Alicja Armatowska[3], Małgorzata Stachowiak[1], Katarzyna Pogoda[4], Anna Niwińska[4], Wojciech Olszewski[5], Zbigniew Nowecki[4], Tomasz J. Sarnowski[6], Elżbieta A. Sarnowska[1,*], Janusz A. Siedlecki[2,*] (**equal contribution)*

[1]Department of Experimental Immunotherapy, Maria Skłodowska-Curie National Research Institute of Oncology , Warsaw, Poland; [2]Department of Molecular and Translational Oncology, Maria Skłodowska-Curie National Research Institute of Oncology , Warsaw, Poland; [3]Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland; [4]Department of Breast Cancer and Reconstructive Surgery, Maria Skłodowska-Curie National Research Institute of Oncology , Warsaw, Poland; [5]Department of Pathology, Maria Skłodowska-Curie National Research Institute of Oncology , Warsaw, Poland; [6]Department of Protein Biosynthesis, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

Contact for the abstract: Iga Jancewicz; iga.jancewicz@pib-nio.pl

Triple negative breast cancer (TNBC) is an especially aggressive subtype of breast cancer (BC) linked with poor prognosis and chemotherapy-resistance. TNBC's aggressiveness is possibly caused by a process called epithelial-mesenchymal transition (EMT). EMT allows cells to acquire invasive phenotype and is driven by transcription factors from SNAIL, Twist and ZEB families. SWI/SNF chromatin remodelling complexes (CRCs) are conserved among eukaryotes. They modify chromatin structure, hence changing expression patterns. Disruption of SWI/SNF CRCs stoichiometry in human leads to development of various cancer types, including BC. This work is focused on the role of BRM (encoded by *SMARCA2*), one of two catalytic subunits of SWI/SNF CRC, in development of TNBC. MDA-MB-231 and MCF7 cell lines were used as research models for TNBC and ER+ BC respectively. MDA-MB-231 with *SMARCA2* overexpression and MCF7 with silenced *SAMARCA2* cell lines were developed and analysed in terms of molecular changes using e.g. Western Blot, RT-qPCR. ChIP-qPCR and RNAseq. Protein-protein interactions were verified using Co-IP and BiFC. Obtained results were compared to available clinical data. Changes of BRM levels in BC cells lead to significant phenotype switch characteristic for EMT process – mainly, downregulation of *SMARCA2* in MCF7 cell line resulted in development of more aggressive characteristics. On the contrary, overexpression of *SMARCA2* in TNBC cell line results in more epithelial cell phenotype. Interestingly, *in vitro* overexpression of SNAIL family proteins in these cells caused downregulation of BRM. However, inhibition of proteasome in the MDA-MB-231 cell line resulted in reversed effect, i.e. upregulation of BRM. IHC analysis of paraffin embedded TNBC samples allowed identification of 3 patient groups based on BRM level. Patients with the lowest BRM level classified for more aggressive tumours.

Financial support: *0229/DIA/2015/44 grant fur IJ by Polish Ministry of Science and Higher Education and UMO-2016/23/N/NZ1/01138 for IJ by National Science Center*

Keywords: SWI/SNF, TNBC, BRM, EMT.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Jabłońska, Joanna: Maintaining of the activity of complex III depends on proper biogenesis of cytochrome c oxidase

J. Grochowska*[1] , M. Grochowski*[2], K. ŁabędzkaDmoch[2], P. Golik[1,2], R. Szczęsny[1]

1. [1] Institute of Biochemistry and Biophysics PAS, Warsaw, Poland,
2. [2] Institute of Genetics and Biotechnology, University of Warsaw, Warsaw, Poland

Contact for the abstract: j.jablonska@ibb.waw.pl, m.grochowski@student.uw.edu.pl

Mitochondria play an essential role in cellular metabolism, mainly by producing majority of the cellular ATP by means of oxidative phosphorylation (OXPHOS). This process depends on OXPHOS complexes localized in the internal mitochondrial membrane. Mitochondrial complexes are dynamic and aggregate in different stoichiometric combinations to form supercomplexes which offer structural and functional advantages. Experiments performed on human cell lines have shown correlation between incorrect formation of complex III or IV, and functional impairment of complex I. Using yeast *Candida albicans* as a model we found that maintenance of the activity of complex III depends on proper biogenesis of complex IV. This link was revealed by targeted deletion of nuclear genes encoding structural components of complex IV as well as inactivation of nuclear-encoded factors which are required for expression of mitochondrial encoded components of this complex. Our data extends view on strong functional/biogenesis interconnection between OXPHOS complexes and supercomplexes formation. Results obtained with the yeast model as well as our attempts to validate these observations in higher Eukaryotes will be presented.

Keywords: mitochondria, oxidative phosphorylation, OXPOS complexes, supercomplexes.

**The authors marked with an asterisk equally contributed to the work.*

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Jurkiewicz, Aneta: MAF1 is involved in the regulation of RNA polymerase III activity in macrophages upon LPS treatment

Aneta Jurkiewicz[1], Hitha Gopalan Nair[1], Abu Musa MD Talimur Reza[1], Damian Graczyk[1]

1. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warsaw, Poland

Contact for the abstract: Aneta Jurkiewicz: a.jurkiewicz20@ibb.waw.pl

Macrophages, cells of the innate immune system, have many biological functions, such as antigen presentation, target cell cytotoxicity, phagocytosis, and regulation of inflammation. Activation of macrophages with lipopolysaccharides (LPS), a major component of the outer membrane of most Gram-negative bacteria, induces rapid transcriptional changes and, within a few hours, transcription of several hundred genes is altered. LPS, recognized by TLR4, induce various signalling pathways and activate several transcription factors such as activator protein-1 (AP-1), IRF3, and NF-κB. Then, transcription factors recruit transcriptional co-activators, for example, histone-modifying enzymes, that change local chromatin structure to enable transcription of target genes, including cytokines and other inflammation mediators. This transcriptional activity is followed by an extensive increase in translation, which in turn requires an increased level of tRNAs, that are transcribed by RNA polymerase III (Pol III). We have previously shown that treatment of macrophages with LPS induces Pol III activity and that NF-κB is involved in this process. However, inhibition of the NF-κB pathway only partially precludes Pol III activation upon LPS treatment, and this suggests that other signalling pathways are involved in the activation of Pol III in these conditions. LPS treatment activates the mammalian target of rapamycin (mTOR) kinase. mTOR, in turn, activates Pol III via phosphorylation and inactivation of its negative regulator, MAF1. Consistently, the results obtained by us imply that the mTOR-MAF1 pathway is a major mechanism of Pol III regulation in macrophages stimulated with LPS. Moreover, changes in MAF1 levels affect the pro-inflammatory functions of macrophages. This phenomenon place MAF1 and Pol III regulation in a broader picture of macrophages biology.

Keywords: macrophages, LPS, MAF1, mTOR.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kanoza, Michał: The role of gingipains, proteolytic enzymes of *P. gingivalis,* in the regulation of TLR3 signaling pathway

Michał Kanoza[1], Anna Gąsiorek[1], Barbara Potempa[2], Anna Golda[1], Jan Potempa[1,2], Joanna Kozieł[1]*

1. Microbiology Department, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland;
2. Department of Oral Immunity and Infectious Diseases, University of Louisville School of Dentistry, University of Louisville, Louisville, Kentucky, United States of America

Contact for the abstract: *joanna.koziel@uj.edu.pl

Toll-like receptors (TLRs) are classified as crucial in the recognition of pathogen-associated molecular patterns (PAMPs) by the host. Among them TLR3, TLR7 and TLR9 are localized intracellularly binding different form of nucleic acids, what leads to the activation of transcription factors, such as NF-κB, AP1, IRF3, and subsequent expression of proinflammatory mediators. In our research we focused on the role of cysteine proteases from *Porphyromonas gingivalis* – gingipains, both arginin (RgpA, RgpB) and lysin specific (Kgp) in the modulation of inflammatory reaction induced by TLR agonists. As a model we applied human gingival keratinocyte cell line (TIGKs). We found that all three gingipains strongly influence the response of cells to Poly (I:C), analogue of dsRNA which activates TLR3 receptor. Obtained data revealed that RgpA and Kgp decreased the expression of proinflammatory mediators (such as IL-6, TNF-α and IFNβ) induced by Poly (I:C) and regulated by NF-κB, AP1 and IRF3 transcription factors. Then, we aimed to analyse the molecular mechanism of above observation showing that gingipains efficiently degrade intracellular adaptor molecule TBK1, which is crucial in the IRF3 signaling pathway. To examine if our observation is biologically relevant we determined the influence of gingipains on the course of keratinocytes infection induced by HSV-1 virus. Obtained data revealed that the presence of gingipains strongly modulates the proliferation of virial particles. Taking together we found that gingipains play an important role in the development of virial infection of gingival keratinocytes via the regulation of inflammatory response executed by the proteolysis of major signaling molecules.

Keywords: TLR3, gingipains, signaling pathway, HSV-1 infection, gingival keratinocytes.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Karpińska, Kamila: Alpha-catulin contributes to neural tube closure in the mouse embryo.

K. Karpińska[1] , C. Cao[2], M. Gielata[1] , V. Vassilev[1] , A. Kobielak[1]

1. Laboratory of the Molecular Biology of Cancer, Centre of New Technologies, University of Wa rsaw, Warsaw, Poland
2. University of Southern California, Los Angeles, California, United States of America

Contact for the abstract: Kamila Karpińska k.karpinska@cent.uw.edu.pl

During the early development of the central nervous system, the neuroepithelial cells undergo dynamic changes in shape. Spatial and temporal changes of the cytoskeletal organization are fundamental to epithelial cell shape changes, and noncetrosomal microtubules assembled along the apicobasal axis and actin filaments and non-muscle myosin II at the apical side are central machineries of cell elongation and apical constriction, respectively. Vinculin and α-catenin are two related proteins that play crucial roles in those processes; the function of their recently characterized homologue alpha-catulin is still poorly understood. Here, using the gene trap system we unveil the function of alpha-catulin during early mouse development. Ablation of alpha-catulin causes defective neural tube closure, due to impairment of both basement membrane assembly and bending of the neural plate. Neural plate superficial cells that normally drive bending of the neural plate by apical constriction, concomitant with apical actin and P-Mlc accumulation, fail to do so in alpha-catulin KOs. Using the 3D model of MDCK cells we showed that removal of alpha-catulin from the cells alters the subcellular distribution of F-actin and apical constriction. These observations suggest that alpha-catulin is a critical determinant of the cellular architecture required for proper neurulationn.

Keywords: alpha-catulin, neurulation, neural tube closure defects, MDCK 3D-model.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kasprzyk, Marta: Understanding the mechanism of oncogene expression regulation by *IGH* enhancers

Marta Kasprzyk[1*], Weronika Sura[1*], Wojciech Łosiewski[1,2], Marta Podralska[1], Marta Kazimierska[1], Tomasz Woźniak[1], Natalia Rozwadowska[1], Agnieszka Dzikiewicz-Krawczyk[1]

1. Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland
2. Poznan University of Medical Sciences

**authors contributed equally to this work*

Contact for the abstract: Marta Kasprzyk marta.kasprzyk@igcz.poznan.pl

Immunoglobulin heavy chain (IGH) locus undergoes several complex rearrangements during B-cell development. VDJ recombination, somatic hypermutation and class switch recombination – all allow for expression of a variety of high affinity antibodies. The intermediates of those processes are DNA double-strand breaks which pose a threat of illegitimate rearrangements. Indeed, characteristic features of B-cell non-Hodgkin lymphomas are recurrent translocations juxtaposing an oncogene (e.g. *MYC, BCL2*) to the immunoglobulin heavy chain (IGH) enhancers: Eμ and 3'regulatory regions (3'RR1, 3'RR2). Survival and proliferation of many B-cell lymphomas depends on the expression of the translocated oncogene. Despite our well established knowledge of the roles of particular IGH enhancers in different steps of B-cell maturation, precise mechanisms of their involvement in regulation of oncogene expression and lymphomagenesis are yet to be determined. The goal of our project is to identify the functional elements in the IGH enhancer regions and enhancer RNAs (eRNAs) transcribed from them, which are essential for oncogene expression and B-cell lymphoma cell growth. We performed a tiling CRISPR/dCas9-KRAB interference screen in Burkitt lymphoma cell lines (BL41 and DG75) with sgRNA library targeting all possible sites in the IGH enhancer regions. Cells were harvested after transduction (T0) and further cultured for 20 population doublings (T1). Changes in abundance of sgRNAs in the cell pool at T1 vs T0 were determined by NGS. We identified 680 sgRNAs at least two-fold depleted in DG75 and 279 in BL41. Sliding window analysis revealed one region in the Eμ and two in the 3RR enhancers whose targeting with CRISPR profoundly inhibited BL cell growth. In parallel, we performed chromatin-associated RNA-Seq to identify eRNAs. We confirmed ongoing transcription at each IGH enhancer and identified several differentially expressed lncRNAs. Validation of the results and determination of their functional relevance are ongoing.

Keywords: B-cell lymphoma, eRNA, CRISPR/dCas9, *IGH* enhancers.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kędzierska, Marta: Effect of silver-doped chitosan-graphene composites on human blood and skin cells

Marta Kędzierska[1], Abdelkrim El Kadib[2], Katarzyna Miłowska[1]

1. Department of General Biophysis, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-001, Lodz.
2. EuromedResearchCenter, Engineering Division, Euro-MediterraneanUniversity of Fes (UEMF), Fes-Shore, Routede SidiHrazem, 30070 Fes, Marocco.

Contact for the abstract: Marta Kędzierska; marta.kedzierska1@unilodz.eu

The antibacterial properties of silver and its compounds have been known for a very long time. Silver due to its biocidal properties is used on a commercial scale in bandages and wound dressings. However, ionic silver is susceptible to complex formation and precipitation, which results in its inactivation. For this reason, they were replaced by NczAg silver nanoparticles. Among the many compounds in the field of medical engineering, attention should be paid to natural polysaccharides, e.g. Chitosan. The tested composites are a combination of chitosan with calligraphic oxide and silver. The most desirable film properties include biodegradability, biocompatibility, bioactivity and non-tendency. Wound healing, stem cell engineering and regenerative medicine were analyzed using graphene-based materials. Due to its mechanical properties, graphene has been used as a material to reinforce hydrogels, biodegradable films and fibers in tissue engineering. Chitosan-graphene hydrogel suits showed a significant improvement in cell adhesion, differentiation and proliferation. It has also been shown that the presence of free electrons in graphene does not affect the proliferation of eukaryotic cells, but it inhibits the multiplication of prokaryotic cells, which prevents the reproduction of microorganisms. The purpose of this study was to check the hemolytic properties of chitosan-graphene biocomposites from Ag. The research was carried out using human erythrocytes. Research shows that the type of modification affects hemolysis to varying degrees. The greatest damage occurs due to the hemotoxic effect of silver ions. In addition, cytotoxicity tests were performed on fibroblasts (BJ line) and keratinocytes (KERTr line). Silver ion-doped composites have been found to be toxic to skin cells causing a significant decrease in viability.

Keywords: chitosan-graphene composites, hemolysis, cytotoxicity, skin cell.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kielczyk, Karolina: MicroRNA induced myogenic differentiation of mouse pluripotent stem cells

Karolina Kielczyk[1], Anita Florkowska[1], Maria A. Ciemerych[1]

1. Department of Cytology, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw

Contact for the abstract: Karolina Kielczyk k.kielczyk@biol.uw.edu.pl

Pluripotent stem cells, such as embryonic stem cells (ESCs), have self-renew capacity and can differentiate into any tissue that builds an organism. Therefore, muscle cells derived from differentiated ESCs hold promise for treating muscle injuries or degenerative diseases. However, so far no efficient protocol for myogenic differentiation of ESCs has been obtained. Our research concerns microRNA as a tool to targeted ESCs differentiation. microRNAs (miRNAs, miRs) are short, single-stranded molecules regulating proliferation and differentiation various types of cells. We aimed to determine the role of miRNAs during *in vitro* myogenic differentiation of ESCs. Our previous study documented the impact of selected miRNAs at differentiation of D3 and B8 mouse ESCs lines. Currently, we wanted to extend our research to the reporter cell lines what would give us the chance to follow these cells after their transplantation into regenerating muscles. Since pluripotent stem cell lines may vary in their reaction to various treatments we have to carefully test them. Thus, we chose two mouse ESCs lines, i.e. H2B-EGFP and 7AC5-YFP, and transfected them with miR145 and miR181, then induced myogenic differentiation through selected sequence of changing media and culture methods. Next, we analysed effect of transient miRNA expression on the level of factors characteristic for: paraxial mesoderm (Pdgfrα), myogenic precursor cells (Pax7) or myoblast and myotubes formation (Myf5, Myog). Additionally, we localised the cells expressing skeletal myosin heavy chains (MyHC) specific for muscle tissue. We observed several differences between 7AC5-YFP and H2B-EGFP cells transfected with miR145 or miR181 at the level of the expression of listed markers. In next step we will test other selected miRNAs, which are promising as a tools to induce ESC myogenic differentiation.

Keywords: ESC, microRNA, myogenic differentiation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kmiołek, Tomasz: Telomeres in patients with frailty syndrome and sarcopenia

Agnieszka Paradowska-Gorycka[1], Tomasz Kmiołek[1], Anna Wajda[1], Diana Hasan[1], Ewa Walczuk[1], Ewa Rzeszotarska[1], Marcin Radkowski[2], Tomasz Targowski[2].

1. Department of Molecular Biology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw
2. Geriatrics Clinic and Polyclinic, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw

Contact for the abstract: Agnieszka Paradowska-Gorycka; agnieszka.paradowska-gorycka@spartanska.pl and Tomasz Kmiołek; tomasz.kmiolek@spartanska.pl

Biological basis responsible for the aging process are still unknown, but many researchers inclined that mitochondria and the length of telomere have a big impact on the aging process. Sarcopenia and frailty syndrome are one of the leading health issues in the older adults. Both conditions are correlated with age and are characterized by progressive loss of muscle. In the present project we analyzed relative telomere length in patients suffered from frailty syndrome and sarcopenia in comparison to healthy subjects. Frailty syndrome is associated with aging and with the increase of vulnerability to additional diseases of elder people. Frailty has been mainly identified as an affliction of elderly people. Development of this syndrome may occur in adults in their early fifties'. Additionally, frailty is characterized by increase rate of apoptosis and inflammation disturbance of calcium homeostasis hormone regulation, and neuronal signals, as well as changes in gene expression. Sarcopenia is a muscle disease, characterized by a gradual loss of skeletal muscle mass and a loss of muscle function. Similar as frailty syndrome, sarcopenia can develop after 50 years There is still a little knowledge on the molecular basis of muscle mass and muscle physiology. Moreover, it is important to recognize the need for biomarkers to distinguish frailty syndrome and sarcopenia. We selected 64 healthy control (HC) aged 40 and 35 HCs aged 28; 8 patients with sarcopenia, 25 patients with frailty syndrome and 18 geriatric patients without sarcopenia and frailty syndrome. Present study revealed significantly lower relative telomerase length in healthy older adults and frailty syndrome when compare to younger healthy subjects. Older female adults and female with frailty syndrome were characterized by longer telomere length when compare to male.

Keywords: Sarcopenia, frailty syndrome, telomere length.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kochanowski, Paweł: The role of normal astrocytes and TGFβ in formation of glioblastoma multiforme invasive front

Paweł Kochanowski[1], Damian Ryszawy†

1. Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Cracow, Poland

Contact for the abstract: Paweł Kochanowski mr.p.kochanowski@gmail.com

The lack of effective treatments for glioblastoma multiforme, the most frequent primary brain tumour, creates a need for new therapeutic approaches. In order to develop new treatments we must understand not only the aetiology but also the factors that regulate progression of already existing tumours. Glioblastoma microenvironment may play crucial role in this phenomenon. Interaction of glioblastoma cells with astrocytes remains poorly understood even though astrocytes are the most abundant brain cells. Some studies suggest that transforming growth factor β may play a crucial role in this interaction. The aim of the study was to analyze the influence of transforming growth factor β, and of the interaction with primary astrocytes, on formation of invasive front of rat glioblastoma cell line F98 in vitro. The obtained results of time-lapse video microscopy prove that factors of astrocytic origin increase motile activity of glioblastoma cells. Moreover, the production of paracrine factors by the astrocytes is modulated by their interaction with glioblastoma cells. Coculture studies indicated that interaction with primary astrocytes, and transforming growth factor β, increase metastatic potential of F98 cells by epithelial-mesenchymal transition related process which was analyzed by immunoblotting and immunofluorescence investigation of SNAI1 levels.

