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ELWIRA ŚLIWIŃSKA
Laboratory of Molecular Biology and Cytometry, UTP University of Science and Technology, al. Kaliskiego 7, 85-789 Bydgoszcz, Poland, e-mail: elwira@utp.edu.pl

Section name:
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IRENEUSZ ŚLESAK
Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Cracow, Poland, e-mail: i.slesak@ifr-pan.krakow.pl

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Lectures/Invited speakers

MANNOSE RECEPTOR IN COLORECTAL METASTASIS TO THE LIVER: MORE THAN WE THINK?

ALBA HERRERO, AITO BENEDICTO, BEATRIZ ARTETA*

Cancer and Translational Group-Tumor Microenvironment team,
Department of Cell Biology and Histology, School of Medicine and Nursing,
University of the Basque Country (UPV/EHU), Spain

The Mannose Receptor (ManR) is a transmembrane protein belonging to the C-type lectin family. In the liver, ManR is involved in the turnover and homeostasis of endogenous molecules, and pathogens. Moreover, ManR possesses a protumoral role by mediating melanoma cells adhesion to the liver endothelium. In an experimental model of colorectal liver metastasis, ManR promotes liver colonization by decreasing the local immune response. In the early steps of liver colonization, the binding of LFA-1 expressed in the tumour cells with its ligand ICAM-1 on LSECs triggers a pro-inflammatory reaction which correlates with an increase in ManR. Such increase has been related to the creation of a pro-tumoral microenvironment by promoting tumour cell adhesion and an increase of pro-inflammatory molecules into the tumour microenvironment mediated by COX-2.

More recently, the implication of ManR in the clearance of denatured-collagen, the existence of a tumour microenvironment where ManR expression and activity is increased, and the observation of a stromal reaction in ManR-promoting conditions prompt us to analyze the implication of this receptor in the remodelling of the stromal compartment during liver metastasis. Either indirect inhibition or direct inhibition of ManR not only reduce the development of liver metastasis but also provoked a reduction in the fibrillar collagen deposition in liver metastatic tissue together with the lower recruitment of pro-fibrotic liver populations. Thus, ManR has more versatile functions at different steps in the metastatic cascade than was initially thought.

* Corresponding author, e-mail: beatriz.arteta@ehu.eus

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Optical Genome Mapping in Routine Human Genetic Diagnostics - its Advantages and Limitations

Paul Dremsek*, Jürgen Neesen

Institute of Medical Genetics, Center for Pathobiochemistry and Genetics, Medical University, Vienna, Austria

Optical genome mapping (OGM) is a molecular method to display large, eukaryotic genomes and their structural features with a high resolution. The method relies on labelling specific sequences of DNA-strands, far longer than in modern sequencing methods. This superior length of the analyzed molecules leads to large contigs covering regions that are otherwise difficult to map. This enables OGM to detect balanced and unbalanced structural variants considered difficult to detect by other current methods. Hence, it promises to detect variants missed by other techniques like Microarray and Whole Exome/Genome Sequencing. However, due to its novelty, not much experience with OGM is available, especially so in regards to its application as a method in clinical human genetics.

Therefore, we tested a novel platform for OGM, the Saphyr System (Bionano Genomics, San Diego, CA, USA) and present our results regarding the feasibility of the method in a diagnostic setting. We tested 14 samples of DNA to confirm structural and numerical chromosomal variants originally detected by other diagnostic means (deletions, duplications, inversions, trisomies and a translocation) and used another 40 samples as a reference.

Most of the variants could be confirmed by OGM (12 of 14). We explore the causes leading to two variants being missed by OGM. Furthermore, we discuss the benefits and limitations of OGM by reviewing the data of all analyzed samples.

In summary, we currently deem OGM as a capable application in routine diagnostics, when embedded into an array of additional techniques.

* Corresponding author, e-mail: paul.dremsek@meduniwien.ac.at

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Transgenerational epigenetic inheritance – the revival of Lamarckianism?