This work was financially supported by Polish National Science Centre (UMO-2015/19/D/NZ3/00273 to D.R.).

Keywords: astrocytes, invasive front, glioblastoma multiforme, epithelial-mesenchymal transition, transforming growth factor β.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kokhan, Anatoli: Changes of platelet electrophysiological properties in response to human myeloperoxidase

Anatoli Kokhan[1], Fyodor Balabin[2], Daria Grigorieva[1], Irina Gorudko[1], Anastasia Sveshnikova[2], Alexey Sokolov[3, 4], Ekaterina Shamova[1]

1. Belarusian State University, Department of Biophysics, Minsk, Belarus
2. Lomonosov Moscow State University, Moscow, Russian Federation
3. Institute of Experimental Medicine, St. Petersburg, Russian Federation
4. Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russian Federation

Contact for the abstract: Anatoli Kokhan rrchypp@gmail.com

Myeloperoxidase (MPO) is enzyme of azurophilic granules of neutrophils, which provides innate immune response to the microbial infection. It is released during neutrophils degranulation, and is one of the key proteins involved in producing HOCl form H_2O_2 and Cl^-. It is known, that immune system can influence the operation of coagulation cascade, contributing to the pathological thrombus formation. Recently it was shown, that MPO can potentiate platelet aggregation. But it remains unknown whether MPO can activate platelets directly and what are the possible mechanisms. In this work we provide evidence that MPO interaction with platelet surface cause changes in cell electrophysiological properties. Platelets where obtained from citrated venous blood by double centrifugation technique. Electrophysiological recordings where performed on EPS 8 patch clamp amplifier using different combinations of pipette solutions. MPO (100 nM) caused hyperpolarization of platelet membrane potential by 10-15 mV. Sometimes occasional oscillations of potential were obtained. To determine the cause of the changes of potential we measured single channel activity in cell-attach mode with applied potential of 150 mV in order to inactivate voltage-gated potassium channels. Without added MPO only occasional channel openings can be seen. Addition of MPO invoked several types of channel openings with conductance ranging from 8 to 20 pS. This pattern of channel activity was different without Cl^- ions in the pipette, suggesting the involvement of chloride channels. Also, in calcium free external saline MPO-induced channel activity was negligible. Treatment of platelets with 20 nM charibdotoxin (BK-channels antagonist) inhibited nearly all MPO-induced channel activity with larger conductance, as well as hyperpolarization of membrane potential. Obtained results suggest direct activation of platelets by MPO through the involvement of calcium gated chloride and potassium ion channels.

Keywords: platelets, myeloperoxidase, patch clamp.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Komorowska, Dominika: Analysis expression of apoptotic genes in MCF-7 cell preincubated with stilbenes and exposure on ionizing radiation

D. Komorowska[1], A. Gajewska[1], A. Rodacka[1]

1. Department of Molecular Biophysics, Faculty of Biology and Environment Protection, University of Lodz, 90-236 Lodz, ul. Pomorska 141/143, Poland

Contact for the abstract: Dominika Komorowska; dominikakomorowska1@gmail.com

Breast cancer is the most commonly diagnosed type of cancer in women. Currently, the basic methods of treatment of breast cancer include surgical tumor removal, chemotherapy and radiotherapy. Radiotherapy is aimed at destroying cancerous changes using ionizing radiation but also is associated with negative effect on normal cells. Therefore, natural compounds (polyphenols) are sought that would protect "normal" cells from the harmful effects of radiation, i.e. they would act radio-protected and sensitize cancer cells to ionizing radiation. The most popular polyphenol and powerful antioxidant is resveratrol (R). The highest concentration of this compound was observed in grapes, berries and peanuts. Naturally occurring hydroxylated analogue of resveratrol is piceatannol (ROH) and its glucoside form is piceid (RG). The antitumor activities of resveratrol are mediated through modulatory effects on gene expression pathways and induction of apoptosis. The aim of this study was to examine whether resveratrol and its derivatives piceatannol and piceid can sensitize breast cancer cells to ionizing irradiation (IR) by increase of induction of apoptosis. The human breast cancer cell line examined was MCF-7 (breast carcinoma). The studies that were performed following 3 hrs preincubation in the presence of resveratrol, ROH and RG (25 µM) and/or IR (doses 2 or 6 Gy) , were the following: test MTT to determine a vibility of cells, induction of apoptosis and analysis expression of apoptotic genes (bcl-2, bax, p53, caspase 3 and caspase8). The obtained results showed that resveratrol and its analogues cause significant increase of expression of proapoptotic gene (bax) in cells exposured on IR.

Keywords: resveratrol, piceatannol, piceid, MCF-7, ionizing radiation, apoptosis.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Konieczny, Igor: Identification of bacterial proteins associated with inorganic polyphosphate (PolyP-ome) in various stress conditions

Sroka M[1], Konieczny I[1]

1. Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland

Contact for the abstract: Igor Konieczny; igor.konieczny@biotech.ug.edu.pl

Inorganic polyphosphate (PolyP) is a ubiquitous polymer of hundreds to thousands of phosphate (P_i) molecules held together by high-energy phosphoanhydride bonds. In bacteria, it functions as an energy and phosphate reservoir, plays an essential role in cell survival, cation homeostasis, stress response, and contributes to sporulation, quorum-sensing, and pathogenesis. In mammals, PolyP appears to play an equally large number of distinct roles, such as stimulation of blood clotting, bone mineralization, mTOR activation, and apoptosis triggering. The molecular mechanism by which such a simple molecule exerts all these diverse functions is likely to be related to its ability to bind to specific proteins, either through ionic interactions or through recently described covalent post-translational modification called lysine polyphosphorylation. However, only a handful of reports have been published so far describing a proteomic analysis of PolyP granules and relevance of PolyP-protein interactions. This was mainly due to technical difficulties with isolation of intact PolyP together with associated partners and limitations of mass spectrometry in polyphosphorylation detection. Here, we report an approach for discriminative isolation of proteins physiologically complexed with PolyP. Using pull-down assays with recombinant PolyP binding domain from *Escherichia coli* PolyP phosphatase (*Ec*PPX) as a bait combined with mass spectrometry, we are able to selectively purify and identify proteins associated with PolyP from bacteria grown under different conditions – both favorable and nonoptimal. The potential targets will be subsequently confirmed by series of in vitro and in vivo experiments. In further steps, we want to characterize the biochemistry and molecular functions of such interaction based on selected candidates from *E. coli* and other bacteria. However, our work will provide a novel tool to study PolyP-omes not only from microorganisms but presumably from eukaryotes as well.

Keywords: inorganic polyphosphate, PolyP-ome, proteomics, mass spectrometry.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kopeć, Zuzanna: The role of Na^+/K^+ ATPase and Na^+/H^+ exchanger in the preimplantation development of a mouse embryo

Zuzanna Kopeć[1], Anna Ajduk[1],

1. Department of Embryology, Institute of Developmental Biology and Biomedical Sciences, University of Warsaw, 02–096 Warsaw, Poland

Contact for the abstract: Zuzanna Kopeć; z.kopec@igbzpan.pl

A blastocyst is the final stage of preimplantation embryo development in mammals. It consists of internally localized inner cell mass (ICM), a group of cells that will form the future fetus body, and trophectoderm (TE), an external layer of cells that is crucial for the embryo implantation. The ICM is adjacent at one side to the TE, and surrounded at the other side by the fluid-filled blastocyst cavity. The formation of this cavity (i.e. cavitation) is one of the key events during the preimplantation embryo development. Here, we show the role of Na^+/K^+ ATPase and Na^+/H^+ exchanger in the process of cavitation. To this end, mouse embryos were cultured with ouabain that blocks Na^+/K^+ ATPase, or EIPA that inhibits Na^+/H^+ exchanger, from various stages of development for 24 hrs or 48 hrs and in some experiments followed by time-lapse imaging. We observed that embryos treated with ouabain from the 4-8 cell stage had lower TE and total cell numbers than control embryos, did not cavitate but degenerated instead. However, ouabain added to the embryo culture from the morula-small blastocyst stage did not affect the embryo development so profoundly: they divided and cavitated similarly to the control. Embryos treated with EIPA, both from the 4-8 cell stage and from the morula-small blastocyst stage had significantly lower TE and total cell numbers than their control counterparts and fail to cavitate/maintain the cavity. In summary, our data indicate that the Na^+/K^+ ATPase pump is more important for the cavity formation than its maintenance. However, the Na/H^+ exchanger activity is important for both, formation and maintenance of the cavity. The obtained results allow for a more accurate understanding of the processes occurring in the first stages of development.

Keywords: blastocyst, Na^+/K^+ ATPase, Na^+/H^+ exchanger, trophectoderm, cavitation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kosyl, Ewa: Preimplantation development of the single blastomeres isolated from the 4-cell mouse embryos

Ewa Kosyl[1], Katarzyna Krawczyk[1], Marek Maleszewski[1]

1. Department of Embryology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Contact for the abstract: Ewa Kosyl; ewa.kosyl@student.uw.edu.pl

In order to sustain full-term fetus development, cells in the mouse blastocysts had to differentiate into three embryonic lineages: trophectoderm (TE), epiblast (EPI), and primitive endoderm (PE). Single blastomeres of the 2-cell mouse embryos differ in their ability to produce EPI cells, so they exhibit unequal developmental potencies. It has been shown, that single blastomeres isolated from the 4-cell mouse embryos (1/4 blastomeres) can develop into blastocysts with inner cell masses (ICMs) consisting of only a few cells or completely devoid of ICM altogether. After transplantation to the recipient mouse, such embryos cannot undergo the complete embryogenesis until term. One of the possible explanations of this issue is an insufficient number of cells contributing to the three cell lineages of the blastocysts. So far it has not been shown, which cell lines can be found in embryos developed from the 1/4 blastomeres. The aim of this study was to investigate the ability of 1/4 blastomeres to differentiate into EPI, PE and TE. To address this question, we divided the 4-cell mouse embryos into single blastomeres and then cultured them for 48 or 72 hours. Next, we identified the three types of cells by immunofluorescence staining and confocal imaging. We observed that isolated 1/4 blastomeres varied in terms of their ability to differentiate into EPI and PE. Some of the 1/4 blastomeres developed into vesicles built only by trophectoderm cells. Blastocysts developed from the 1/4 blastomeres cultured for 48 hours often had ICMs consisting only of EPI cells. ICMs of the blastocysts derived from the 1/4 blastomeres cultured for 72 hours were more often composed of both EPI and PE than in 48-hours-cultured 1/4 blastomeres. Taken together, our data suggests that an insufficient number of PE cells is one of the major factors limiting the developmental potential of single 1/4 blastomeres.

Keywords: 1/4 blastomeres, blastocyst, trophectoderm, epiblast, primitive endoderm.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kovalchuk, Vasylyna: SLC6A14 – an amino acid transporter B0,+, exclusively interacts with SEC24C as a cargo recognizing COPII element

Vasylyna Kovalchuk[1], Łukasz Samluk[1], Barbara Juraszek[1,] Dominika Jurkiewicz[1], Sonja Sucic[2], Michael Freissmuth[2], Katarzyna A. Nałęcz[1]

1. Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland
2. Institute of Pharmacology and the Gaston H. Glock Research Laboratories for Exploratory Drug Development, Center of Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria

Contact for the abstract: Vasylyna Kovalchuk; v.kovalchuk@nencki.edu.pl

SLC6A14 , a plasma membrane amino acid transporter $B^{0,+}$ ($ATB^{0,+}$), is a Na/Cl dependent transporter for neutral and basic amino acids. It is up-regulated in several types of cancers. Trafficking starts to the plasma membrane with leaving the endoplasmic reticulum (ER) and interaction with a cargo recognizing protein SEC24 within Coatomer II (COPII) complex**.** We studied trafficking of ratSLC6A14 in a heterologous expression system in HEK293 cells. Western blot, deglycosylation and immunofluorescence demonstrated that the substantial amount of fully glycosylated SLC6A14 appears at the cell surface after 48h. Only one of four SEC24 paralogues SLC6A14 co-precipitated with SEC24C isoform and has an interaction with SEC24C was confirmed by proximity ligation assay. Co-localization of endogenous SLC6A14 with SEC24C was observed in MCF-7 breast cancer cells. Part of the overexpressed $ATB^{0,+}$ is directed to proteolysis, a process significantly reversed by a proteasome inhibitor bortezomib. Analysis of SLC6A14 amino acid sequence and detected specificity for SEC24C confirms a hypothesis proposed for a neurotransmitter transporters branch of SLC6 family that a hydrophilic residue at +2 position downstream of the ER export "RI" motif determines interaction with C isoform of SEC24 proteins and promotes further trafficking to Golgi and plasma membrane. There is an equilibrium between export from ER and degradation mechanisms in case of overexpressed transporter. *This work was financed with Polish National Science Centre Grant No. 2012/B/NZ3/000225, with the EU Horizon 2020 Research and Innovation Programme under Marie Sklodowska-Curie Grant Agreement No. 665735 (Bio4Med) and with the funds from the Polish Ministry of Science and Higher Education as part of the 2016–2020 funds for the implementation of international projects (Agreement No. 3548/H2020/COFUND2016/2) and grants of the Austrian Science Fund FWF (P31255 and SFB35-10 to Sonja Sucic and Michael Freissmuth, respectively).*

Keywords: Amino acid transporter; ER export; SAR1; SEC24 proteins; SLC6A14.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kozak, Katarzyna: NtZIP11 – a novel protein involved in plant response to elevated Zn level

Katarzyna Kozak, Anna Papierniak, Małgorzata Palusińska, Karolina Maślińska and Danuta Maria Antosiewicz

1. Department of Plant Anatomy and Cytology, Faculty of Biology, University of Warsaw, 1 Ilji Miecznikowa Str., 02-096 Warsaw, Poland

Contact for the abstract: Katarzyna Kozak; katarzyna.kozak@biol.uw.edu.pl

Phytoremediation uses plants to remove pollutants from the soil, water or air. In previous studies, we noticed that tobacco (*Nicotiana tabacum* var. Xanthi), a species considered for phytoremediation, possessed an ability to store high concentrations of Zn in leaves without symptoms of toxicity. Consequently, the existence of "Zn-storage cells" within the palisade parenchyma of tobacco leaves has been shown, and their role in the protection of neighbouring non-accumulation cells from Zn toxicity was assigned. In this study, we identified genes encoding proteins potentially involved in the accumulation of metals in "Zn-storage cells" – one of which was *NtZIP11*. It was cloned and the protein was classified as a plasma membrane Zn importer. *NtZIP11* expression was upregulated in plants exposed to Zn excess, the highest transcript level was detected in the leaves [1.]. Moreover, Zn accumulation pattern in tobacco leaves (Zinpyr1 staining) was compared with the tissue-specific expression of *NtZIP11p::GUS* determined by histochemical staining. The *NtZIP11*p::*GUS* transgenic lines were grown on medium with and without Zn excess. Cross-sections through leaf blades were subjected to either GUS or Zinpyr1 staining. The patterns of blue staining (GUS activity) and green staining (Zinpyr1 fluorescence, indicating the amount of Zn) were the same. Staining was seen primarily in the groups of palisade mesophyll cells and in veins, which suggests that expression of *NtZIP11* (upregulated by Zn excess) takes place in “Zn storage cells” containing a high amount of Zn. In conclusion, it is probable that NtZIP11 participates in Zn uptake into the “Zn-storage cells”. It might be a strategy allowing storage of a large amount of Zn in the leaf without causing harmful effects on a whole organ. References: [1] Kozak et. al., Environmental and Experimental Botany 157 (2019) 69-78. Funding: Polish Science Centre (NCN), HARMONIA-6 call (2014/14/M/NZ3/00527).

Keywords: NtZIP11, zinc, tobacco, transporter.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kozak, Tamara: The combined application of Newcastle disease virus and cytostatics against prostate cancer cells with different malignancy *in vitro*

Tamara Kozak [1,2], Alexandra Lykhova [1], Nazar Vydasov [1]

1. RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NASU, Kyiv, Ukraine;
2. Educational and Scientific Centre Institute of Biology and Medicine, Taras Shevchenko National University of Kyiv, Ukraine

Contact for the abstract: Tamara Kozak; kozaktamara.work@gmail.com

At the present day, it is a huge problem that some types of prostate cancer (PC) can be insensitive to hormone therapy and/or can be resistant to chemotherapy or develop this ability. It`s obvious, that we need to find alternative ways except to traditional cytostatic drugs (taxanes) for such cases. Combinations of rarely used for PC chemotherapy and a virus with cancer cells target can be an interesting remedy for *in vitro* research. Newcastle disease virus (NDV) is used for cancer complex treatment since this virus isn`t harmful to the human organism and can stimulate the synthesis of antitumor and immunomodulatory cytokines. Our research aim was *in vitro* study the impact of the NDV LaSota vaccine strain in combination with vincristine or cisplatin (CP) on the viability of human PC cells with different malignancy. We observed that low malignancy LNCaP cells (hormone-sensitive) were more susceptible to applied chemotherapy compared to high malignancy PC-3 cells (hormone-resistant). Regarding the virus, both cell lines were receptive similarly. Provided complex action of NDV and CP on PC cells, the six from thirteen combinations of agents have the additive/synergetic effect on LNCaP cells (for example, 10 HAU/mL NDV showed 69,33±2,89% live cells; 0,31 ug/mL CP – 89,27±1,50% and in combined mode NDV/CP – 35,19±2,20%), and only two - for PC-3 cells. In contrast, the combined influence of NDV and vincristine on these cells was more successful – seven combinations of agents on LNCaP cells lead to decrease the number of live cells on 3-22% (compared with monomode) and all combinations on PC-3 cells reduce the number of live cells on 10% (compared with monomode) and have the additive/synergetic effect. These results can become a base for further investigations of complex treatment with NDV and vincristine on PC, especially in the case of high malignancy form.

Keywords: prostate cancer, Newcastle disease virus, vincristine, cisplatin.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kulig, Kamila: Comparison of *Candida albicans* extracellular vesicles produced at different culture conditions

Kamila Kulig[1], Justyna Karkowska–Kuleta[2], Maria Rąpała–Kozik[2], Andrzej Kozik[1]

1. Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow
2. Department of Comparative Biochemistry and Bioanalytics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow

Contact for the abstract: Kamila Kulig; kamila.kulig@uj.edu.pl

Extracellular vesicles are produced by different pathogenic microorganisms and probably play a role of important carriers for virulence factors. So far, the production of extracellular vesicles has been described for bacterial species such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* as well as some fungi such as *Saccharomyces cerevisiae* and *Cryptococcus neoformans*. *Candida albicans* is a commensal yeast-like fungus that in healthy individuals belongs to physiological microbiota; however, in patients with impaired immune system or with disbalanced microbiota composition, it becomes a dangerous pathogen causing diseases of variable severity, ranging from relatively mild superficial infections of skin and mucous membranes to life-threatening systemic candidiases. This yeast exploits different mechanisms to infect the host cells, including the morphological dimorphism, phenotypic switching, formation of biofilms, production of hydrolytic enzymes and adhesins and the formation of extracellular vesicles. The present work aimed at a refinement of methods for the isolation and characterization of *C. albicans* extracellular vesicles. Extracellular vesicles produced by *C. albicans* were obtained by a chromatographic separation (gel filtration) of the concentrated supernatant collected after fungal growth in the form of yeast cells in different culture media and conditions. The gel filtration allowed us to obtain fractions containing fungal extracellular vesicles and fractions with proteins secreted to the extracellular environment. The concentration of proteins and lipids was compared in both fractions and particular proteins were identified with the use of tandem mass spectrometry. Inside the extracellular vesicles, proteins involved in the adhesion, virulence and cell wall biosynthesis were identified, including glucan-1,3-beta-glucosidase Bgl2, secreted beta-glucosidase Sun41, cell-surface mannoprotein Mp65 and Pir1. Further studies of the production of extracellular vesicles by *C. albicans* are important to better understand the mechanisms of virulence of these fungi and find more effective ways to prevent candidiases in the future.

Keywords: *Candida albicans*, extracellular vesicles, virulence factors, pathogenesis.

This work was supported in part by the National Science Centre of Poland (grant no. 2016/23/B/NZ6/00089 awarded to A.K.).

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kurkova, Anastasia: Epidemiology of serotypes of *S. pneumoniae* in patients older 18 years in Russia: in healthy carriers, patients with acute otitis media, pneumonia and invasive pneumococcal disease.

Anastasia Kurkova, Alexander Muravyev

1. Smolensk State Medical University

Trustee: Professor Roman S. Kozlov, corresponding member of RAS, MD, MSc, DSc

S. pneumoniae is a major cause of global morbidity and mortality. There are 97 serotypes of pneumococcus and its distribution varies by age, disease syndrome, disease severity, geographic region and over time. Due to increasing pneumococcal resistance, vaccination is the most common mechanism to prevent the burden of pneumococcal infection. To determine the spectrum of serotypes of *S. pneumoniae* in patients older 18 years in Russia. Collection of strains of *S. pneumoniae* from healthy carriers and patients with different forms of pneumococcal infection were provided by the participating laboratory. Strains were sent to reference laboratory of the Institute of Antimicrobial Chemotherapy of Smolensk State Medical University (IAC, Smolensk). All strains were re-identified using classic bacteriologic methods and MALDI-TOF Biotyper. Molecular typing of *S. pneumoniae* was performed using Real-Time PCR. Descriptive statistics was used to estimate the coverage of PCV13 and PPV23. We analysed 500 strains of *S. pneumoniae*. There were 74 strains from healthy carriers. The most common were serotypes 19F (21.62%), 6AB (18.92%), 3 (14.86%), 23F (10.81%) and 11AD (8.11%). The coverage of PCV13 was 75.67% and of PPV23 – 85.13%. 71 strains were detected from middle ear fluid in patients with AOM. The predominant serotypes were 19F (11.27%), 3 (9.86%), 6AB and 11AD (each 8.45%), 22F and 23F (each 5.63%), 14, 15AF and 23A (each 4.23%). The coverage rates for PCV13 and PPV23 were 47.90% and 61.98%, respectively. The most frequent serotypes among 311 respiratory samples from patients with pneumonia were 19F (14.15%), 6AB (11.25%), 3 (9.97%), 14 (5.79%), 23F (5.47%), 11AD (4.5%) and 22AF (4.18%). PCV13 covers 53.38% of these serotypes, while PPV23 covers 67.52%. There were obtained 44 strains of *S. pneumoniae* from patients with IPD. Serotypes 3 (20.45%), 22 AF (9.09%), 19 F, 23F and 6 AB (each 6.82%), 12, 15AF and 9 NL (each 4.55%) were mainly determined. The coverage of PCV13 and PPV23 was 52.26% and 70.45%, respectively. The most common serotypes of *S. pneumoniae* in adults were 19F (14.2%), 3 and 6AB (each 11.6%), 23F (6.4%), 11AD (5.4%), 14 (5%). In general, PPV23 covers the majority of serotypes of *S. pneumoniae*, however PCV13 covers a lower number of serotypes, that further can lead to its ineffectiveness in adult population.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kuzmicz-Kowalska, Katarzyna: Regulation of neural progenitor survival by the morphogens Shh and BMP

Katarzyna Kuzmicz-Kowalska[1], Catherine Gilbert[1], Anna Kicheva[1]

1. Institute of Science and Technology Austria, Klosterneuburg, Austria

Contact for the abstract: Katarzyna Kuzmicz-Kowalska; katarzyna.kuzmicz@ist.ac.at

In the developing vertebrate neural tube a dorsoventral pattern of neural progenitor subtypes is specified by opposing morphogen gradients of Sonic hedgehog (Shh) and Bone Morphogenetic Proteins (BMP). In addition, these morphogens have been implicated in regulating progenitor proliferation, differentiation and apoptosis as well as neural tube size. However, their precise contribution in controlling neural tube growth is still poorly understood. To address this question, we are using mouse genetics and *ex vivo* chick and mouse assays to alter Shh and BMP signaling in a temporally controlled manner. Our preliminary data in chick embryo culture and chick explants indicates that indicates inhibition of either Shh or BMP signaling results in increased cell death, while activation of either pathway promotes cell survival in a concentration dependent manner. Consistent with this, mouse mutants with different levels of Shh activity have progressively higher levels of cell death, but no defects in progenitor proliferation. Altogether, our observations suggest a model in which the probability of cell death depends on levels of spatially distributed inhibitor of apoptosis controlled jointly by levels of Shh and BMP signaling. To test this and identify the underlying molecular mechanism, we are currently investigating potential downstream targets within the cell survival machinery using genetic and genomic approaches. Altogether, our results point to a novel function of the opposing morphogen gradient system in tissue size regulation besides their function in cell fate specification. These results will provide insight into the role of morphogens in coordinating tissue growth with pattern formation.

Keywords: neural tube, apoptosis, morphogens, growth.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Kwiatkowska, Monika: Subcellular localization of mRNAs encoding mitochondrial proteins in zebrafish

Monika Kwiatkowska[1,2], Barbara Uszczyńska-Ratajczak[1], Sreedevi Sugunan[2,3], Silvia Carbonell-Sala[4], Maciej Migdał[2], Cecilia Winata[2], Agnieszka Chacińska[3,5]

1. Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan, Poland
2. International Institute of Molecular and Cell Biology, Warsaw, Poland
3. Centre of New Technologies, University of Warsaw, Poland
4. Centre for Genomic Regulation, Barcelona, Spain
5. International Institute of Molecular Mechanisms and Machines PAS, Warsaw, Poland

Contact for the abstract: Monika Kwiatkowska; mkwiatkowska@ibch.poznan.pl

Despite the presence of mitochondrial DNA, the vast majority of mitochondrial proteins (~99%) are encoded by the nuclear genome. Therefore, the biogenesis of functional mitochondria relies on the synthesis and import of those proteins in their precursor form into the organelle. The vast majority of mitochondrial proteins can be imported into mitochondria in a post-translational manner, after completing their synthesis in the cytosol. Therefore, for decades, this type of import was considered the main route for mitochondrial proteins. At the same time, the evidence for alternative routes has been long debated. Although in eukaryotes proteins are mainly synthesized in the cytosol, subcellular structures like endoplasmic reticulum (ER) or mitochondria can exploit localized protein synthesis, designed to meet the local needs of the cell. Moreover, it has been shown that in living cells the translation of nuclear-encoded proteins can be combined with their import into mitochondria. Although the mechanisms and machinery of mitochondrial protein import have been well described, the cytosolic stage of this process remains poorly understood, especially in higher eukaryotic organisms. To address these limitations, we investigated the subcellular localization of nuclear-encoded mRNAs coding mitochondrial proteins using Illumina and long-read Oxford Nanopore Technology (ONT) RNA sequencing. Our results suggest that post-translational import likely to be the main route for mitochondrial proteins, whereas the co-translational import is rather a specialized path that restricts itself to transport mainly large and evolutionary conserved proteins, frequently possessing transmembrane domains. Additionally, the ONT sequencing results allowed us to detect new splicing events and extend existing zebrafish genome annotation.

Keywords: zebrafish, mitochondria biogenesis, mRNA subcellular localization, RNA-seq.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Langari, Ariana: Morphology of red blood cells derived from women with early pregnancy loss. Atomic force microscopy study

Ariana Langari[1], Velichka Strijkova[1,2], Sashka Krumova[1], Ina Giosheva[1,3], Emil Gartchev[3], Alexey Savov[3], Kameliya Kercheva[3], Stefka G. Taneva[1], Svetla Todinova[11] Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences,

Acad. G. Bonchev Str. Bl. 21, 1113 Sofia, Bulgaria

1. Institute of Optical Materials and Technologies "Acad. Yordan Malinovski", Bulgarian Academy of Sciences, Acad. G. Bontchev Str. 109, 1113 Sofia, Bulgaria
2. University Obstetrics and Gynecology Hospital "Maichin Dom", 2 Zdrave Str., Sofia, Bulgaria

Contact for the abstract: Ariana Langari; arianalangari@abv.bg

The morphology of human red blood cells (RBCs) is an important indicator of cells functionality and health. In the present study we combine optical and atomic force microscopy to evaluate the alteration of RBCs morphology during cells aging in women with early pregnancy loss, as compared to healthy pregnant and parous women. The freshly isolated RBCs from women with early pregnancy loss do not differ significantly from those of healthy pregnant subjects, as well as parous women, and the biconcave disc shape is the predominant morphology. Along the cell aging path RBCs undergo different morphological alterations and in the end of the follow-up period of 50 days the spiculated and crenated cells become the dominant morphologies. A time-dependent trend for the reduction of the RBCs size and membrane roughness is observed. The kinetics of these changes differs for the three studied groups – they occur at a much earlier stage for early pregnancy loss women than for the control ones. Furthermore, the roughness and the size of RBCs from those women diminishes exponentially, while linearly for both the control parous and the pregnant women. Our results provide an evidence for accelerated aging of RBCs derived from women with early pregnancy loss as compared to healthy controls. Data might serve as a basis for the development of morphometric algorithm to distinguish between different pathological conditions during early pregnancy.

Keywords: atomic force microscopy, red blood cells, cells aging, early pregnancy loss.

Acknowledgements:

This work is supported by grant КП-06-Н21/4, Competition for financial support for basic research projects – 2018, National Science Fund (S.T.).

Research equipment of Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science was used in this investigation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Latoszek, Ewelina: Role of CacyBP/SIP in the regulation of mutant huntingtin aggregation

Ewelina Latoszek[1], Magdalena Czeredys[1], Jan Ludwiczak[2], Stanisław Dunin-Horkawicz[2], and Jacek Kuźnicki[1]

1. International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Neurodegeneration, Poland
2. Structural Bioinformatics Laboratory, Centre of New Technologies, University of Warsaw, Poland

Contact for the abstract: Ewelina Latoszek; ewelina.latoszek@hotmail.com

Huntington's disease (HD) is a neurodegenerative disease caused by the expansion of CAG triplets (encoding glutamine, Q) in the huntingtin gene (HTT). In the striatum of YAC128 mice, the model for HD, we observed an increased level of CacyBP/SIP dimers comparing to control mice (Czeredys et al., 2013). These results may suggest that the physiological function of CacyBP/SIP is disturbed in this model. It is known that CacyBP/SIP participates in the ubiquitin-mediated degradation of β-catenin as a component of the ubiquitination complex. Therefore our aim was to verified the role of wild-type CacyBP/SIP and its mutants in the dimerization domain on ubiquitination of mutant HTT. Using in silico methods such as Rosetta and Molecular Dynamics we predicted mutations that should stabilize (K21T and T30R_S33E) CacyBP dimerization domain and then introduced them into CacyBP/SIP. As a cellular model of HD, we used the HEK293 line, which was transfected both with plasmids encoding mutant HTT with 72Q-RFP or wild-type HTT with 25Q-RFP (as a control) and with plasmids containing wild-type CacyBP or its dimerization mutants. With the application of fluorescent microscopy in cells overexpressing both CacyBP and mutant HTT we found the decreased number and size of aggregates as compared to control. Using immunoprecipitation and western blot analysis we detected that CacyBP/SIP decreases protein level of mutant HTT and increases its ubiquitination. We also found that CacyBP/SIP dimerization mutants are less active in the inhibition of mutant HTT aggregation by CacyBP and mutant HTT is less ubiquitinated in their presence. Our results indicate that CacyBP/SIP is responsible for inhibition of mutant HTT aggregation by facilitating its ubiquitination. The dysregulation of CacyBP/SIP dimers could be potential pathology mechanisms of HD.

Keywords: Huntington's disease, huntingtin, CacyBP/SIP, dimerization, aggregation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Leszczyński, Paweł: The role and regulatory mechanism of Prdm3 in neuronal differentiation

Paweł Leszczyński[1], Magdalena Śmiech[1], Aamir Salam Teeli [1] Hiroaki Taniguchi[1]

1. Institute of Genetics and Animal Breeding, Laboratory of Genome Editing and Transcription Regulation, Polish Academy of Science, Jastrzębiec, Poland

Contact for the abstract: Paweł Leszczyński; p.leszczynski@ighz.pl

Prdm (PRDI-BF1(positive regulatory domain I-binding factor 1) and RIZ1 (retinoblastoma protein-interacting zinc finger gene 1) homologous domain containing) transcription factors are a group of proteins that have received considerable attention due to their importance in regulating the development of several organs including brain. Prdm3 plays an important role in hematopoiesis but the function in the neural development is poorly understood. In our study, we examined the role and regulatory mechanism of Prdm3 in neurogenesis. Using CRISPR/Cas method, we first generated *Prdm3* gene knockout in P19 cell line (frequently used as a model of neurogenesis). *Prdm*3 knockout caused premature neural differentiation of P19 cells, but also increased non-neural cells proliferation. In order to elucidate the mechanism of Prdm3 expression, we selected a potential regulatory region using *in silico* analysis. From many indicated factors, we focused on the Gata proteins and retinoic acid (RA)-dependent signaling. The functions of Gata factors are well recognized in the development of endodermal organs, but their impact on the development of the neurons is poorly defined. It is known that RA activates *Prdm3* expression and can exert synergistic effect with Gata2, but this mechanism has not been studied in Prdm3-dependent neurogenesis. We found that Gata6 expression is increased during neurogenesis. Based on luciferase reporter assay, we confirmed Gata-dependent activation of Prdm3 regulatory element. Moreover, our experiments identified a synergistic effect of the RA stimulation and Gata6 factor on the activity of Prdm3 regulatory element. To determine the RA-dependent mechanism, we focused on RA receptors (RARs) and we found that RARα and RARβ augmented the activity of Prdm3 regulatory element. Our study indicated that Prdm3 plays an essential role in neuronal differentiation and its expression is synergistically regulated by Gata6 and RA signaling pathway.

Keywords: neurogenesis, Prdm3, P19 cells, Gata6, retinoic acid.

The study was financially supported by the National Science Centre, Poland (grant no. 2017/25/N/NZ3/01886).

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Lučinskaitė, Ieva: Antioxidant Activity and Organic acid Changes in *Picea abies (L). H. Karst* Needles After Treatment With Cold Plasma and Electromagnetic Field

Ieva Lučinskaitė[1*], Kristina Laužikė[2], Vytautas Čėsna[1], Vaida Sirgedaitė – Šėžienė[1]

1. Institute of Forestry, Lithuanian Research Centre for Agriculture and Forestry, Liepų str. 1, LT-53101 Girionys, Kaunas District, Lithuania
2. Institute of Hirticulture, Lithuanian Research Centre for Agriculture and Forestry, Kauno St. 30, Babtai, LT-54333 Kaunas District, Lithuania

***Contact for the abstract:** Ieva Lučinskaitė; ieva.luc@gmail.com

Woody plants residues contains a large amounts of polyphenols that represent the main group of secondary metabolites in plants which are useful for human health. *Pinacea* species are considered as a natural source of antioxidant compounds and characterized by their pharmaceutical and nutraceutical properties. Pre-sowing seed treatment with new alternative technologies (cold plasma or electromagnetic methods) induce not only plant growth but also substantial increase in amount of biologically active compounds. HPLC and spectrofotometric methods were used in order to identify the change of organic acids concentration and antioxidant activity (DPPH and ABTS) in the needles of 7 half-sib families of *Picea abies* in response to seed treatment with physical stressors: cold plasma for 1 and 2 min (CP1, CP2) and electromagnetic field for 2 min (EMF2). The results showed that the concentration of organic acids, DPPH and ABTS depended on treatment duration and the *Picea abies* genotype. The higher increase of DPPH and ABTS was determined in 541 half-sib family of *Picea abies* after 1 minute of CP1 exposure (26.050 mg g^{-1} and 30.386 mg g^{-1}, respectively comparing with control). The data of research indicate the statistical significant increase of malic acid (0.810 mg g^{-1}), succinic acid (4.064 mg g^{-1} comparing with control), citric acid (0.181 mg g^{-1} comparing with control), oxalic acid (0.032 mg g^{-1} comparing with control) and ascorbic acid (3.321 mg g^{-1} comparing with control) in 541 half-sib family of *Picea abies* after CP1 exposure. Cold plasma showed stimulating effect on various biological processes in *Picea abies* needles. For this reason it could use as an innovative technology in agriculture, ecology and industry of food. The results indicate that the plant genetic selection also plays an important role for the further research of use of physical stressors in the manufacture of nutritional supplements.

Keywords: Norway spruce, physical stressors, cold plasma, genetic selection.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Łagosz-Ćwik, Katarzyna*: Porphyromonas gingivalis* alters DNA methylation in gingival fibroblasts – implications for the pathogenesis of periodontitis

Katarzyna Łagosz-Ćwik[1], Sławomir Dudek[1], Aleksander Grabiec[1]

1. Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Contact for the abstract: Katarzyna Łagosz-Ćwik; kasia.lagosz@doctoral.uj.edu.pl

Periodontitis is a chronic inflammatory disease caused by the presence of pathogenic bacteria in the periodontium. The anaerobic bacterium *Porphyromonas gingivalis* plays a key role in driving the chronic inflammation. Interactions between *P. gingivalis* and structural gingival cells, such as fibroblasts, significantly contribute to the pathogenesis of periodontitis. However, little is known about the role of epigenetic mechanisms, in particular DNA methylation, in the pathogenesis of periodontitis. Here we examined the effects of *P. gingivalis* on DNA methylation and expression of DNA methyltransferases (DNMTs) in primary human gingival fibroblasts (GFs). Challenge with bacteria induced *DNMT1* expression 24 hours post-infection which translated into increased methylation level of the *MyD88* promoter. Interestingly, we noted the opposite profile of changes in DNA methylation in a chronic model of infection that we established to study long-lasting effects of *P. gingivalis*. *DNMT1* expression and global DNA methylation in pre-infected GFs stimulated with TNF for 24 hours at 7th day after removal of bacteria were decreased. We also observed elevated IL-6 and IL-8 secretion in pre-infected GFs stimulated with TNF at different time points and this trend was maintained up to 7 days after infection. To gain more insight into the consequences of altered DNA methylation pattern in *P. gingivalis*-challenged GFs, we investigated the influence of a DNMT1-specific inhibitor (decitabine) on GF antimicrobial responses and internalization of bacteria. Decitabine caused an increase of bacterial adherence and we hypothesize that it may be attributed to *MyD88* promoter hypomethylation that we observed. We also showed increased expression and secretion of the antimicrobial chemokine CCL20 in *P. gingivalis*-infected GFs. Collectively, our data show that DNA methylation profile is differentially regulated immediately upon infection and in a chronic infection model. Future studies will be aimed at unraveling the consequences of altered *MyD88* promoter methylation in GF response to *P. gingivalis*.

Keywords: *Porphyromonas gingivalis*, gingival fibroblasts, DNA methylation, epigenetics.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Maksymowicz, Małgorzata: Intracellular trafficking and signaling of lymphotoxin β receptor (LTβR)

Małgorzata Maksymowicz[1], Magdalena Banach-Orłowska[1], Marta Miączyńska[1]

1. Laboratory of Cell Biology, International Institute of Molecular and Cell Biology, Warsaw, Poland

Contact for the abstract: Małgorzata Maksymowicz malgorzata.m.maksymowicz@gmail.com

Tumor Necrosis Factor Receptor Superfamily (TNFRS) members are responsible for maintenance of the immune system homeostasis. One of them, lymphotoxin β receptor (LTβR) mediates inflammatory responses in various cell types. At the cellular level, upon ligand binding, the receptor activates the pro-inflammatory NF-κB and AP-1 pathways. Yet, the intracellular distribution of LTβR, the routes of its endocytosis and their connection to the signaling activation were not characterized. Here, we investigated the contribution of LTβR internalization to its signaling potential. We depleted the regulators of different endocytic routes (clathrin-mediated, dynamin-dependent or clathrin-independent). This resulted in the impairment of LTβR internalization, indicating that this receptor uses multiple entry pathways. Cells deprived of clathrin and dynamins exhibited enhanced activation of canonical NF-κB signaling represented by increased degradation of IκBα inhibitor and elevated expression of LTβR target genes. We also demonstrated that clathrin and dynamin deficiency reduced to some extent LTβR-triggered activation of the non-canonical branch of the NF-κB pathway.