Sigrid Hoyer-Fender

Institute for Zoology and Anthropology, Developmental Biology, Georg-August-University of Göttingen, Germany

Transmission of a genetic trait from one cell generation to the next beyond the sheer nucleotide sequences is defined as epigenetic inheritance. Epigenetic effects were first observed in a strain of the fruit fly *Drosophila melanogaster* by a mosaic eye colour phenotype. It was figured out that the chromosomal position of the *white* gene, which is essential for the red colour of the eye in wild-type flies, dictates its activity and causes the mottled eye phenotype, a phenomenon that becomes known as position effect variegation, in short PEV. Whether the gene becomes activated or suppressed is first stochastic but when established is stably inherited to daughter cells. Position effects are also responsible for the variable expression of chromosomally integrated transgenes. Thus, gene expression is not only controlled by cis-regulatory sequences and trans-activating factors but also by chromosomal context. Chromatin is assembled and structured by histone proteins that are, additionally, subjected to diverse post-translational modifications (PTMs). Histone PTMs specify the chromatin state and its activity, e.g. di- and trimethylation of lysine 9 of histone H3 (H3K9) correlate with transcriptional repression, whereas methylation of H3K4 specifies active genes. Histone PTMs are finally interpreted by the binding of specific proteins or protein complexes eventually regulating gene activity. However, since histone PTMs are dynamically regulated by specific enzymes that either attach ('writers') or remove ('erasers') a specific modification, the environment has a strong impact on the enzymatic activities. Although exposure to external factors has an inherited epigenetic effect in several species, demonstration of true transgenerational epigenetic inheritance (TEI) is hard to prove. TEI is the transmission of a phenotype caused by an environmentally induced epigenetic modification in the parental generation to those offspring that were never exposed to the signal that had triggered the change. The fruit fly *Drosophila melanogaster* seems to be an adequate model to explore epigenetic inheritance and TEI. The presentation will give an overlook of epigenetic inheritance in diverse species and its causes. Finally, own results investigating the transmission of an induced epigenetic change in the fruit fly *Drosophila melanogaster* will be presented.

* Corresponding author, e-mail: shoyer@gwdg.de

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Oral Presentations

NITRIC OXIDE-DEPENDENT RELEASE OF MACROPHAGE EXTRACELLULAR TRAPS (METS) BY BONE MARROW-DERIVED MACROPHAGES

DOMINIKA DRAB*, MICHAL SANTOCKI, ELZBIETA KOLACZKOWSKA

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Extracellular trap formation is the mechanism by which innate immune cells, including macrophages, release extracellular DNA decorated with nuclear and granular proteins. Macrophage extracellular traps (METs) are used to catch, immobilize and kill invading pathogens. However, mechanisms involved in MET formation are still fragmentary and mainly studied on macrophage cell lines or macrophages directly isolated from the organism but not on primary naïve cells, hence, we aimed to study mechanisms of their formation by bone marrow-derived macrophages (BMDMs) upon stimulation with lipopolysaccharide (LPS) and zymosan. In particular, we focused on involvement of nitric oxide in MET formation. In our study, BMDMs were obtained from the bone marrow immature cells by culturing them in conditioned medium comprising of macrophage colony-stimulating factor (M-CSF) derived from the mouse fibroblast L929 cell line cultured for 10 days. BMDM differentiation lasted 7 days and was verified by flow cytometric analysis of specific macrophage surface markers (F4/80 and CD11a). Then BMDMs were stimulated with LPS and/or zymosan to induce METs. Some cells were pretreated with nitric oxide synthase (NOS) inhibitors (L-NAME, 1400W) or nitric oxide donor (SNAP) or peroxynitrite. After overnight stimulation, METs were visualized by fluorescence/confocal microscopy upon immunocytochemical staining detecting MET proteins, histones (H2A.X) and MMP-9 attached to extracellular DNA. We showed that BMDMs were able to produce METs, as NOS inhibitors (L-NAME, 1400W) halted the MET formation by BMDMs upon stimulation with LPS, and SNAP and peroxynitrite induced MET release. Thus we postulate that MET formation is reactive nitrogen species dependent.

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* Corresponding author, e-mail: dominika.drab@doctoral.uj.edu.pl

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NEW INSIGHTS ON THE CORPUS LUTEUM PHYSIOLOGY: VASPIN EFFECT ON LUTEAL STEROID/PROSTAGLANDIN SYNTHESIS, ANGIOGENESIS, AND PROLIFERATION/APOPTOSIS

PATRYCJA KUROWSKA[1*], EWA MŁYCZYŃSKA[1], MONIKA DAWID[1], JOELLE DUPONT[2], AGNIESZKA RAK[1]

[1]*Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*INRAE, Unité Physiologie de la Reproduction et des Comportements, Nouzilly, France*

Corpus luteum (CL) is a transient endocrine gland formed from ovulated follicles and is also a site of the rapid remodeling, growth, differentiation, and death of cells; CL synthesizes progesterone (P4), essential for pregnancy maintenance and ovarian periodicity, which plays a central role in the regulation of the estrous cycle. Defects in CL function lead to infertility, miscarriages, and ovarian cycle disorders. The aim of this study was to examine the effect of vaspin on porcine luteal cell function including steroid/prostaglandin synthesis, angiogenesis, proliferation, apoptosis, and explain molecular mechanism of this processes. Vaspin plays important roles in inflammation, obesity, and regulates ovarian follicles physiology.