Keywords: endocytosis, lymphotoxin β receptor, NF-κB pathway, cell biology

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Maciak, Karolina: The c.1495 T>C and c.1529 G>A mutations of the red blood cell pyruvate kinase gene are responsible for life-threatening hereditary chronic anemia

Karolina Maciak[1], Anna Adamowicz-Salach[2], Alicja Siwicka[2], Jarosław Poznański[1], Monika Góra[1], Beata Burzyńska[1]

1. Institute of Biochemistry and Biophysics, Polish Academy of Sciences
2. Department of Pediatrics, Hematology and Oncology, Medical University of Warsaw

Contact for the abstract: Karolina Maciak; kmaciak@ibb.waw.pl

An accurate diagnosis of congenital hemolytic anemias is sometimes difficult but it is crucial for appropriate care and treatment. Red cell pyruvate kinase deficiency (PKD) is a glycolytic defect causing congenital non-spherocytic hemolytic anemia. PKD is transmitted as an autosomal recessive trait, the clinical symptoms occurring in homozygous and compound heterozygous patients. The clinical features of PKD are highly variable, varying from mild to very severe neonatal anemia which can lead to death in the neonatal period. The aim of our study was to establish the molecular characteristics of severe hemolytic anemia in three brothers. Two brothers have been under the care of the outpatient department of hematology since birth due to hemolytic anemia of unknown etiology. The RBC parameters and biochemical tests did not clearly indicate the cause of the anemia. The third brother died shortly after birth because of massive hemolytic anemia. Parents of the patients were not anemic. Using NGS sequencing we detected two different mutations in the PKLR gene in the probands. One mutation (c.1529 G>A) leads to the replacement of arginine by glutamine at position 510 (R510Q). It is the most common mutation of the PKLR gene in Northern and Central Europe. The second mutation (c.1495 T>C) which predicts a serine for proline substitution at position 499 (S499P) is a novel mutation. Sanger sequencing confirmed the presence of the mutations c.1529 G>A in the mother and c.1495 T>C in the father, both in heterozygotic condition. Bioinformatics analysis confirmed the deleterious effect of both mutations. Molecular modeling also suggested that the structural changes induced by the R510Q and S499P substitutions have a direct impact on the stability of the enzyme. To conclude, in some cases of congenital hemolytic anemias molecular characterization of the defect by NGS is essential for establishing of the proper diagnosis.

Keywords: congenital hemolytic anemia, pyruvate kinase deficiency, WES.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Mandal, Pratik Kumar: Deciphering the unique proteostatic and metabolic adaptations of RPMs to altered iron levels

Pratik Kumar Mandal[1], Patryk Ślusarczyk[1], Gabriela Jędruszewska[1], Wojciech Pokrzywa[1] and Katarzyna Mleczko-Sanecka[1]

1. International Institute of Molecular and Cell Biology, Warsaw, Poland

Contact for the abstract: Pratik Kumar Mandal; pmandal@iimcb.gov.pl

Corresponding author. Email: wpokrzywa@iimcb.gov.pl, kmsanecka@iimcb.gov.pl

Iron is an essential micronutrient and iron-containing protein complexes are required for various biological processes. In mammals, erythropoiesis utilizes the highest amount of iron for heme synthesis to synthesize haemoglobin in over 360 billion RBCs daily. The splenic red pulp macrophages (RPMs) recycle about 5 million senescent RBCs by erythrophagocytosis each second. A critical factor for the functioning of RPMs is the autophagy-lysosomal system. The RBCs-containing phagosome merges with lysosomal vesicles (forming the erythrophagolysosome) where senescent RBCs are degraded. After phagocytosis of senescent RBCs, haemoglobin is degraded into heme and globin by proteolytic system. It was largely unknown if the rates of RBCs uptake and digestion are regulated by fluctuating iron availability, and specifically if these processes may be affected by iron deficiency. Surprisingly, our preliminary data revealed that RPMs possess specialized mechanisms that enhance the rate of RBC removal under iron-deficient (ID) conditions, a phenomenon that likely contributes to the adaptation of the whole organism to limited iron supplies. We found that in a mildly anemic mouse model of dietary iron deficiency, RPMs exhibit enhanced lysosomal activity, suggesting their higher potential for digestion of RBCs. Strikingly, iron-depleted RPMs exhibit enhanced mitochondrial membrane potential and increased mitochondria mass, indicative of accelerated oxidative metabolism and elevated energetic demands. Such a boost of metabolic activity in iron-deficient RPMs is unique in comparison to other cell types which typically 'slow down' their metabolism when iron levels drop. We hypothesized that this well-orchestrated response of RPMs to iron deficiency likely contributes to the 'adaptation' of the whole organism to limited iron supplies.

Keywords: Macrophages, Iron Homeostasis, Anemia.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Maślińska, Karolina: Search for mechanisms regulating the efficiency of Zn/Cd translocation to shoots

Karolina Maślińska[1], Anna Barabasz[1], Małgorzata Palusińska[1], Katarzyna Kozak[1] and Danuta Maria Antosiewicz[1]

1. Faculty of Biology, University of Warsaw, 1 Ilji Miecznikowa Str., 02-096 Warsaw, Poland

Contact for the abstract: Karolina Maślińska; k.maslinska@student.uw.edu.pl

The regulation of Zn homeostasis is closely related to the homeostasis of other metals by common pathways. This research aimed at understanding the role of the NtZIP4 protein in the phenomenon of Cd-depended stimulation of Zn translocation to shoots in tobacco. The RNAi plants with the reduced *NtZIP4* mRNA level were generated. Plants (transgenic and wild-type) were grown on medium containing various concentrations of Zn and Cd. Whole roots and shoots were collected to determine both metals concentrations by AAS. The ratio of Cd shoot-to-root concentration in transgenic plants decreased by ~ 50% (as compared with the wild-type). In plants exposed to 0,25µM Cd the concentration of Zn increased (relative to those grown without Cd). This research showed that NtZIP4 participates in the regulation of the efficiency of Zn/Cd-dependent translocation to shoots of both metals.

Keywords: zinc, cadmium, NtZIP4, tobacco.

The study was financially supported by OPUS-8 no. 2014/15/B/NZ9/02303 by the National Science Centre, Poland.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Michniak, Katarzyna: The localization of YAP and cell polarity factors in preimplantation rabbit embryo

Katarzyna Michniak, Elżbieta Wenta-Muchalska, Katarzyna Filimonow, Anna Piliszek

In the early mammalian embryo, the first lineage choice is that between the trophectoderm (TE) which will form embryonic part of the placenta, and the inner cell mass (ICM), which will give rise to the embryo proper and yolk sac. Correct differentiation of TE lineage is essential for successful implantation and pregnancy. It was confirmed that the Hippo pathway controls the first embryo lineage specification in the early mouse embryo. In the inside cells, the Hippo pathway is active, leading to phosphorylation and degradation of the transcription factor YAP by cell polarity protein complex. In the outside cells, inactive Hippo allows for YAP nuclear translocation which in turn activates trophectoderm specific transcription factors expression. Our preliminary data show that the rabbit is a useful model to study trophectoderm development in non-rodent mammalian species. In contrast to the mouse, cavitation in the rabbit embryo starts at the 64-cell stage, which affects the ratio of inside and outside cells. We have confirmed that the main component of the Hippo pathway, YAP is present in the rabbit embryo at 3.5 dpc. It is differentially localized in ICM (cytoplasmic) and TE (nuclear), suggesting that, similar to the mouse, TE cell fate is related to Hippo pathway activity. Here we present localization of YAP and cell polarity factors PKC, P-ERM, beta-catenin and f-actin in the rabbit embryo from the late morula to the expanded blastocyst stage. Curiously, we found that important adhesion protein E-cadherin is not localized in a polarized manner in the rabbit embryo.

This work was funded by Narodowe Centrum Nauki (SONATA BIS nr 2017/26/E/NZ3/01205).
Deposited in PMC for immediate release.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Mierzejewski, Bartosz: Muscle interstitial progenitor cells and their differentiation potential

Mierzejewski Bartosz[1], Grabowska Iwona[1], Jackowski Daniel[1], Irhashava Aliksandra[1], Michalska Zuzanna[1], Stremińska Władysława[1], Jańczyk-Ilach Katarzyna[1], Ciemerych Maria Anna[1], Brzóska Edyta[1]

1. Department of Cytology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Contact for the abstract: Bartosz Mierzejewski; bmierzejewski@biol.uw.edu.pl

Skeletal muscles regeneration relays on stem cells called satellite cells. In injured muscles, satellite cells become activated, start to proliferate, differentiate in myoblasts, which further fuse to form myotubes and myofibers. However, many other cell types present in muscle support muscle regeneration and some of them manifest myogenic potential similar to that one of satellite cells. In present study we used CD146 marker to isolate interstitial progenitor cells from murine hind limb muscles. Their localization in muscle, expression of selected markers, clonogenic, myogenic, chondrogenic, and osteogenic potential *in vitro* were examined and compared with results obtained for satellite cell-derived myoblasts and bone marrow-derived mesenchymal stromal cells. Moreover, heterotopic transplants of examined cell populations were performed and their ability to differentiate was analyzed. Our results showed that CD146+ muscle interstitial progenitor cells are localized outside basal lamina of myofibers, express nestin and do not express Pax7. These cells were also myogenic but failed to follow chondrogenic and osteogenic program, both *in vitro* and *in vivo*. We conclude that CD146+ cells obtained from murine hind limb skeletal muscles represent muscle interstitial progenitor cells which differ from satellite cells and show clonogenic and myogenic potential.

Keywords: mouse**,** skeletal muscle regeneration, differentiation, satellite cells, mesenchymal stromal cells, interstitial cells, CD146.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Minina, Elena: Differential gene expression analysis in patients with bronchial asthma on the viral infection background

E.S. Minina[1], A.K. Kakoichankava[1,2], I.S. Minin[3]

1. Vitebsk State Medical University, Vitebsk, Belarus
2. Nativita, Minsk, Belarus
3. Digital Vision Labs LLC, Moscow, Russia

Contact for the abstract: Elena Minina; l.mnn1551@gmail.com

In the course of our study, an analysis of 56 nasal lavage cell transcriptomes obtained from the Gene Expression Omnibus database (GSE30326, GSE51392) was performed. The study included patients with exacerbation of bronchial asthma (BA) on the picornavirus infection background (n=32), patients with BA and healthy people (without allergic diseases) whose isolated epithelial cells were stimulated with Polyinosinic:polycytidylic acid (n=24). For the analysis of transcriptome data, Transcriptome Analysis Console (TAC) Software, ThermoFisher was used. Analysis of the transcriptome data of the patients with BA and healthy people when modeling a viral infection revealed activation of VEGFA-VEGFR2 signaling pathway, IL-18 signaling pathway, regulation of Toll-like receptor signaling pathway, interferon alpha/beta signaling pathway, type II interferon signaling pathway (IFNG), decreased activation of ciliary landscape and cell cycle signaling pathways. It should be noted that the significance of these pathways was more pronounced in healthy people. During exacerbation of BA on the picornavirus infection background compared with the state after virus-induced exacerbation of BA (after 7-14 days), overexpression of a number of genes involved in VEGFA-VEGFR2 signaling pathway, IL-18 signaling pathway, regulation of Toll-like receptor signaling pathway, interferon alpha/beta signaling pathway, type II interferon signaling pathway (IFNG) was observed. Thus, the functional analysis of the nasal lavage cell transcriptomes in patients with BA and picornavirus infection and in the model «BA + viral infection» demonstrates the activation of signaling pathways aimed at mobilizing the adaptive antiviral immune response system associated with increased production of interferons, activating the Toll-like receptors and interleukin-18. Moreover, in comparison with healthy people (without allergic diseases), immunosuppression is observed in patients with BA on the viral infection background.

Keywords: transcriptome, bronchial asthma, viral infection.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Mioduszewski, Łukasz: Polyglutamine aggregation and phase diagram in a coarse-grained model

Łukasz Mioduszewski[1,2], Marek Cieplak[1]

1. Institute of Physics, Polish Academy of Sciences, Warsaw, Poland
2. Faculty of Mathematics and Natural Sciences, Cardinal Stefan Wyszyński University in Warsaw, Poland

Contact for the abstract: Łukasz Mioduszewski; l.mioduszewski@uksw.edu.pl

When polyglutamine tracts present in some proteins get longer than a specific threshold, they can cause neurodegenerative diseases, including Huntington disease. There are many hypotheses trying to explain this phenomenon, ranging from amyloid formation, through cytotoxicity of oligomers, to knot formation in monomers that prevents their digestion by proteasome. We simulate many polyglutamine chains (total system size is 1800 residues) of length 20 (below threshold), 40 and 60 (above threshold) in various temperatures and densities. This allows us to construct a phase diagram, based on the number of contacts between different chains, and on the relative orientation between them: we introduce an "order parameter" that allows us to distinguish between random orientation in high temperatures from amyloid-like ordering of chains in low temperatures. We study how clusters of chains connected together form and break, and what is the population of amyloids, oligomers and single polyglutamine chains in various conditions. The molecular dynamics simulation model represents each residue as one pseudo-atom and includes implicit solvent. It was parameterized for intrinsically disordered proteins, like polyglutamine.

Keywords: Polyglutamine, Neurodegeneration, Coarse-graining, Modeling, Simulations, Aggregation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Mrozowicz, Justyna: Effect of vitamin D supplementation on liver transcriptome in rats

Justyna Mrozowicz[1], Alicja Wierzbicka[1], Barbara Szymczyk[2], Małgorzata Świątkiewicz[2], Anna Koseniuk[1,] Maria Oczkowicz[1]

1. National Research Institute of Animal Production, Department of Animal Molecular Biology.
2. National Research Institute of Animal Production, Department of Animal Nutrition and Feed Science.

Contact for the abstract: Justyna Mrozowicz; justyna.mrozowicz@izoo.pl

Vitamin D is widely recognised as a potent modulator of bone calcification, immunity, and many human diseases like cancer, diabetes, autoimmune disorders or depression. Analysis of pathways activated after vitamin D supplementation could help to understand the molecular mechanisms engaged in vitamin D action. Thus, we aimed to establish the effect of vitamin D supplementation on liver transcriptome using 3`quant mRNA sequencing. The experiment was carried on 12 male 4-months old Wistars rats divided into two groups: one received no supplementation while the other group was supplemented with 5000 U/Kg of feed for three months. After RNA isolation, twelve libraries of 3`quant mRNA QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen) were created and sequenced on Illumina Hiscan 2500. Bioinformatic analysis was performed on Bluebee platform, which uses DESeq2 software for Differentially Expressed Genes (DEGs) identification. We observed little effect of vitamin D supplementation on the liver transcriptome. Using standard criteria (1.5 fold change, p-adjusted<0.05), no DEGs were identified in our data. Nonetheless, we performed Gene Set Enrichment Analysis (GSEA) of the whole dataset and identified several enriched Gene Ontology terms and pathways. Among the most enriched KEGG pathways, we identified "Signalling pathway regulating pluripotency of stem cells" (FDR=0.02 Normalized Enrichment Score (NES) =1.9), "TGF beta signalling pathway" (FDR=).01, NES=1.9, Steroid biosynthesis FDR=0.06, NES=-1.8, while among the Panther pathways we identified "Vitamin D metabolism and pathway" (FDR=0.24, NES=-1.66) and GABA-B receptor II signalling (FDR=0.016, NES=1.9). Enriched Biological Processes included: "exocrine system development", "heart development", "dendrite development". Our results suggest that three months supplementation with 5000 U of vitamin D may have a subtle impact of cholesterol biosynthesis and developmental processes in young rats.

Keywords: Vitamin D, rats, transcriptome, RNA-seq.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Nowacka, Marta: A three-dimensional microenvironment affects chemosensitivity of ovarian cancer cells

Nowacka M.[1], Januchowski R.[1]

1. Department of Histology and Embryology, Poznan University of Medical Sciences, Święcickiego 6 St., 61-781 Poznań, Poland.

Contact for the abstract: Marta Nowacka; marta.nowacka@student.ump.edu.pl

Epithelial Ovarian cancer (EOC) is the leading cause of death in gynecological malignances. Standard treatment of EOC is a combination of aggressive cytoreductive surgery followed by chemotherapy. However, such treatment is insufficient and diseases recurrent in 80 percent of patients. The main reason of recurrences is primary or acquired drug resistance. The drug resistance phenomenon can be driven by cellular mechanisms such as: an overexpression of Pgp and BCRP proteins; or mechanisms specific to cancer tissue. Tissue specific mechanisms are related to tumor microenvironment and expression of Extracellular Matrix (ECM) molecules. Three dimensional (3D) in vitro cancer models can provide cell and ECM organization with higher biological relevance when compared to typical two dimensional (2D) monolayers. *The aim of this study was* to evaluate the role of extracellular matrix molecules in development of drug resistance in ovarian cancer. We characterized 3D in vitro models of A2780 EOC cell line and 2 sublines resistant to paclitaxel (A2780P1 and A2780P2). We compared gene and protein expression with 2D cell cultures and tested their efficacy as models for evaluating chemoresistance. The 3D cell culture was more resistant to paclitaxel in comparing with its 2D counterpart. Resistance increase was more significant in 3D resistant sublines (A2780P1, A2780P2) than in sensitive variant A2780. Gene expression profile was not altered in examined 2D and 3D cell cultures. Examined cell cultures differed in spheroid morphology. In conclusion, three dimensional structure of the tumor might play crucial role in drug resistance. 3D cell culture exhibited more relevant conditions to in vivo systems and represented more reliable tool to estimate drug sensitivity.

Keywords: 3D cell cultures, ovarian cancer, drug resistance, ECM.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Osredek, Ivana: Detection and validation of personalized therapeutic TCRs for adoptive T cell therapy in glioblastoma patients

Ivana Osredek[1], Chin Leng Tan[1], Edward Green[1], Michael Platten[1]

1. German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ)

Contact for the abstract: Ivana Osredek; i.osredek@dkfz-heidelberg.de

Immune checkpoint inhibitors have shown promising results in patients whose tumors have many mutations, but less so in cancers such as glioblastoma, which has a low somatic mutational load and limited intratumoral infiltration of immune cells. For such tumors, identifying the patient's T cell receptors (TCR) that target their tumor antigens and reintroducing them transgenically as part of adoptive cell therapy, has led to complete remission in a small number of patients. Such personalized therapy is clearly the future yet realizing this requires the intercalation of bioinformatics and molecular biology into existing clinical practice; in particular, during the critical first step, namely the identification of tumor-reactive TCRs. To detect and visualize those T cells that are tumor-reactive, we performed single-cell combined RNA and TCR sequencing using the 10X Chromium platform. We established a bioinformatics pipeline written in R utilizing the tidyverse packages to combine cutting-edge bioinformatics tools for single-cell analysis such as Seurat and scater with published repertoire analysis tools ALICE/OLGA and Cell Ranger. We also compared different approaches of data normalization, scaling and cell clustering and how they affect conclusions of biological relevance. In addition, we used the VDJdb database of validated CMV/HIV/EBV reactive TCRs to exclude these 'off-target' TCRs from further analysis. Having refined our target TCR list, we then cloned the sequences into an S/MAR DNA delivery vector, a new generation on gene expression delivery vectors, for functional validation using a NFAT reporter assay platform established in the lab.

Keywords: immunotherapy, single cell sequencing, glioblastoma, tumor reactive TCRs.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Parisi, Sofia: Towards a molecular understanding of the role of RAB33B in hereditary skeletal dysplasia

Sofia R. Parisi[1] and Jeremy C. Simpson[1]

1. School of Biology and Environmental Science & Conway Institute of Biomolecular and Biomedical Research, University College Dublin (UCD), D04 N2E5 Dublin, Ireland

Contact for the abstract: Sofia Parisi; sofia.parisi@ucdconnect.ie

Smith-McCort dysplasia (SMC) is a rare hereditary disease resulting in severe skeletal abnormalities during the development of affected individuals. Recently, three different point mutations in highly conserved regions of RAB33B gene, resulting in amino acid substitutions, have been reported. The molecular and cellular basis for SMC is not known, but, at the cellular level, it may be due to Rab33b dysfunction. Small GTP binding proteins of the Rab family, including Rab33b, are the master regulators of membrane trafficking, suggesting that the cause of SMC may be linked with altered membrane flux in cells. The Rab33b protein mainly localises to the Golgi complex and regulates transport between this organelle and the endoplasmic reticulum, as well as playing a role in Golgi homeostasis. To date, few interactors of Rab33b have been identified, among them are the Golgi protein GM130, and the endosomal proteins Rabaptin-5 and Rabex-5. Rab33b has also been implicated in autophagy, suggesting an intriguing link between its role in membrane traffic and the wider homeostasis of cell membranes regulated through autophagy. Our preliminary data suggest that the defective Rab33b proteins can no longer attach to the Golgi apparatus, resulting in consequences for membrane trafficking events. This project aims to provide a more detailed molecular understanding of the Rab33b protein, and its molecular link to SMC. Specifically, our research is addressing the functional cellular consequences of Rab33b mutants in terms of protein stability and GTPase activity; the effects of Rab33b mutations on Golgi function; the interaction network of Rab33b and the contribution of Rab33b to autophagy.

Keywords: Membrane trafficking, Rab GTPases, Rab33b, Smith-McCort dysplasia.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Pawelec, Paulina: Insight into the influence of histone deacetylase inhibitor Givinostat on CNS inflammatory response after neonatal hypoxia-ischemia

Pawelec Paulina[1], Ziemka-Nalecz Malgorzata[1], Zalewska Teresa[1]

1. Neuro Repair Department, Mossakowski Medical Research Centre, PAS, A. Pawinskiego 5 Str. 02-106 Warsaw, Poland

Contact for the abstract: ppawelec@imdik.pan.pl

Inflammation is an important contributing factor to the evolution of hypoxic-ischemic brain injury in neonates. Moreover, the inflammatory response is mainly directed by the activation of resident in the CNS microglial cells together with infiltrating cells of the peripheral immune system (leukocytes, monocytes/macrophages). Both type of cells produce soluble inflammatory molecules (cytokines, chemokines), as well as reactive oxygen and nitrogen species, what is a cause of persistent neuronal injury. It occurred recently that the treatment of adult animals after experimental brain ischemia with histone deacetylase inhibitor (HDACi) – Givinostat (ITF 2357), is associated with anti-inflammatory action. Our present investigation was to determine the influence of Givinostat on brain injury in immature animals. Seven-day-old rat pups were used to unilateral carotid artery ligation followed by 60 min of hypoxia (7.6% O_2). Givinostat (5 and 10 mg/kg b.w.) was administered in a 5-day regime with the first injection given immediately after hypoxic exposure. The damage of the ipsilateral hemisphere was evaluated by immunohistochemistry 14 days after the insult. For identification the type of microglia, we used markers labeling pro-inflammatory M1 (ED1/interleukin-1 beta) and anti-inflammatory M2 phenotype (ED1/arginase-1). Furthermore, the effect of Givinostat on expression of cytokines and chemokines in the rat brains at 24h, 72h and 5 days after HI were assessed by Luminex assay. The data suggest that Givinostat administration did not promote the conversion of the microglia phenotype from inflammatory M1 to anti-inflammatory M2. The action of Givinostat is expressed only by the decrease of pro-inflammatory molecule MIP-1α, 72 h after ischemic insult. Summarizing, it is necessary to conduct further research to completely explain the effect of this histone deacetylase inhibitor on inflammatory processes after hypoxic-ischemic insult in immature animals.

Keywords: Givinostat, hypoxia-ischemia, microglia, inflammation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Pedor, Jenni K.: Towards a simplified workflow for tRNA-seq library preparation

Jenni K. Pedor[1], Pavlína Gregorová[1], L. Peter Sarin[1]

1. The RNAcious Laboratory, Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Finland

Contact for the abstract: Jenni K. Pedor; Jenni.Pedor@helsinki.fi

During the last decade, groundbreaking discoveries have cemented the multifaceted role of transfer RNAs (tRNAs) as regulators of translation and cellular function. As tRNA research gains momentum, several high-throughput sequencing methods for the quantitative analysis of tRNA isoacceptors in cells have emerged. However, the strong secondary structure and rich post-transcriptional modification of most tRNA molecules pose significant challenges for reverse transcriptases, thus hampering library preparation and introducing quantification biases. Current approaches rely on demethylation and processive next generation reverse transcriptases, such as TGIRT, to overcome these problems. Nonetheless, demethylation only removes one type of modification while introducing the need for a control library. Here, we suggest a simplified pipeline for tRNA library preparation that omits the demethylation step and uses an alternative thermostable group II intron reverse transcriptase, MarathonRT, which is capable of reading through modifications and highly structured sequences. In addition, we will incorporate β-elimination into the workflow to provide information on the ratio of charged vs. uncharged tRNAs in the cells. This improved protocol will reduce the time and cost of tRNA-seq library preparation while providing additional information on the charge state of the tRNA pool.

Keywords: tRNA seq, reverse transcription, group II intron maturase, β-elimination.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Peachonkina , Elizaveta: The effect of Rose bengal photosensitizer on the accumulation of astaxanthin in *Haematococcus pluvialis* cells

Pechenkina E.I., Samovich T.V., Kozel N.V.

1. Institute of Biophysics and Cell Engineering, National Academy of Sciences of Belarus, 27 Akademicheskaya Str., 220072 Minsk, Belarus

Contact for the abstract: Elizaveta Peachonkina; eliza9214504@mail.ru

The microalga *Haematococcus pluvialis* is one of the most promising natural sources of astaxanthin, – a carotenoid pigment widely used in food, pharmaceutical industries and in cosmetology due to its extremely high antioxidant activity, which exceeds that of β-carotene and vitamin E. In the cells of *H. pluvialis*, the content of astaxanthin is up to 3-5% of the dry mass of algae. We used the ability of photosensitizer dyes, in particular, Rose bengal xanthene dye (RB), to generate reactive oxygen species in the light to create conditions for the accumulation of astaxanthin in *H. pluvialis* cells. RB has both one of the highest quantum yields of singlet oxygen generation and the absorption spectrum with a maximum in the green region, which is the most favorable spectral region for the induction of photooxidative stress in plant systems. When using the BR photosensitizer as an additional inducer of carotenogenesis in high-intensity light at low concentrations of 0.1–0.5 μM in the culture medium, an increase in the dry weight of the haematococcus and the number of cells in suspension compared with the action of high-intensity light alone (control) were detected. The observed increases in dry weight and number of cells are significant and exceeded control by 41 and 56% respectively. The use of low concentrations of the photosensitizer during the action of high-intensity light also leads to an increase in the production of astaxanthin more than 20% compared to the action of high-intensity light alone. We suggest that the increase the yield of astaxanthin when a RB photosensitizer is added to the *H. pluvialis* culture medium during treatment with high-intensity light is associated with the signaling properties of singlet oxygen, which may be the primary agent in the transduction of a signal that triggers increased synthesis of astaxanthin in *H. pluvialis* cells.

Keywords: *Haematococcus pluvialis* cells, astaxanthin, Rose bengal, singlet oxygen.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Pietrzak, Bernadeta: Triclocarban induces apoptosis and impairs autophagy in neuronal cell cultures

B. Pietrzak[1], A. Wnuk[1], J. Rzemieniec[1], K. Przepiórska[1], M. Kajta[1]

1. Maj Institute of Pharmacology, Polish Academy of Sciences, Department of Experimental Neuroendocrinology, Laboratory of Molecular Neuroendocrinology, Smetna Street 12, 31-343 Krakow, Poland

Contact for the abstract: Bernadeta Pietrzak; pietrzak@if-pan.krakow.pl

Triclocarban is a phenyl ether classified contaminant widely used as a compound of cosmetics or detergents and continually discharged into the environment. According to epidemiological data, exposure to environmental pollutants is strongly correlated with an increased risk of several diseases including nervous system illnesses. Nevertheless there was no data linking effects of triclocarban to apoptosis or autophagy which are involved in neurodevelopmental and neurodegenerative diseases. Therefore the aim of this study was to investigate the impact of triclocarban on apoptosis and autophagy in primary neuronal cell cultures. In light of obtained results triclocarban leads to loss of the mitochondrial membrane potential and induces caspase-3 activity. Furthermore, Hoechst 33342 and calcein AM staining visualized apoptotic nuclei formation and impaired cell survival in triclocarban-treated neocortical cells. In addition, triclocarban dysregulates expression of autophagy related genes and causes a time-dependent inhibition of autophagy related mRNA and protein expression levels. To sum up, obtained results provide evidence that triclocarban has a capacity to induce apoptosis and impair autophagy in neurons at the early developmental stages.

Keywords: apoptosis, autophagosomes, triclocarban, primary neurons, environmentally persistent chemicals.

This study was financially supported by grant no. 2015/19/B/NZ7/02449 from the National Science Centre of Poland. Presented abstract is a part of the manuscripts Kajta et al. (2019, 2020) https://doi.org/10.1007/s12035-018-1285-4, https://doi.org/10.1016/j.scitotenv.2019.134818.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Pitek, Marcin: Helix I of the DnaJ J-domain is responsible for its remarkable stability

Marcin Pitek[1], Łukasz Nierzwicki[2], Milena Stolarska[1], Igor Grochowina[1], Jacek Czub[2], Jaroslaw Marszalek[1,3], Bartłomiej Tomiczek[1]

1. Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland
2. Department of Physical Chemistry, Gdansk University of Technology, Gdansk, Poland
3. Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin, United States of America

Contact: Marcin Pitek; pitekmarcin22@gmail.com

J-domain proteins (JDPs) are regulatory partners of Hsp70 chaperones, which are required for stimulation of Hsp70s' ATPase activity and recruitment of their substrates. JDPs guide Hsp70s' substrates to various molecular processes such as protein folding, trafficking and degradation. The evolutionary conserved J-domain, a common feature of all JDPs, serves as a primary Hsp70 interaction site and is required for Hsp70 ATPase activity stimulation. The role of helices II and III of J-domain, which are directly involved in interaction with Hsp70, has been thoroughly investigated. Conversely helix I, positioned away from Hsp70-J-domain interface has gained little attention. Here, we investigated the role of helix I of the J-domain in stabilizing the J-domain fold of DnaJ (bacterial JDP). We compared thermodynamic effects of substitutions of residues located at helix I to remaining residues forming a hydrophobic core of the J-domain. First, we approximated the J-domain stability change upon mutations reducing sidechain volume while maintaining its hydrophobic character utilizing efficient statistical approach. Our results hinted the existence of correlation between side chain volume reduction and decrease of J-domain stability. Next, we verified the thermodynamic effects of chosen mutations by free energy alchemical calculations. Results corroborate significant contribution of residue L10 of helix I to J-domain stabilization. We then investigated the stability of an interface formed by helices II and III of the J-domain by Replica-Exchange Umbrella Sampling simulations. Results obtained for the wild-type J-domain of DnaJ showed a deep energy well consistent with available experimental stability measurements. The same approach was then applied to helix I deletion variant, which was chosen based on previous estimations of mutations effects. This change resulted in halving the ΔG of interface formation. These results underline the importance of helix I for stabilization of the J-domain fold and understanding the way helical bundles form stable structures in general.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Poczta, Anastazja: New cladribine analogues as compounds with increased antileukemic properties- summary of *in vitro* study

Anastazja Poczta[1], Aneta Rogalska[1], Małgorzata Łukawska[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. ŁUKASIEWICZ Research Network-Institute of Biotechnology and Antibiotics, 5 Staroscinska St., 02-516 Warsaw, Poland

Contact for the abstract: Anastazja Poczta; anastazja.poczta@unilodz.eu

Haematological malignancies are currently one of the most common causes of death among patients of various age groups. A chemotherapeutic agent that is currently used to treat lymphoid malignant tumors is cladribine (CLA, 2′-deoxyadenosine). The aim of the study was to elucidate the mechanism of action of cladribine derivatives containing a formamidine group at position 6 (CLA-FDM, CLA-FPAZ, CLA-FPIR, CLA-FPIP, CLA-FHEX, and CLA-FMOR) using acute monocytic leukemia (THP-1), acute promyelocytic leukemia (HL-60) and acute lymphoblastic leukemia (MOLT-4). All tested compounds were synthesised in the ŁUKASIEWICZ Research Network-Institute of Biotechnology and Antibiotics, Warsaw. Cladribine derivatives were evaluated for cytotoxicity (XTT assay), genotoxicity (measurement of phosphorylation of H2AX, alkaline comet assay with proteinase K post-treatment following assessment of the cell cycle) and the ability to induce apoptosis (intracellular calcium concentration, caspase-3/7 activity and increased sub-G1 cell population) in comparison with the parent drug. The role of ATR in dCK activation in response to cladribine derivatives was also investigated. CLA derivatives were highly effective against leukemic cells, causing DNA fragmentation, and inducing DNA-protein cross-links, showing high cytotoxicity against leukemic cells and low toxicity towards normal cells. CLA derivatives increased the levels of intracellular calcium ions, caspase-3/7 and the percentage of sub-G1 apoptotic cells and blocked cells in the S phase of the cell cycle to a greater extent than free CLA. CLA-FMOR showed the highest efficacy in performed *in vitro* study.

Keywords: apoptosis, cladribine analogues, cytotoxicity, dCK , genotoxicity.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Pristupa, Kristina: The content of low molecular weight antioxidants in transgenic plants *Nicotiana tabacum* under heavy metal salts conditions

Pristipa K.V.[1], Kukulyanskaya T.A.[1], Khramtsova E.A.[1]

1. Belarusian State University, Minsk, 220030

Contact for the abstract: Kristina Pristupa; kristina.pristupa@mail.ru

Increase plant resistance to adverse environmental factors is one of the key challenges facing scientists. The intensity of free radical oxidative processes in plants increases under abiotic stress; the non-enzymatic components of the antioxidant defense are activated, and prevent the development of free radical oxidative processes. The development of abiotic stress leads to the formation of excess ethylene in plants. One way to reduce it is to create transgenic plants that carry the bacterial *acdS* gene encoding 1-aminocyclopropane-1-carboxylate deaminase in their genome. This enzyme catalyzes the destruction of the ethylene precursor. The goal of research was study the effect of heavy metals in soil on the content of non-enzymatic antioxidants in non-transgenic and transgenic *Nicotiana tabacum* plants carrying the *acdS* gene of bacteria *Pseudomonas putida* B-37. It was shown that the lowest content of antioxidants in plants was observed in the soil without the introduction of metals. It was established that the least sensitivity of plants to soil contamination with metal salts was detected when lead (II) ions were introduced into the soil. The maximum content of phenolic compounds (PC), flavonoids occurred during soil treatment with chromium (VI) ions: in non-transgenic plants the content of PC increased by 80%, the content of flavonoids increased by 153% compared to the control series. The total content of PC and flavonoids in transgenic plants increased by 2.3 and 2.8 times, respectively, compared with the control series. The content of vitamins C and E in non-transgenic plants increased by 1.9 and 2.1 times, respectively, when Cr^{6+} ions were introduced into the soil, and in transgenic plants increased by 2.2 and 2.5 times, respectively, compared with the control series.

Keywords*:* Antioxidant system, low molecular weight antioxidants, *acdS* gene, *Nicotiana tabacum*.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Przepiórska, Karolina: Neurotoxic effects of DDE are mediated *via* stimulation of apoptosis and autophagy

Karolina Przepiórska[1], Agnieszka Wnuk[1], Joanna Rzemieniec[1], Bernadeta Pietrzak[1], Małgorzata Kajta[1]

1. Maj Institute of Pharmacology, Polish Academy of Sciences

Contact for the abstract: Karolina Przepiórska; przepior@if-pan.krakow.pl

Dichlorodiphenyldichloroethylene (DDE) is a toxic compound that belongs to the group of organochlorines. DDE is a product of metabolic degradation of the commonly used pesticide DDT. The pesticide was previously used worldwide in agriculture but nowadays it is still used in South America, Africa and Asia to control disease-vectors responsible for malaria and zika virus diseases. DDE remains in the environment because of its resistance to degradation and ability to bioaccumulate in the food chain. Population studies suggest that exposure to DDE is associated with mental and psychomotor retardation, impairment of cognitive skills, autism and attention deficit and hyperactivity disorder (ADHD)-like behaviors as well as Alzheimer's and Parkinson's diseases. The aim of present study was to evaluate the effects of DDE isomers on neuronal cells in primary cultures. We showed for the first time that a neurotoxic effect of DDE involves not only apoptosis but also autophagy. It has been evidenced by increased expression levels of autophagy-related genes such as *Becn1*, *Map1lc3a*, *Map1lc3b* measured by qPCR. The DDE-induced patterns of mRNA expression were reflected by alterations in the protein levels (ELISA) as well as increased detection of autophagosomes. To verify the involvement of autophagy in neuronal cell death we used specific mRNA silencing particles i.e., siRNAs. The cells transfected with *Becn1* and *Atg7* siRNAs were less vulnerable to DDE than wild-type cells. In summary, we identified new mechanisms of neurotoxic action of DDE which, in addition to inducing apoptosis and neurotoxicity, stimulate autophagy in mouse brain neurons.

This study was supported by the National Science Center grant NCN no. 2015/19/B/NZ7/02449 and the statutory fund of the Maj Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland.

Keywords: Autophagy, DDE, neurotoxicity, primary neuronal cell cultures.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Rabiasz, Alicja: Searching for novel genes involved in the pathogenesis of primary ciliary dyskinesia (PCD)

Alicja Rabiasz, Patrycja Kaźmierczak, Michał Witt, Ewa Ziętkiewicz

1. Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland

Contact for the abstract: Alicja Rabiasz; alicja.rabiasz@igcz.poznan.pl

Ciliopathies are a group of genetic disorders caused by defects of primary or motile cilia, evolutionary conserved organelles present on the surface of many eukaryotic cells. Primary ciliary dyskinesia (PCD) is a key example of a ciliopathy caused by the dysfunction of motile cilia, whose symptoms include respiratory tract distress, hearing impairment, male infertility, and *situs inversus*. PCD is predominantly inherited as an autosomal, recessive disease. Until now, over 40 genes have been reported to be involved in PCD pathogenesis. Some of these genes encode proteins that form cilia ultrastructure, others encode cytoplasmic proteins that are responsible for cilia assembly. Mutations in the currently known PCD causative genes explain only ~70% of PCD cases. Thus, to obtain a complete picture of the genetic basis of PCD, it is necessary to search for novel genes involved in the pathogenesis of PCD. Like other similar endeavors, these studies require identification of candidate genes, for example by NGS-based genetic screening studies in PCD patients, followed by functional analysis of the candidates to prove their involvement in cilia motility. Our team has performed the whole-exome next generation sequencing (WES) in 120 unrelated Polish PCD patients with no mutations in the most frequently involved PCD causative genes. Besides revealing the presence of unknown mutations in some of the less studied PCD genes, new mutations were found in genes that were previously not described as associated with PCD. Based on *in silico* analysis, several candidate were selected. The role of this genes in motile cilia biology is presently being studied using RNA interference (RNAi) in the ciliated flatworm, *Schmidtea mediterranea.*

The project is supported by the National Science Center, grant no 2018/29/N/NZ5/00810.