In vitro study of luteal cells showed that vaspin at doses 1 - 100 ng/ml increased both P4 secretion and steroidogenic enzymes: CYP11A1 and HSD3B protein expression *via* 78-kDa glucose-regulated protein (GRP78) receptor and protein kinase A (PKA). Moreover, vaspin stimulated PKA and mitogen activated kinase (ERK1/2) phosphorylation. We noted that vaspin increased prostaglandin PGE2/PGF2 secretion and its receptors PTGER1/PTGFR, as well as levels of angiogenic factors: VEGFA, FGF2, ANGPT1 and receptors VEGFR1/2 expression *via* GRP78 and ERK1/2. Additionally, vaspin in a time-dependent manner stimulated cell proliferation and decreased apoptosis *via* GRP78 and ERK1/2. Differences between groups were analysed by one-way ANOVA followed by Tukey's post hoc test ($n=4$, $p<0.05$).

This study provides evidence that vaspin by regulation of steroid/prostaglandin synthesis, angiogenesis, proliferation, and apoptosis is a new local regulator in the porcine CL.

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* Corresponding author, e-mail: patrycja.kurowska@uj.edu.pl

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REGULATION OF INFLAMMATORY-MEDIATED CHEMERIN EXPRESSION

KAMILA KWIECIEŃ*, PAWEŁ MAJEWSKI, JOANNA CICHY, MATEUSZ KWITNIEWSKI

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

White adipose tissue is no longer considered as a passive energy storage, but well-described active endocrine organ. It produces a variety of regulatory proteins collectively called adipokines. Chemerin, encoded by *RARRES2* gene, is a member of adipokine family and has immunomodulatory, chemotactic, antibacterial and metabolic functions. These pleiotropic actions of chemerin require multilevel regulation of its' secretion. It has been shown that chemerin expression is up-regulated in adipocytes but not hepatocytes after stimulation with proinflammatory cytokines. However, mechanisms that control the inflammatory-mediated chemerin expression still remains obscure.

Our previous studies showed that *RARRES2* promoter proximal region -252/+258 bp is involved in constitutional chemerin expression but do not respond to cytokine stimulation in luciferase reporter assays. Recent computational analysis of publicly-available ChIP-Seq database show that distal fragment of *RARRES2* promoter located -6000/-7000 bp can bind several inflammatory-mediated transcription factors in adipocytes. However, dual-luciferase reporter assay shows no significant up-regulation of luciferase activity in cytokine-stimulated 3T3-L1 cell line transfected with reporter construct containing *RARRES2* distal fragment.

In order to determine the transcription factors involved in inflammatory-mediated chemerin expression, various TF inhibitors were used prior to interleukin-1 and oncostatin M stimulation of 3T3-L1 cell line. Together our findings suggest that chemerin up-regulation following IL1- and OSM stimulation may be mediated through activation of NF B and STAT3, but also point that inflammation-responsive transcription factor binding sites are located outside of the investigated fragments or that they require other cis-regulatory elements.

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Mechanizmy regulacji ekspresji chemeryny w mysich adipocytach i hepatocytach pod wpływem cytokin prozapalnych. The study was supported by BMN2020 WBBiB UJ No. PSP: N19/MNS/000037 (KK).

* Corresponding author, e-mail: kamila.kwiecien@uj.edu.pl

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FUNCTIONAL LINKS BETWEEN EXPRESSION PATTERN OF ATP-BINDING CASSETTE TRANSPORTERS AND DRUG RESISTANCE WITHIN HUMAN GLIOBLASTOMA MULTIFORME (GBM) CELLS