Keywords: motile cilia, primary ciliary dyskinesia, RNA interference, *Schmidtea mediterranea.*

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Radziwonik, Wiktoria: A *PRNP* mutation (p.Pro102Leu) associated with rare Gerstmann-Sträussler-Scheinker disease: case report

Radziwonik Wiktoria[1], Karpinski Marek[2], Elert-Dobkowska Ewelina[1], Sulek Anna[1]

1. Laboratory of Molecular Basis of Neurodegenerative Diseases, Department of Genetics, The Institute of Psychiatry and Neurology in Warsaw, Poland
2. Neurogenetics Unit, Department of Neurology, Jagiellonian University Hospital in Cracow, Poland

Contact for the abstract: Wiktoria Radziwonik; wiktoriaradziwonik@gmail.com

Gerstmann-Sträussler-Scheinker disease (GSS) is a rare prion disease with autosomal dominant mode of inheritance (MIM#137440). A 32 year-old man with progressive lower limb weakness, gait ataxia, imbalance, dysarthria, rigidity, thinning of the corpus callosum and cerebellar and brainstem atrophy was admitted to genetic counseling unit with suspicion of spinocerebellar ataxia for further diagnostic work-up. Genetic testing for the most common types of spinocerebellar ataxias SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, SCA36, DRPLA were negative. Implementation of custom-designed NGS panels (I-III) gave the opportunity to expand the genetic diagnostics of rare neurodegenerative disorders. Panel I contains probes for coding sequences for 152 known genes associated with spinocerebellar ataxias and hereditary spastic paraplegias; panel II – 118 genes related to amyotrophic lateral sclerosis and frontotemporal dementias, HD-like phenotype, Parkinson's disease, Alzheimer's disease, dementias, dystonias, leukodystrophies; panel III – 89 genes causing neuromuscular disorders: muscular dystrophies, myasthenias, myotonias, myopathies. The patient was referred to panel I, but the analysis did not identify any putative variants, which could explain his phenotype. The panel have been revealed only 4 variants with uncertain clinical significance: p.Gly921Ser in *ITPR1* gene (dominant); p.Arg448Trp in *TDP1* (recessive); p.Ile1525Thr in *ATM* (recessive); and p.Ala469Val in *RAB3GAP2* (recessive). We obtained results of genetic testing of the patient's 1st degree cousin affected with similar symptoms, in whom mutation c.305C>T (p.Pro102Leu) in *PRNP* gene was identified. Sanger sequencing revealed the same mutation in our patient (*PRNP* gene was included in panel II only). Due to some significant limitations of technology (e.g. possibility of detecting a large number of gene variants whose role in the pathogenesis of the disease is not conclusive), cooperation between a molecular geneticist and a neurologist may be necessary to make a diagnosis in some complicated and unusual cases.

Keywords: spinocerebellar ataxias (SCA), Gerstmann-Sträussler-Scheinker disease, Next Generation Sequencing, custom-designed panels.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Reut, Veronika: Effect of lactoferrin, elastase and lysozyme on neutrophil respiratory burst

Veronika Reut[1], Daria Grigorieva[1], Alexey Sokolov[2], Irina Gorudko[1]

1. Department of Physics, Belarusian State University, Minsk, Belarus
2. Institute of Experimental Medicine, Saint Petersburg, Russia

Contact for the abstract: Veronika Reut; ReutVE@bsu.by

The first line of innate immunity against pathogens provided by neutrophil functions, e.g. generation of reactive oxygen species (ROS) during respiratory burst, formation of neutrophil extracellular traps and degranulation with releasing antimicrobial proteins. Many of latters play an important role in inflammation. However, recent observations have shown that neutrophil secretory proteins can modulate neutrophil activation. Studying of effects of lactoferrin, protein of specific granules, elastase, protein of azurophilic granules, and lysozyme, which is present in both types of granules, on neutrophils respiratory burst was the aim of present work. Gallocyanine (C.I.51030) was used as a sensitive fluorescent sensor for registration ROS production by neutrophils (λ_{ex}=360 nm, λ_{em}=490 nm). Respiratory burst was induced by well-known stimuli – N-formyl-Met-Leu-Phe (500 nM). To describe this process: v – the slope of the initial linear portion of fluorescence intensity curve, and h – changes in fluorescence intensity of the solution compared to the background level at 7 min were used. It was found that in the absence of stimulus neutrophil secretory proteins themselves are not able to induce neutrophil ROS production. However, it was shown that lactoferrin and elastase acts as priming agents, e.g. potentiate fMLP-induced ROS production by neutrophils: 1) preincubation of neutrophils with lactoferrin resulted in increase of h (~170-180%) at 250 and 500 mg/L and v (~150%) at 750 mg/L concentration of the protein; 2) strong increase of both h and v (about twice of control) was observed when 10-50 nM of elastase was added to neutrophils. Lysozyme did not affect the activity of neutrophils until concentration 250 mg/L.

Keywords: Gallocyanine, neutrophils, respiratory burst, granule's proteins.

This work was partly supported by grants RFBR (grant 18-515-00004), BRFBR (grant B18R-058) and MD-1901.2020.4.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Romanowska, Kamila: The significance of *HIF-3α* gene DNA methylation in colorectal cancer

Kamila Romanowska[1], Agnieszka A. Rawłuszko-Wieczorek[2], Paweł P. Jagodziński[3]

1. Department of Head and Neck Surgery, Poznan University of Medical Sciences, The Greater Poland Cancer Centre, Poznan, Poland
2. Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
3. Department of Biochemistry and Molecular Biology, Poznan University od Medical Sciences, Poznan, Poland

Contact for the abstract: Kamila Romanowska; kamromanowska@gmail.com

Colorectal cancer (CRC) is one of the most common cancer type in the world. Hypoxic conditions during formation of CRC support the development of more aggressive phenotype. The cellular response to hypoxia is regulated by hypoxia-inducible factors (HIFs) that allow cancer cells to adapt to adverse conditions. HIF is a heterodimeric complex composed of an oxygen-induced HIF-α and constitutively expressed HIF-β which mediate the primary transcriptional response to hypoxic stress. There are three identified isoforms of the HIF-α subunit: HIF-1α, HIF-2α, HIF-3α. The first two are particularly well described in the literature, while HIF-3α function is much less understood. Interestingly, the expression level of the *HIF-3α* in CRC may be modulated by epigenetic mechanism- DNA methylation within its promoter region. The purpose of this study was to verify the hypothesis about the effect of DNA methylation on the expression of the *HIF-3α* gene and the effect of this epigenetic regulation on the cellular response in hypoxic conditions. We investigated the presence of DNA methylation in the *HIF-3α* gene in established colorectal cancer cell lines. Changes in DNA methylation were positively correlated with *HIF-3α* gene expression at the mRNA level. Moreover, we observed an increase of *GLUT1* and *VEGF* transcripts under hypoxic conditions in DLD-1 and HT-29 cell lines infected with lentiviral molecules containing sh-*HIF-3α*. Our findings present that methylation-induced epigenetic silencing of *HIF-3α* might be involved in fine-tunning hypoxia response in CRC.

Key words: colorectal cancer, hypoxia, hypoxia-inducible factor 3α, DNA methylation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Rzeszotarska, Ewa: The role of microRNAs and their target genes in rheumatoid arthritis

A. Paradowska-Gorycka[1], T. Kmiołek[1], **E. Rzeszotarska[1]**, A. Wajda[1], E. Walczuk[1], E. Kuca-Warnawin[2], K. Romanowska-Próchnicka[3,4], B. Stypinska1, D. Majewski[5], P. P. Jagodzinski[6], A. Pawlik[7]

[1]Department of Molecular Biology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland; [2]Department of Pathophysiology and Immunology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland; [3]Department of Connective Tissue Diseases, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland; [4]Department of Pathophysiology, Warsaw Medical University, Warsaw, Poland; [5]Department of Rheumatology and Internal Medicine, Poznan University of Medical Science, Poznan, Poland; [6]Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan, Poland; [7]Department of Physiology, Pomeranian Medical University, Szczecin, Poland

Contact for the abstract: Agnieszka Paradowska-Gorycka; agnieszka.paradowska-gorycka@spartanska.pl and Ewa Rzeszotarska; ewa.rzeszotarska@spartanska.pl

Rheumatoid arthritis (RA) and osteoarthritis (OA) are chronic diseases, which affect joints. MicroRNAs (miRNAs), small non-coding RNAs, play an important role in determining and maintaining the expression of genes involved in Th17 differentiation, nevertheless the expression of miRNA in Treg is essential for maintaining immune homeostasis. The imbalance between Th17 and Treg cells and abnormalities in the production of cytokines can lead to the development of the inflammatory process and autoimmunity. The active form of RA may be caused by a shift in the balance of the immune system towards subpopulations of pro-inflammatory T cells (mainly Th17). Therefore, we studied if miRNAs, by affecting the expression of transcriptional factors associated with the differentiation and functioning of Th17/Treg cells can participate in the inflammatory process in RA patients. We examined if miRNAs influence the development and course of RA and thereby if they can act as potential biomarkers of disease activity. We analyzed 14 RA, 11 OA patients and 15 healthy subjects. We studied expression of transcriptional factors: SOCS1, SMAD3, SMAD4, STAT3, STAT5a and microRNAs (miR-24, -26a, -126, -146a, -155) in Treg and Th17 cells by using qPCR (QuantStudio 5, ThermoFisher). In RA Treg cells we noticed the following positive correlations of (miR-155–SMAD4; miR-155, miR-31–SMAD3; miR-26–SOCS1) and negative correlations (miR-126, -26–STAT5a). In RA Th17 cells we observed positive correlations between miR-155–STAT3; miR-26–SOCS1, STAT3 and SMAD3. We also presented the correlation between miR-31 and SMAD3 and between miR-26 and SOCS1 in RA Treg cells. RA patients have a higher expression level of miR-24 in Tregs when DAS28> 5.1. RA patients have a higher expression level of miR-31 in Th17 cells when DAS28≤ 5.1. RA patients with positive rheumatoid factor (RF) have higher miR-146a level in Treg cells.

Keywords: Rheumatoid arthritis, osteoarthritis, microRNA, transcriptional factors.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Serwach, Karolina: Study of the interaction between endogenous STIM proteins and NR2B subunit of NMDA receptor

Karolina Serwach[1], Joanna Gruszczynska-Biegala[1]

1. Mossakowski Medical Research Centre Polish Academy of Sciences, 02-106, 5 Pawińskiego Street, Warsaw, Poland

Contact for the abstract: Karolina Serwach; kserwach@imdik.pan.pl

STIM1 and STIM2 proteins are calcium (Ca^{2+}) sensors localized in the endoplasmic reticulum (ER). STIMs play a role in Store-Operated Ca^{2+} Entry (SOCE), which is described as Ca^{2+} influx into cellular cytosol in response to Ca^{2+} depletion from ER. Interaction of STIMs with Ca^{2+} channel-Orai1 is crucial for SOCE in non-excitable cells. In neurons STIMs activate Orai, TRPC channels, AMPA receptors and inhibit voltage-gated Ca^{2+} channels. Recently, our studies revealed the interaction between endogenous STIMs and NMDA2 receptor in rat cortical neurons (Gruszczynska-Biegala et al. 2020, *Cells*). We have shown that thapsigargin-mediated SOCE requires direct interaction between STIMs and NMDA2 receptor subunits. The aim of our present study is to determine whether NMDA-induced activation of NMDA receptor also influences the interaction between STIMs and NR2B subunit of NMDA receptor (NR2B) in rat cortical neurons. We have conducted immunofluorescent staining in rat cortical neurons using anti-NR2B and anti-STIM1 or anti-STIM2 antibodies after activation of NMDA receptor by NMDA for 1, 5, 15 or 30-minutes. Our preliminary data confirmed that both STIM proteins co-localize with NR2B. The 15-minute time point proved to be the most suitable for further research. To support these findings, we have performed co-immunoprecipitation experiment using anti-NR2B, anti-STIM1 and anti-STIM2 antibodies. Preliminary data showed that activation of NMDA receptor for 15 minutes increases the association of endogenous NR2B with STIM2, but decreases it with STIM1. Our results suggest that there is a direct interaction between NR2B and STIMs. In addition, activation of NMDA receptor by NMDA may influence the formation of NR2B-STIM1 and NR2B-STIM2 complexes in rat cortical neurons. The data also show that in neurons STIM1 has a different function than STIM2. However, further research in this field is needed with greater replication of these experiments. Supported by funds from the National Science Centre (2017/26/E/NZ3/01144, J.G-B).

Keywords: STIM proteins, NMDA2B receptor subunit, SOCE, Ca^{2+} signalling.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Sierpowski, Mateusz: Vaspin has stimulatory effect on human syncytiotrophoblast BeWo cells proliferation but not on cytotrophoblast JEG-3 cells. Preliminary studies

Sierpowski Mateusz, Mlyczyńska Ewa, Rak Agnieszka

1. Department of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Poland

Contact for the abstract: Sierpowski Mateusz; mateusz.sierpowski@student.uj.edu.pl

Vaspin is a serine protease inhibitor (serpin A12) that belongs to adipokines and exhibits insulin-sensitizing and anti-inflammatory potential. Molecular mechanism of vaspin action may be explained by interaction with the GRP-78 receptor, known of transducing proliferative signals and present on the surface of trophoblastic cells. Recent data indicated vaspin production by the ovarian and placenta cells but the role of serpin A12 in the placenta has been not described. The aim of this study was to examine protein expression as well as dose- and time-dependent effect of vaspin on proliferation of human cyto- and syncytiotrophoblast cells, JEG-3 and BeWo, respectively. JEG-3 represents extravillous trophoblast, while BeWo villous one. Both cell lines derive from human choriocarcinoma and are used as a model of placenta function. JEG-3 and BeWo cells (4×10^3 cells per well on 96-well plate) were cultured with increasing concentrations of vaspin (0.01; 0.10; 1.00; 10.00; 100.00 ng / mL) for 24, 48 and 72 hours. Vaspin protein levels in cell lysates were detected by Western blotting. Cell proliferation was measured using alamarBlue Assay (cat no. DAL 1025), whereas statistical analysis was performed using Kruskal-Wallis test. Vaspin protein expression was detected in both cell lines – JEG-3 and BeWo. We observed that vaspin stimulated significantly BeWo cells proliferation in time- and dose-dependent manner: differences in measured absorbance were detected after 24 and 48 hours and the strongest effect was observed in cells treated with 0.10 and 1.00 and 10.00 ng / mL of vaspin ($p<0,01$). However, in JEG-3 we observed no effect on cell proliferation. Vaspin, by increasing BeWo cells proliferation, is a new regulator of placental function including the growth and development of the placenta. Our preliminary studies have to be confirmed to molecular mechanism of vaspin action in the placental cells.

Keywords: vaspin, placenta, trophoblast, proliferation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Słabońska, Joanna: Mechanism of inhibition of ATP synthase by oligomycin and bedaquiline

Joanna Słabońska[1], Antoni Marciniak[1], Jacek Czub[1]

1. Gdańsk University of Technology, Faculty of Chemistry, Department of Physical Chemistry, ul. Gabriela Narutowicza 11/12, 80-233 Gdańsk, Poland

Contact for the abstract: Joanna Slabonska; joanna.slabonska@pg.edu.pl

F1Fo-ATP synthase is an enzyme present in all living organisms, including the mitochondria of yeast and mammals, as well as plant thylakoid and bacterial cells. The transmembrane Fo region consists of the stationary subunit a and the rotating c-ring, allowing proton translocation. To elucidate the mechanism of proton transport through Fo is vital for any future research aimed at using Fo as a molecular target for therapeutics. Here, we try to determine the mode of action of two known Fo inhibitors - bedaquiline and oligomycin - in the transmembrane region of the ATP synthase. By using molecular dynamics simulations, we observe the effect of ligand binding to the c-ring to identify the mechanism of inhibition of ATP synthase. We first establish the most favorable protonation states of bedaquiline and glutamic acid, the c-ring residue responsible for the binding and transfer of protons. By considering all possible combinations of states, we observed that neutral bedaquiline has a considerable affinity for the protonated cGlu59 on the c-ring of Mycobacterium tuberculosis. In contrast, simulations of oligomycin bound to mitochondrial Fo confirm specific binding to deprotonated cGlu59. Using enforced rotation protocols, we investigate the effect of inhibitor binding on c-ring rotation and proton transfer.

Keywords: ATP synthase, bedaquiline, oligomycin, inhibition.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Stachowiak, Małgorzata: Analysis of expression of vitamin D receptor and 1-α-hydroxylase in clear cell renal cell carcinoma

Stachowiak M.[1], Rusetska N.[2], Jancewicz I.[1], Szymanski M.[3], Siedlecki J.A.[2], Sarnowska E.[1]

1. Department of Experimental Immunotherapy, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw
2. Department of Molecular and Translational Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw
3. Pharmacology Department, Pharmaceutical Research Institute, Warsaw

Contact for the abstract: Małgorzata Stachowiak; malgorzata.stachowiak@pib-nio.pl

Vitamin D regulates expression of some important genes through vitamin D receptor (VDR) activation, which is a transcription factor. Aberration in distribution and vitamin D metabolism can lead to cancer development. Kidneys are main organs, where bioactive vitamin D is created. Therefore, the expression of vitamin D receptor and 1-α-hydroxylase (CYP27B1) was investigated in clear cell renal cell carcinoma (ccRCC). Expression of VDR and CYP27B1 was evaluated by immunohistochemistry on the patient tissue with ccRCC, using anti-VDR and anti-CYP27B1 antibodies. There was 116 patients examined for VDR and 93 – for CYP27B1 expression. Human ccRCC cell line (A498) was supplemented with different vitamin D concentrations, followed by immunocytochemistry analysis. Downregulation of VDR and CYP27B1 protein expression was observed in tumor tissue compare to control. The level of staining in cancer tissue is notably decreased. Both proteins are localized in cytoplasm. Immunocytochemistry on A498 cell line with vitamin D showed cytoplasmic and nuclear localization of VDR. The ratio of receptor distribution changes in response to vitamin D in favor of nuclei. Downregulation of VDR and CYP27B1 expression in ccRCC suggests that vitamin D metabolism is involved in tumor pathogenesis. Subcellular VDR localization in healthy and tumor cells confirms problems with vitamin D metabolism in Polish population. Furthermore, VDR nuclear localization in A498 cells depends on vitamin D concentration, which suggests that VDR nuclear translocation impacts on receptor activation.

Keywords: VDR, ccRCC, CYP27B1, kidney.

Financial support: Ministry of Education and Science within the Diamond Grant project no. 0139/DIA/2017/46.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Strycharz, Justyna: Expression of histone deacetylase (SIRT1) and acetylotransferases (PCAF, CBP, EP300) is changed by hyperglycemia during differentiation of human visceral preadipocytes

Justyna Strycharz[1], Ewa Świderska[1], Adam Wróblewski[1], Janusz Szemraj[1], Józef Drzewoski[2], Agnieszka Śliwińska[3]

1. Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland
2. Central Teaching Hospital of the Medical University of Lodz, Lodz, Poland
3. Department of Nucleic Acid Biochemistry, Medical University of Lodz, Lodz, Poland

Contact for the abstract: Justyna Strycharz; justyna.strycharz@umed.lodz.pl

Type 2 diabetes (T2DM) is associated with hyperglycemia-triggered epigenetic imbalance along with deregulation of adipogenesis and accelerated visceral fat accumulation. Histone acetylation and deacetylation directly impacts chromatin remodeling, thus having an impact on expression of numerous genes involved in adipogenesis and T2DM pathogenesis. Therefore, we aimed to examine the impact of hyperglycemia on expression of histone and non-histone deacetylase (SIRT1) and acetylotransferases (PCAF, CBP, EP300) in visceral pre-/adipocytes (HPA-v). 23-day-long cell culture was conducted in either normoglycemic (N) or hyperglycemic conditions (H) (30mM) and involved three stages : preadipocytes' proliferation (N / H), differentiation (NN / HH) and maturation (NNN / HHH). Expression was measured using Taqman probes and normalized to RPLPO and UBC expression level via 2^-ΔCt method. Statistics was conducted using t-test and ANOVA. Our results suggest that mature adipocytes cultured in normoglycemia exhibit reduction of PCAF and CBP in comparison to preadipocytes (NNN vs N), with no changes of SIRT1 and EP300 levels. Hyperglycemic conditions triggered upregulation of PCAF and SIRT1 in mature adipocytes while compared to preadipocytes (HHH vs H), but CBP downregulation in differentiated and mature adipocytes (HH vs H, HHH vs H). Moreover, EP300 expression was increased in differentiated adipocytes (HH vs H), followed by downregulation in mature adipocytes (HHH vs HH) upon hyperglycemia. Considering the impact of hyperglycemia in comparison to normoglycemia, we found the decrease of PCAF and SIRT1 in preadipocytes (H vs N). Moreover, hyperglycemia triggered EP300 upregulation and CBP downregulation in differentiated adipocytes (HH vs NN) along with PCAF expression increase in mature cells (HHH vs NNN). Summarizing, our data suggests that hyperglycemia impacts expression of genes associated with histone de-/acetylation, and thus, chromatin remodeling. This may, in turn, promote changes in expression of numerous diabetes-related genes in pre/adipocytes, leading to further deregulation of adipogenesis, fat accumulation and T2DM progression.

Keywords: hyperglycemia, visceral adipocytes, SIRT1, histone acetylotransferases.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Sukhaveyeva, Sviatlana: Dependence of expression of genes involved in ethylene signaling and biosynthesis on gravistimulation in tomato leaves

Sukhaveyeva S.V.

1. Institute of Biophysics and Cell Engineering National Academy of Sciences of Belarus, Minsk, Belarus

Contact for the abstract: Sviatlana Sukhaveyeva; suhoveevalmbc@mail.ru

Plants as sessile organisms have developed specific growth reactions to deal with numerous changes in their environment. These reactions, referred to as tropisms, cause the movement of plant structures in response to various stimuli. Gravitropism is the movement (directional growth) of plants in response to one of the fundamental forces on earth: gravity. Different parts of a plant respond differently to gravity: roots grow toward it (positive gravitropism); aerial parts of a plant grow away from it (negative gravitropism). It is well-known a role of phytohormone auxin in development of gravitropic response. However, the role of other phytohormones, including ethylene, on gravitropism in plants has not been studied in detail, especially at the transcriptomic level. The aim of this research was to evaluate influence of gravistimulus on expression of genes associated with biosynthesis and signaling of ethylene such as ACS, EBF, BRU and some genes of SAUR-family. Sensitivity to gravistimulus was determined in the apical leaves of tomato plants. For gravistimulation the experimental groups of tomatoes were turned 90° so their stems were horizontal and exposed at different time intervals. One of the experimental groups was additionally treated with ethephon, the direct ethylene source. RT-PCR was used to quantify mRNA level of target genes. It was found that under gravistimulation expression of SAURs, BRU1 and ACS was increased, while expression of EBF1 was decreased. At the same time, under additional treatment of gravistimalated plants with ethephon expression of SAURs, BRU1 and ACS was reduced and expression of EBF1 was increased. These results suggest that the changes in expression of target genes involved in signaling and biosynthesis of ethylene could be important for development of negative gravitropic response in plant leaves.

Keywords: gravitropism, ethephon, tomato (*Lycopersicum esculentum* L.), gene expression.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Surowiec, Magdalena: The effect of peptidylarginine deiminase from *Porphyromonas gingivalis* on interactions between *Candida albicans* biofilm and host proteins

Magdalena Surowiec[1], Justyna Karkowska-Kuleta[1], Joanna Kozieł[2], Barbara Potempa[3], Jan Potempa[2, 3], Andrzej Kozik[4], Maria Rąpała-Kozik[1]

1. Department of Comparative Biochemistry and Bioanalytics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, Gronostajowa 7, 30-387 Krakow, Poland
2. Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, Gronostajowa 7, 30-387 Krakow, Poland
3. Department of Oral Immunology and Infectious Diseases, University of Louisville School of Dentistry, 501 S. Preston St, Louisville, KY 40202 USA
4. Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, Gronostajowa 7, 30-387 Krakow, Poland

Contact for the abstract: Magdalena Surowiec; magdalena.surowiec@doctoral.uj.edu.pl

Porphyromonas gingivalis is an anaerobic, pathogenic bacterium present in the oral cavity is a major cause of periodontitis. Moreover, the impact of *P. gingivalis* on occurrence of other serious diseases as reumathoid arthritis or Alzheimer's disease was also indicated. *Candida albicans* is an opportunistic pathogen, a part of human physiological microflora, which can cause candidiasis in humans with decreased immunity. Both of these microorganisms have the ability to form mixed biofilms, structures important in virulence and colonization in host organism. The aim of this study was to examine the impact of bacterial secretory protein peptidylarginine deiminase (PPAD) on interactions between *C. albicans* biofilms and human plasma proteins: plasminogen and kininogen. PPAD catalyzes the reaction of citrullination – conversion of arginine residue to citrulline in peptides. In result, citrullination can modify properties of proteins, due to the changes in protein charge or functionality. In this study the citrullination of fungal surface-exposed proteins and human plasma proteins was investigated with the use of mass spectrometry analysis. The binding of citrullinated human proteins to unmodified *C. albicans* cells and binding of unmodified kininogen or plasminogen to fungal cells treated with PPAD were tested with the microplate enzyme-linked ligand sorbent assays. Significant reduction of plasminogen binding to fungal cells modified by bacterial enzyme was indicated, however citrullination of *C. albicans* proteins did not significantly affect the level of kininogen binding. Furthermore, the binding level of citrullinated human proteins to unmodified *C. albicans* cells was unchanged when compared to binding of unmodified kininogen or plasminogen. These observations can contribute to a better understanding of the process of pathogenesis of diseases related to activity of PPAD in case of mixed-species biofilms. This work was supported by the National Science Centre of Poland (grant number 2015/17/B/NZ6/02078 to MRK).

Keywords: biofilm, plasminogen, kininogen, candidiasis, periodontitis, citrullination.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Szczepanek, Monika: Growth analysis of two melanoma spheroids cell line on U-shape microplates

Monika Szczepanek, Ewa Stępień

1. Department of Medical Physics Marian Smoluchowski Institute of Physics, Jagiellonian University, Łojasiewicza 11, 30-348 Kraków, Poland

Contact for the abstract: Monika Szczepanek; monika.szczepanek@uj.edu.pl

The aim of the research is to develop the optimal method of melanoma spheroid culture as a model for investigation on the effectiveness of Boron Neutron Capture Therapy (BNCT). Radiation therapy is commonly used for treatment of the advanced stages of cancer, but it can damage healthy cells in the cancer vicinity. Therefore, BNCT can be used as a treatment which could decrease side effects of radiation therapy and make it more effective due to selectivity of the therapy. Melanoma is one of the most aggressive type of skin cancer. Treatment is effective only on early stages of disease, however rapid growth, metastases and various of responses to used therapy makes it ineffective. Therefore, melanoma is a subject for the intensive investigation and a good model for laboratory experiments. Nowadays, in cancer research 3D cell culture like spheroids are employed. Spheroids are unable to mimic the structure, organization, microenvironment and drug resistance which are observed in solid tumors. Two cell lines of melanoma with different grade of malignance (WM115 - primary tumor, WM266-4 -metastasis) were cultured as spheroids in different cell densities (500 – 3000 cells/well). In the first step of research, dynamics of the growth were evaluated by the analysis of the diameter of spheroids. The shape of the spheroids were characterized by analyzing solidity, circularity and Feret diameter using a dedicated macro in the imageJ software (FIJI). Diameter of spheroids during culturing were increasing from 408,6 to 1035,1 µm and from 260,0 to 1004,1 µm for WM115 and WM266 respectively. In the next step, the viability of the spheroids was tested using the flow cytometry method. Viability of both cell lines ranged from 85.3% to 97.6% within different days of culture. This analysis will help to select the appropriate growth condition of spheroids for future tests of neutron radiation influence on melanoma.

Keywords: spheroids, 3D culture, melanoma, BNCT.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Szymaszek, Patryk: Spectrophotometric characterisation and research of the usefulness of potential highly selective luminescent sensors for the determination of metal ions

Patryk Szymaszek[1], Paweł Fiedor[1] Joanna Ortyl[1], Anna Chachaj-Brekiesz[2]

1. Cracow University of Technology, Faculty of Chemical Engineering and Technology, Laboratory of Photochemistry and Optical Spectroscopy, Warszawska 24, 31-155 Cracow, Poland
2. Jagiellonian University, Faculty of Chemistry, Ingardena 3, 30-060 Kraków

Contact for the abstract: Patryk Szymaszek; szymaszek.patryk@gmail.com

The development of analytical tools for the identification and quantification of metal ions is of great interest to scientists dealing with environmental protection, medicine or cell biology. Metal ions are essential for the proper functioning of a single cell and the whole organism. For this reason, visualization of the location and degree of oxidation will allow to determine their biological role and to understand the disorders and diseases caused by the disturbance of specific ions. The development of new fluorescent sensors focuses mainly on obtaining probes that will be highly selective and specific for one particular type of ion. Several methods of iron ion detection such as absorption atomic spectroscopy, voltamperometry or colorimetry are known, but fluorimetry is the most popular of these methods. The use of fluorimetry in combination with a fluorescent chemosensor allows the determination of metal ions functions in living organisms. By combining it with microscopic imaging, fluorescent sensors can be a powerful tool for determining the presence and concentration of ions. Although there are many sensors available that interact specifically with ions, the photostability of sensors is an uncut problem. In addition, sensors specific to such paramagnetic ions as iron ions have low selectivity in the presence of other paramagnetic ions such as Cu^{2+}, Ni^{2+}. For this reason it is important to develop high selectivity sensors with one of them. Bearing in mind the need to develop new selective fluorescence sensors, new derivatives of 2-amino-4,6-diphenylpyridine-3-carbonitrile were tested as fluorescence probes. The influence of the formation of inclusion complexes of the tested sensors with cyclodextrins on their solubility was also tested. Sensor sensitivity was tested using Infinite 200 PRO NanoQuant multi-level microplate reader by Tecan. The measurement consisted in recording the fluorescence spectrum of samples located in individual wells of the plate at a given wavelength.

Keywords: probe, sensor, luminescence, fluorescence, metal ion, cyclodextrin.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Takalchyk, Yuliya: Cell therapy for brain injury

Yuliya Takalchyk, Svetlana Pashkevich, Vladimir Kulchitcky

1. Institute of Physiology, National Academy of Sciences of Belarus

Contact for the abstract: Yuliya Takalchyk stukachyulya@gmail.com

To apply cellular technology, it is necessary to develop effective methods for stem cell implantation. Perineural spaces of the cranial nerves are a promising way of stem cell delivery. The olfactory and trigeminal nerves were used in this study to identify the best option for mesenchymal stem cell implantation, depending on the location of the injury in the brain. Rats were modeled by local destruction of the sensorimotor zone (anterior cranial fossa) or cerebellar cortex (posterior cranial fossa). Then the animals were injected with mesenchymal stem cells in the region of the olfactory or trigeminal nerve for their migration to the area of injury. Microscopic examination of brain tissue and adjacent areas showed that the introduction of mesenchymal stem cells through the olfactory nerve is effective in trauma to the anterior cranial fossa. In case of injury in the posterior cranial fossa, stem cells must be inserted through the trigeminal nerve. Assessment of the condition of the animals after modeling the injury and the introduction of mesenchymal stem cells was performed in the test "Horizontal Bar" and "Elevated Plus Maze". Test results on the horizontal bar showed that the most effective recovery process was found in rats with injected stem cells after injury modeling. Seven days after the operation, in animals that were not injected with stem cells, the test results on the horizontal bar were lower than in the control group. Similar results were obtained when testing rats with brain injury in the Elevated Plus Maze. The perineural migration of mesenchymal stem cells along the olfactory or trigeminal nerve fibers to the destroyed areas of the brain is accompanied by a more effective restoration of coordination and motor activity in such rats compared to those that were not given mesenchymal stem cells after injury.

Keywords: mesenchymal stem cells, brain injury, perineural migration, cranial nerves.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Tempes, Aleksandra: The role of µ2-adaptin serine 45 phosphorylation in clathrin-mediated endocytosis

Aleksandra Tempes[1], Agnieszka Brzozowska[1], Anna Malik[1,2], Ewa Sitkiewicz[3], Tomasz Węgierski[1], Katarzyna Misztal[1], Michał Dadlez[3], Jacek Jaworski[1]

1. International Institute of Molecular and Cell Biology, Warsaw, Poland
2. Nencki Institute of Experimental Biology, Warsaw, Poland
3. Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland

Contact for the abstract: Aleksandra Tempes; atempes@iimcb.gov.pl

Eukaryotic cells employ numerous endocytic routes for the internalization of various types of cargo. One of them, in which AP2 adaptor protein complex (AP2 complex) plays a central role, is clathrin-mediated endocytosis (CME). AP2 consists of four subunits: α2-adaptin, β2-adaptin, µ2-adaptin, and σ2-adaptin. This complex is responsible for recruitment clathrin molecules to the plasma membrane. Despite the importance of AP2 for CME, regulation of its function is not fully understood, especially concerning posttranslational modifications of AP2 subunits. Data obtained in our laboratory pointed out the modulatory role of mTOR/p70S6 kinase pathways in the CME process. Our study aims to understand the molecular mechanism underlying this regulation.To assess functional interaction between mTOR/p70S6 kinase pathway and AP2 as well as to identify AP2 phosphorylation sites, we used pull-down and immunoprecipitation experiments followed by in vitro kinase assays and mass spectrometry. Effects of newly identified post-translational modifications of AP2 on CME were evaluated by examining formation and internalization of clathrin-coated pits in cells expressing wildtype or mutated µ2-adaptin using TIRF microscopy, transferrin internalization and proximity ligation assays. Our experiments revealed that (*i*) µ2-adaptin interacts with p70S6 kinase, (*ii*) µ2-adaptin is phosphorylated in p70S6 kinase-dependent manner at serine 45 (S45), (*iii*) S45 phosphorylation affects lifetime distribution of clathrin-coated pits, clathrin recruitment, transferrin (CME cargo) internalization rate and interaction of transferrin receptor with AP2. Based on these data, we conclude that p70S6 kinase-dependent phosphorylation of S45 of µ2-adaptin likely affects conformational changes of AP2 and by this mean contributes to the early stages of CME.

Keywords: clathrin-mediated endocytosis, AP2, p70S6 kinase, phosphorylation.

This work has been supported by Polish National Science Centre grants 2016/21/B/NZ3/03639 and 2017/25/N/NZ3/01280

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Twardowski, Piotr: In-vitro testing of degradation tags in search of novel antibiotics

Piotr Twardowski[1], Maria Klimecka[1], Anna Antosiewicz[1], Matylda Izert[1,] Maria Górna[1]

1. Structural Biology Group, Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, Poland;

Contact for the abstract: Piotr Twardowski; pk.twardowski@student.uw.edu.pl

The targeted protein degradation is gaining momentum as a new approach in drug discovery in areas such as cancer and neurodegenerative diseases. However, this approach was not yet used as an antimicrobial agent as bacteria lack E3 ubiquitin ligase, usually used in such research. In order to perform targeted degradation, so called degraders are used. Degraders are bifuncional molecules composed with two ligands, one binding to the protease and the second one binding to the target protein. Here, we tried to take the first step in creation of such degraders in bacteria by assessing which of the tested degradation tags (parts binding to proteases) shows the highest efficiency. We conducted a study of degradation of eGFP combined with ten degradation peptide tags, derivatives of naturally occurring ssrA tag, in presence of bacterial proteases from AAA+ family ClpXP alongside with ATP regeneration system and an adaptor protein sspB. For measurements of decrease of eGFP fluorescence plate reader was used. The findings from our research illustrate how modifications impact the rate of eGFP degradation. Moreover, we performed the optimization of measurements by establishing the most efficient ratio of proteases and choosing the most effective composition of ATP regeneration system. The findings make a crucial step in further research for synthesis of an antimicrobial degrader.

Keywords: protein degradation, PROTAC, degradation tag, ClpXP.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Tyrakowska, Małgorzata: Structure-function analysis of anti-viral protein IFITM3

Alicja Chmielewska PhD[1], Małgorzata Tyrakowska[1], Anna Szczepińska[1], Krystyna Bieńkowska-Szewczyk[1]

1. Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk

Contact for the abstract: Małgorzata Tyrakowska; malgosia.agnieszka2@gmail.com

Despite rapid development in antiviral research, viral infections remain a threat to humankind and a global cause of death. Novel approaches for prevention and treatment of viral diseases require extensive understanding of mechanisms of infection and host immune responses. Vertebrates' cells have evolved to defend from viral infections by various pathways of innate and adaptive immune responses. IFITM (*interferon-induced transmembrane*) proteins are expressed during viral infection as effectors of innate response. Their expression is stimulated by interferon – signaling protein triggering a cellular "anti-viral state". IFITM1, IFITM2 and IFITM3 proteins inhibit viral infection mainly by blocking virus entry into the cell. IFITM3 has been proved to inhibit numerous viruses e.g influenza A virus, Ebola virus and severe acute respiratory syndrome-related coronavirus. We showed that IFITM3 was able to inhibit efficiently tick-borne encephalitis virus (TBEV). To analyze the structure-function relationship in IFITM3 protein, I constructed a panel of cell lines stably producing different mutated versions of IFITM3 protein, including mutations of several posttranslational modification sites. A549 cells were transduced with retroviral vectors carrying mutated IFITM3 genes to obtain stable expression of mutated proteins. Obtained cells were tested in TBEV infectivity assay. In conclusion, we show the importance of particular IFITM3 regions and posttranslational modification sites in IFITM3 protein antiviral functions.

Keywords: IFITM3, TBEV, restriction factor, antiviral activity.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Uśpieński, Tomasz: The role of the ubiquitin-proteasome system (UPS) in Hedgehog signaling pathway

Tomasz Uśpieński[1], Paweł Niewiadomski[1]

1. Center of New Technologies, University of Warsaw

Contact for the abstract: Tomasz Uśpieński; t.uspienski@cent.uw.edu.pl

The fine balance between protein synthesis and degradation is necessary for homeostasis of every cell. The ubiquitin-proteasome system (UPS) is the key pathway responsible for protein degradation in Eukaryotes. The essential specificity of UPS is achieved owing to a cascade of three enzymes, ubiquitin activation enzyme, ubiquitin conjugating enzyme and ubiquitin ligase. The UPS regulates multiple cellular processes such as cell cycle, gene expression, cellular signaling, and carcinogenesis. The Hedgehog pathway, which is vital for proper development of higher eukaryotes, is among signaling pathways regulated by the UPS. Dysregulation of this pathway leads to many developmental abnormalities and may result in cancer, such as medulloblastoma, the most common brain tumor in children. Thus, modulation of Hedgehog signaling, including through the UPS, may prove to be an attractive therapeutic option in oncology. The UPS was shown to negatively regulate the Hedgehog pathway. In the absence of a signal, partial degradation of Gli2 and Gli3 transcription factors, known as proteasomal processing, leads to the formation of their repressor forms (GliR). GliR are then translocated into the nucleus where they repress target gene expression. However, our results suggest that the proteasome activity may be also required for the maximal target gene induction by activated Gli2 upon pathway activation. Therefore, the proteasome has a dual role in the Hedgehog signaling – it is necessary for Gli repressor formation, but is also critical for Gli activation.

Keywords: Gli, Hedgehog, UPS.