MACIEJ PUDEŁEK*, JAROSŁAW CZYŻ

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

Gliomas constitute a subtype of the most malignant brain neoplasms. Among them, glioblastoma multiforme (GBM; IV-grade brain tumor; WHO) represents the greatest challenge for the current neurooncology. Anatomical and physiological constraints (incl. blood-brain barrier) and development of GBM chemoresistance result in a limited efficiency of GBM chemotherapy and subsequent tumor recurrences. GBM chemoresistance is strongly related to the activity of ABC transporters (ATP-binding cassette transporters). They act as membrane-associated and/or intracellular pumps, which effectively remove cytotoxic xenobiotics (incl. cytostatic drugs) from intracellular spaces. Their activity can initiate the expansion of drug-resistant tumor cell lineages in stress conditions. In the present study, we determined the role of individual ABC proteins in xenobiotics efflux and chemoresistance of human GBM cells. For this purpose, we verified the expression pattern of ABCB1, ABCC1 and ABCG2 proteins in T98G and U87 human GBM cells using ImageStream®X cytometry and fluorescence microscopy. Subsequently, esiRNA was applied to down-regulate the expression of individual transporter levels, followed by time-lapse calceinAM efflux analyses. Finally, we examined the sensitivity of GBM cells to an array of cytostatic drugs (with MTT test) and traced their long-term microevolution under doxorubicin stress with a special emphasis on ABCB1 expression changes after drug ablation. We show that the basal levels of ABC transporters are strongly associated with the resistance of GBM cells to cytostatic drugs. However, an ectopic ABC down-regulation does not increase cellular chemosensitivity. Collectively, these data indicate that chemotherapeutic stress activates the alternative systems of chemoresistance that facilitate the microevolution of drug-resistant GBM lineages.

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* Corresponding author, e-mail: maciej.pudelek@doctoral.uj.edu.pl

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MILD AND ASYMPTOMATIC COVID-19 CONVALESCENTS HAVE LONG-TERM ENDOTYPE OF IMMUNOSUPPRESSION ASSOCIATED WITH MYELOID DERIVED SUPPRESSOR CELLS OF GRANULOCYTE ORIGIN AND GRANULOCYTE DYSFUNCTION

IZABELA SIEMIŃSKA[1*], KAZIMIERZ WĘGLARCZYK[1], MARCIN SURMIAK[2], DOROTA KUROWSKA-BARAN[3], MAREK SANAK[2], MACIEJ SIEDLAR[1] AND JAREK BARAN[1]

[1]*Department of Clinical Immunology, Jagiellonian University Medical College, Wielicka 265, 30-663 Krakow, Poland*

[2]*Department of Internal Medicine, Jagiellonian University Medical College, Skawińska 8, 31-066 Krakow, Poland*

[3]*Department of Clinical Microbiology, Laboratory of Virology and Serology, University Children's Hospital, Wielicka 265, 30-663 Krakow, Poland*

The SARS-CoV-2 virus infection (COVID-19) is associated with severe lymphopenia and impaired immune response, including the expansion of myeloid-derived suppressor cells (MDSCs). These cells have been described both in infections and cancer and are known for their immunosuppressive activity. In the case of COVID-19, long-term complications have been frequently observed (long-COVID). In this context we aimed to investigate the immune response of COVID-19 convalescents after a mild or asymptomatic course of disease.

We enrolled 13 convalescents who underwent a mild or asymptomatic infection with SARS-CoV-2, confirmed by a positive result of the (COVID-19) PCR test and 13 healthy donors without SARS-CoV-2 infection in the past. Whole blood was used for T cell subpopulation and MDSCs analysis. MDSCs and normal density granulocytes (NDGs) were sorted out by FACS and used for T cell proliferation assay with autologous T cells activated with anti-CD3 mAb. Serum samples were used for the detection of anti-SARS-CoV-2 neutralizing IgG and GM-CSF concentration.

Our results showed that in convalescents, even 2 months after infection, an elevated level of MDSCs of granulocyte origin (PMN-MDSCs) is still maintained in the blood, which correlates negatively with the level of CD8 + and double-negative T cells. Moreover, PMN-MDSCs showed a trend in affecting the production of anti-SARS-CoV-2 neutralizing antibodies. Surprisingly, our data showed that in addition to PMN-MDSCs also NDGs from convalescents inhibit proliferation of autologous T cells. Additionally, in convalescents sera we detected significantly higher concentrations of GM-CSF.

We conclude, that in mild and asymptomatic COVID-19 convalescents PMN-MDSCs and granulocyte dysfunction are responsible for a long-term endotype of immunosuppression.