This work was supported by the OPUS grant 2017/27/B/NZ3/01163 from the National Science Centre.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Wielento, Aleksandra: Toll-like Receptor 2 Activation Is Enhanced by the Citrullinated *Porphyromonas gingivalis*Surface Proteins

Aleksandra Wielento[1], Jan Potempa[1,2]

1. Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Cracow, Poland
2. Department of Oral Immunology andInfectious Diseases, School of Dentistry, University of Louisville, Louisville, United States of America

Contact for the abstract: Aleksandra Wielento; aleksandra.wielento@doctoral.uj.edu.pl

Porphyromonas gingivalis(*Pg*) is the keystone oral pathogen implicated in development and progression of periodontitis. One of numerous virulence factors of *Pg* is peptidylarginine deiminase (PPAD), an enzyme, which enables this pathogen to citrullinate proteins and peptides, both host- and bacterium-derived.Previous studies showed that PPAD is essential to stimulate the proinflammatory response in host cells. Moreover, our results suggested citrullinated fimbriae as the vital factor enhancing inflammation. This *Pg* surface component is a ligand for Toll-like Receptor 2 (TLR2).To verify our hypothesis and uncover other factors crucial in the TLR2 activation we used a reporter system in transfected cell line overexpressing the TLR2 receptor. Cells were infected with various *Pg* strains and their mutant deficient in different surface proteins. The highest activation of the TLR2 receptor was observed for the ATCC33277 strain, while the W83 and 381 strains elicited weak activation. Catalytic inactivation of PPAD, deletion of gingipains or fimbriae in the ATCC33277 strain caused decrease in TLR2 activation. TLR2 was also activated by outer membrane vesicles (OMV) isolated from ATCC33277 and W83 *Pg* strains and their PPAD^{C351A} mutant strains. Interestingly, activation by both ATCC strains was stronger than that induced by W83 strains and there were no significant differences in cell response triggered bywt and PPAD mutant of the same strain. Treatment with purified ATCC33277 major fimbriae with/without *Pg* standard/ultrapure LPS induced TLR2 activation as well, while treatment with any type of LPS did not enhance the signal. In conclusion, the presented results imply that major *Pg* virulence factors activating TLR2 are bacterial cell surface proteins citrullinated at the C-terminus by PPAD, especially major fimbriae. Moreover, gingipains activity is crucial for this activity apparently by releasing C-terminal Arg residues. Furthermore, *Pg* activated TLR2 in the strain-dependent manner, which suggests that type of fimbriae plays the key role in this process.

Keywords: *Porphyromonas gingivalis,* PPAD, citrullination, TLR2, fimbriae.

This work was supported by grant from the National Science Centre, Poland UMO-2018/30/A/NZ5/00650.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Wierzbicka, Alicja: Preliminary study on the effect of vitamin D dietary supplementation on *vdr*, *cyp2r1* and *cyp27b1* transcripts in selected tissues of young rats by gender

Alicja Wierzbicka[1], Maria Oczkowicz[1], Justyna Mrozowicz[1], Barbara Szymczyk[2], Małgorzata Świątkiewicz[2], Anna Koseniuk[1]

1. National Research Institute of Animal Production, Department of Animal Molecular Biology.
2. National Research Institute of Animal Production, Department of Animal Nutrition and Feed Science.

Contact for the abstract: Alicja Wierzbicka; alicja.wierzbicka@izoo.krakow.pl

The expression of the genes responsible for vitamin D metabolism occurs in many organs, not only in the liver or kidney. This means that vitamin D, depending on the body's needs, can be metabolised locally, i.e. biologically activated in various organs and tissues. This fact suggests that vitamin D affects the functioning of many organs and tissues. The experiment was carried out on 36 Wistar rats. In the experiment, the animals were divided into three feeding groups consisting of 6 males and 6 females. The animals were fed unlimited with feeds, which differed only in the content of vitamin D3 (cholecalciferol). Genetic material isolated from selected rats tissues subjected to the experiment was used to determine the Relative Quantity (RQ) of the *vdr*, *cyp2r1*, and *cyp27b1* transcripts. The study was carried out on RealTime QuantStudio ™ 7 Flex PCR system. Preliminary studies have shown that the effect of different supplementation is less significant than the differences between the sexes in particular nutrition groups.The most spectacular differences in both the dose-effect and gender were observed in one of the major metabolising organs- the liver. Significant differences in *cyp27b1* expression were observed in the liver under the influence of various supplementation doses in males and females. In contrast, gender differences in *vdr* expression in the liver were highly significant between each of the nutritional groups. Also, analysis of *cyp27b1* transcripts in fat showed significant and highly significant gender differences in all dietary groups. In addition, highly significant gender differences in *cyp2r1* expression were observed in the brains in non-vitamin D supplemented animals. The analysis of the relative quantities of tested gene transcripts showed significant differences in the way vitamin D metabolites in males and females both in the major metabolising organs and at the local level, i.e. in individual organs.

Keywords: Vitamin D, rats, transcriptome, gender.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Wilga, Magdalena: Comparison of expression of genes encoding subunits of chromatin remodeling complex SWI/SNF in different lines of clear cell renal cell carcinoma

Magdalena Wilga[1*], Anna Maassen[1*], Joanna Kosior[1], Joanna Szarkowska[2], Janusz A. Siedlecki[2], Elzbieta Sarnowska[2], Tomasz J. Sarnowski[1]

1. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
2. Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland

[*]equal contribution

Contact for the abstract: Magdalena Wilga; magdalena.wilga@ibb.waw.pl

Recent studies provide an ample of evidences for the important role of epigenetics in cancer biology. The evolutionarily conserved SWI/SNF complexes are responsible for ATP-dependent chromatin remodeling. They play essential role in processes relevant to cancer development, like DNA repair and regulation of genes involved in cellular processes such as proliferation, transformation, differentiation, adhesion, etc. It is currently estimated that up to 20% of human cancers demonstrate SWI/SNF aberrations. In several tumor subgroups, e.g. in clear cell renal cell carcinoma (ccRCC), SWI/SNF mutations are early mutational events and can be considered as a factor that initiates and drives tumor development. In 40% of ccRCC cases the mutation in *PBRM1* gene encoding non-core subunit of SWI/SNF complexes was found. Modification in stoichiometry of complex composition may cause loss of correct SWI/SNF function or gaining of new, improper function leading to de-regulation of important cellular processes. In our research we evaluated expression of genes encoding core and non-core subunits of SWI/SNF complex in clear cell renal cell carcinoma A498, 786-O (primary) and CAKI-1 (metastasis) cell lines. Additionally, we used RPTEC line - normal kidney tube epithelial cells as a control. We notified alterations of expression level of various SWI/SNF complex subunits between normal and cancer cells on both, transcript and protein levels. Given the substantial differences in the SWI/SNF subunit abundance between various ccRCC cell lines we postulate that the impairment of SWI/SNF function may play an important role not only in ccRCC development but also its progression.

Keywords: SWI/SNF chromatin remodeling complex, ccRCC.

Financial support: Polpharma Scientific Foundation 5/XVII/18 (TJS)

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Wojtyś, Marta Ilona: Effect of NaCl on the oligomeric state of wild-type, C- and N-His-tag variants of adenylosuccinate synthetase from *Helicobacter pylori*

Marta Ilona Wojtyś[1,2], Beata Wielgus-Kutrowska[1], Ante Bubić[3], Ivana Leščić Ašler[3], Maria Winiewska-Szajewska[1], Elżbieta Katarzyna Jagusztyn-Krynicka[2], Marija Luić[3], Agnieszka Bzowska[1]

1. Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland
2. Department of Bacterial Genetics, Institute of Microbiology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096, Warsaw, Poland
3. Division of Physical Chemistry, Ruđer Bošković Institute, POB 180, Bijenička cesta 54, HR-10002 Zagreb, Croatia

Contact for the abstract: Marta Ilona Wojtyś; lyczekmarta@biol.uw.edu.pl

Purine metabolism is the growth-limiting step for all cells, prokaryotic and eukaryotic. There are two metabolic routes, which organisms can use to obtain purine nucleotides, the indispensable building blocks of DNA and RNA - *de novo* and salvage pathways. Some organisms, including pathogenic bacterium *Helicobacter pylori*, lack enzymes of *de novo* purine synthesis route and therefore enzymes of salvage pathway are potential drug targets against such pathogens. Human infections with *H. pylori* induce both acute and chronic gastritis and peptic ulcer and approximately 1% of infections cause gastric cancer. New drugs and new therapies are necessary to overcome the antibiotic resistance of this pathogen. We have chosen the purine salvage pathway enzyme, namely adenylosuccinate synthetase as a possible target for the development of antimicrobials against this pathogen. As the first step to analyze its stability we characterized its oligomeric state in different buffers. The recombinant wild type enzyme from the *H. pylori* 26695 strain was purified to homogeneity using a three-step chromatographic procedure, while C- and N-His-tag versions were obtained by affinity chromatography using Ni-based and Co-based resins. All three enzyme variants have similar catalytic activity in a salt-free medium. Analytical ultracentrifugation with absorbance detection shows that addition of NaCl to the protein storage buffer significantly affects its oligomeric state, at least when the enzyme is present in the low concentration (70 □g/mL and lower). Presence of salt favors the monomeric form while when there is no salt dimer prevails. Interestingly, influence of salt concentration is strikingly different for three enzyme variants studied. C-His-tag AdSS is the most sensitive to the presence of salt, the N-His-tag version is the most resistant, while the behavior of the wild type enzyme places it between its tagged counterparts. This result indicates that tags can significantly change the properties of proteins.

Keywords: *Helicobacter pylori*, adenylosuccinate synthetase, oligomeric state, analytical ultracentrifugation.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Wróbel, Karolina: Accumulation of double-stranded RNA in the nucleoplasm of HeLa cells

Karolina Wróbel[1,2], Kamila Affek[1], Aleksander Chlebowski[1,2], Andrzej Dziembowski[2] & Roman Szczęsny[1]

1. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
2. International Institute of Molecular and Cell Biology, Warsaw, Poland.

Contact for the abstract: Karolina Wróbel; karolina.wrobel@ibb.waw.pl

Double-stranded RNA (dsRNA) is the duplex type of RNA that plays essential roles in diverse biological processes among which the best known and common is RNA interference, involving short double-stranded RNA molecules. Under physiological conditions in human cells endogenous dsRNA molecules with longer double-stranded fragments (more than 30 base pairs), and therefore not acting as mediators of RNA interference, can be found almost exclusively in mitochondria as a product of bidirectional transcription of mitochondrial DNA, and levels of which can be significantly increased upon depletion of components of mitochondrial degradosome. Unlike mitochondrial dsRNA, to date, very little is known about possibilities of increase of dsRNA amount in human nucleus, which under physiological conditions fluctuates at a very low level. In our laboratory we performed a genome-wide siRNA screen focused on searching of abnormalities in dsRNA levels in human cells. We found that depletion of certain nuclear proteins indeed resulted in strong accumulation of dsRNA in the nucleoplasm of HeLa cells. Our preliminary data indicate that the accumulation of those molecules is likewise related to the cell cycle. Interestingly, observed dsRNAs are not newly transcribed molecules and we hypothesize that they are not exclusively products of RNA polymerase II transcription. Our progress toward understanding the molecular basis of the observed phenomenon will be presented.

Keywords: double-stranded RNA, nuclear RNA processing, cell cycle.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Wróblewski, Adam: Hyperglycemia induces expression changes of miR-26a-5p and its target, *IL6* gene, during the differentiation of visceral adipocytes.

Adam Wróblewski[1], Justyna Strycharz[1], Ewa Świderska[1], Janusz Szemraj[1], Józef Drzewoski[2], Agnieszka Śliwińska[3]

1. Department of Medical Biochemistry, Medical University of Lodz
2. Central Teaching Hospital of the Medical University of Lodz,
3. Department of Nucleic Acid Biochemistry, Medical University of Lodz,

Contact for the abstract: Adam Wróblewski; adam.wroblewski@stud.umed.lodz.pl

Interleukin 6 (IL-6) is mostly known as a pro-inflammatory cytokine mainly produced in macrophages and mediating acute phase response. IL-6 acts also as an adipokine and anti-inflammatory myokine. IL-6 may affect insulin signaling through insulin receptor substrate (IRS), however, its role in the development of impaired glucose metabolism (IGM) is still unclear since its actions depend on tissue and other cytokines. Obesity contributes to IGM and type 2 diabetes. Adipocytes are constantly renewed from preadipocyte reservoir, possibly exposed to chronic hyperglycemia (HG). Recent study on murine model evidenced regulation of *IL6* expression by miR-26a-5p. Aim of the study was to evaluate miR-26a-5p and *IL6* mRNA and protein expression in adipocytes differentiated in normoglycemia (NG) and in chronic HG. Human visceral preadipocytes (HPA-v, ScienCell Research Laboratories), have undergone adipogenesis in NG and chronic HG (30mM) in three stages of cell culture: preadipocyte proliferation (5 days), adipocyte differentiation (11 days), and adipocyte maturation (6 days). mRNA and miR-26a-5p expression levels were measured using qPCR method (TaqMan probes), whereas IL-6 protein levels were estimated using ELISA assay (Cloud-Clone). One-way ANOVA and t-tests were used for statistical analysis. During the adipogenesis in NG *IL6* mRNA expression decreased after differentiation with unchanged protein levels. However, mature adipocytes had increased protein levels whereas mRNA levels remained unchanged. In HG-treated adipocytes, both *IL6* mRNA and protein levels were downregulated after differentiation and upregulated after maturation. Adipocytes differentiated in HG had lower protein levels than untreated adipocytes instead of unchanged mRNA levels. HG-treated mature adipocytes had higher *IL6* mRNA levels than untreated adipocytes with consistent decrease of miR-26a-5p levels. HG could augment inflammation and affect insulin signaling through the changes of *IL6* and miR-26a-5p expression profiles in adipocytes. Agreeing changes in miR-26a-5p and *IL6* mRNA levels support potential regulation of *IL6* expression. Financed from NCN grant number 2015/17/B/NZ7/03019.

Keywords: miR-26a-5p, IL-6, visceral adipocytes, adipogenesis, hyperglycemia, inflammation, insulin signaling, diabetes.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Yerofeyeva, Anna-Maria: Effects of adipose-derived mesenchymal stem cells on local nociception and tendon regeneration at Achilles tendon injury model in rats

A.-M. V. Yerofeyeva [1], I. P. Zhavoronok[1], O. A. Antipova[1], E. L. Ryzhkovskaya[1], T. E. Kuznetsova[1], I. B. Vasilevich[2], S. V. Pinchuk[2], I. D. Volotovsky[2], A. Yu. Molchanova[1]

1. Institute of Physiology of National Academy of Sciences of Belarus, Minsk, Republic of Belarus
2. Institute of Biophysics and Cellular Engineering of National Academy of Sciences of Belarus, Minsk, Republic of Belarus

Contact for the abstract: Anna-Maria Yerofeyeva; amyerofeyeva@zoho.eu

Currently, tendon injuries are one of the most common diagnoses in orthopedics and sports medicine and often lead to disability and chronic tenalgia. Conservative methods of treatment do not allow complete functional tendon repair and elimination of pain and swelling. Due to their immunomodulatory and regenerative properties, adipose-derived mesenchymal stem cells (ADMSCs) are considered as a suitable candidate for tendon injury treatment strategies development. Therefore, in present study, potential antinociceptive and regenerative effects of locally administered ADMSCs investigated in experimental model of Achilles tendon injury. Achilles tendon injury of Wistar rats was modeled by clamping (trauma without transection). Allogenic transplantation of 0.25×10^6 ADMSCs into the site of injured tendon was done at 2 regimens: on the 1st day after surgery and twice on the 1st and 3rd day after surgery. The limb circumferences were measured daily for 28 days after trauma. Nociceptive responses to mechanical stimulus were evaluated on days 0, 7, 14, 21 and 28 after trauma, and some animals were sacrificed for histological studies. Experimental Achilles tendon injury resulted in mechanical allodynia that was detectable from 7th day after surgery and caused edema of the injured paw. A single local injection of 0.25×10^6ADMSCs induced a decrease of the thresholds of nociceptive response to a mechanical stimulus and did not affect the severity of edema of the injured limb throughout the whole period of investigation. On the contrary, administering the same dose of ADMSCs twice (with 2 days gap) effectively abolished nociceptive sensitivity but did not contribute to a decrease of edema of the injured limb, probably due to increased proliferation of granulation tissue. Findings of physiological tests correlate with histological characteristics of injured tendons. The regimen of ADMSCs transplantation can strongly impact onto their physiological effects and should be considered.

Keywords: Mesenchymal stem cells, adipose tissue, tendon injury, nociceptive sensitivity.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Ziółkowska, Sylwia: Newly synthesized derivative of aziridine and hydrazone exerts cytotoxic effects against HT-29 colorectal adenocarcinoma cell line in combination with BioMarine® Medical fish-liver oil mix

Sylwia Ziółkowska[1], Piotr Czarny[1], Adam M. Pieczonka[2], Janusz Szemraj[1]

1. Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland
2. Department of Organic and Applied Chemistry, Faculty of Chemistry, University of Lodz, Lodz, Poland

Contact for the abstract: Sylwia Ziółkowska; sylwia.ziolkowska@stud.umed.lodz.pl

Aziridines are nitrogen-containing, three-membered ring heterocycles while hydrazones have a structure with formula R1R2C=NNH2. Many natural aziridine alkaloids show biological activities such as antibacterial, antimicrobial or anticancer, whereas the hydrazones demonstrate above properties and also anti‑inflammatory activity. The combination of hydrazone structure with aziridine ring may potentially lead to compounds with biologically effective character. The aim of study was to examine the anticancer activity of new synthesized aziridine and hydrazone derivatives against human colorectal adenocarcinoma HT-29 cell line. In the experiments the cells were incubated for 24h with six new synthetized compounds in eighteen concentrations ranging from 0,01 µg/mL to 1 mg/mL. Next, the cells were subjected to MTT assay. According to obtained results, one compound, i.e. ARA12, exhibited the anticancer properties. Thus, HT-29 were incubated for 24h with 50 and 120 µg/mL ARA12, and subjected to flow cytometry, to evaluate the apoptosis level and distribution of cell cycle phases. Additionally, the effect of BioMarine® Medical fish-liver oil mix containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was investigated. The oil mix was added alone or in combination with ARA12 in concentrations of 20 or 50 µg/mL for both FFA. Statistical analysis was performed by one-way ANOVA and Tukey's post-hoc test. Obtained results demonstrated that cell viability was significantly decreased in concentration of 150 µg/mL only in case of ARA12. Although ARA12 did not induce apoptosis by itself, it exhibited this property in combination with fish-liver oil. Interestingly, number of cells in phases G2/M and S was reduced in both ARA12 alone and in combination with oil but not in oil alone. To conclude, ARA12 reduced HT-29 cells viability. It influenced cell cycle phases; however it did not induce apoptosis.

Keywords: colorectal cancer, cell viability, apoptosis, cell cycle.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

Żydziecki, Aliaksandr: Comparison of two renaturation methods and their refolding yields for therapeutically relevant recombinant $LysK_{CA}$ and bovine α-interferon

[1] Aliaksandr Żydziecki, [1]Uladzimir Prakulevich, [1]Mikhail Sholukh

1. Belarusian State University, Minsk, Belarus

Contact for the abstract: Aliaksandr Żydziecki; Zhydzetski@gmail.com

Many recombinant proteins being secreted in transformed microbial host cells are often deposited in insoluble inactive aggregates called inclusion bodies (IBs). It in turn requires the solubilization and reactivation of the target protein whereby conducting the refolding process. In this study we compared refolding yield of the recombinant endolysin $LysK_{CA}$ and bovine α-interferon (rbIFN- α) by two methods – conventional refolding via dilution and matrix-assisted refolding (MAR) in dependence on the purity of given proteins. For both renaturation approaches we used the same refolding buffer selected by the screening method for each protein. We found out that the more the solubilizate of the target protein contaminated with other proteins and nucleic acids the less refolding yield irrespective of the refolding method and vice versa. The effectiveness of refolding method has been shown to depend on the hydrophobicity and domain organization of protein molecule. The refolding yield of rbIFN-α via dilution and MAR was 26.96±3.82% and 0%, and for $LysK_{CA}$ 29.5±6.7% and 28.2±3.75% respectively. We supposed that hydrophobic parts of one-domain rbIFN-α irreversibly interact with the resin matrix while two-domain $LysK_{CA}$ (and three-domain LysK) can refold with one part of molecule but bound with the sorbent by another. Moreover, protein being immobilized on a resin less affected by different external factors on its refolding compared to the renaturation via dilution which yield considerably depends on the presence of stabilizing additives, pH and redox potential of the refolding buffer, dilution factor and other conditions. Also we ascertained that if MAR is applicable for protein it occurs immediately, nearly with the same yield after 20 min, 1 h and 24 h. Refolding by dilution in our case highly depended on the renaturation time and was 0% after 20 min and 1 h while the maximum yield of rbIFN-α was observed after 24 h.

Keywords: recombinant endolysin $LysK_{CA}$, recombinant bovine α-interferon, matrix-assisted refolding, refolding by dilution.

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.