* Corresponding author, e-mail: i.gorska@doctoral.uj.edu.pl

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Poster Presentations

KINETICS OF TUMOR DERIVED EXTRACELLULAR VESICLES (TEVS) ENGULFMENT BY HUMAN BLOOD MONOCYTES AND THP-1 CELLS

ANETA ANDREASIK[1], ANNA ALWANI[1*], MAGDALENA OŻÓG[2] AND MONIKA BAJ-KRZYWORZEKA[1*]

[1]*Department of Clinical Immunology, Jagiellonian University Medical College, Wielicka 265, 30-663 Krakow, Poland*

[2]*School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, ul. Jedności 8, 41-200 Sosnowiec, Poland*

Monocytes are phagocytic cells that can recognize and engulf pathogens, apoptotic cells, and other particles present in their proximity. During tumor progression, monocytes are exposed to the Tumor-derived Extracellular Vesicles (TEVs), small membrane vesicles released by tumor cells. The mechanisms of TEVs engulfment are not fully understood however kinetics of the uptake process is relevant to predict the bioavailability and half-life of TEVs in body fluids. This study was designed to compare the dynamics of TEVs engulfment by human monocytes and monocytic cell line (THP-1).

Monocytes were isolated from peripheral blood leukocytes of healthy donors by counter-current elutriation. THP-1 cells were cultured according to ATCC recommendation. TEVs were isolated by ultracentrifugation from the supernatants of colon cancer cell line (HCT116) culture. TEVs were labeled with SytoRNA green dye. Monocytes and THP-1 cells were incubated with labeled TEVs in different ratios for 30min to 18 hours at different temperatures.

The obtained results indicated that monocytes engulf TEVs faster and more efficiently than THP-1. After 2h incubation app. 80% of monocytes and 20% of THP-1 cells showed green fluorescence from TEVs. The efficiency of uptake increased after overnight incubation. The uptake of TEVs was almost completely inhibited at 4°C. In summary, TEVs are internalized by monocytic cells mostly via phagocytosis. Monocytes engulf and restore TEVs very rapidly however the phagocytic potential of THP-1 is lower, which should be considered when selecting an experimental model for TEVs internalization.

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* Corresponding author, e-mail: anna.alwani@doctoral.uj.edu.pl; monika.baj-krzyworzeka@uj.edu.pl

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MICROPARTICLES FROM SEPTIC INDIVIDUALS IMPACT EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) BUT NOT NITRIC OXIDE (NO) PRODUCTION BY MURINE MACROPHAGES

ANNA BIAŁA*, WERONIKA ORTMANN, ELŻBIETA KOŁACZKOWSKA

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Extracellular vesicles (EVs) are diverse population of nano-micrometer, enclosed membrane structures containing various bioactive molecules, namely lipids, proteins and fragments of genetic material. EVs formation/composition is dependent on their subtype, and as key players in intercellular communication they are released by any cell type under either *in vivo* or *in vitro* conditions. Microparticles (MPs) contribute to numerous pathological processes such as systemic inflammation aka sepsis. Herein we aimed to determine if the microparticles (secreted in different body compartments) collected from mice with liposaccharide (LPS)-induced sepsis (endotoxemia) affect effector functions of murine macrophages (RAW 264.7). MPs were isolated from blood plasma and exudative/inflammatory fluid from the peritoneal cavity of mice with endotoxemia. In particular, we evaluated macrophage viability, adhesion to the surface, mitochondrial activity and reactive oxygen (ROS) and reactive nitrogen (RNS) species production. Additionally, expression of inducible nitric oxide synthase (iNOS) was studied by immunocytochemistry. MPs (independently of their origin/concentration) up-regulated macrophage viability/numbers but did not impact other parameters except of iNOS. The iNOS expression was increased by LPS and furthermore by its combination with MPs originating from peritoneal cavity. Surprisingly, however, MPs had no impact on NO release independently of their origin. We hypothesize that MPs secreted in peritoneal cavity might be more heterogeneous in terms of transported cargo e.g. due to the fact that they are released also by non-blood related cell types. In line with this, the cargo might impact NO production by antioxidant/antinitrosant activity or L-arginine scavenging.

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* Corresponding author, e-mail: anna.biala@doctoral.uj.edu.pl

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THE EFFECT OF 5-FLUOROURACIL ON 2-OXOGLUTARATE DEHYDROGENASE (2-OGDH) IN THE RAT HEART

JUSTYNA BIELEŃ[1*], MICHAŁ JURCZYK[2], VERONIKA ALEKSANDROVYCH[2], ANNA GAWĘDZKA[1], PAULINA STACH[2], JAGODA DRĄG[1], KRZYSZTOF GIL[2], MAŁGORZATA KNAPIK-CZAJKA[1]

[1]*Department of Biochemical Analytics, Jagiellonian University Medical College, Medyczna 9 30-688 Krakow, Poland*

[2] *Department of Pathophysiology, Jagiellonian University Medical College, Czysta 18, 31-121 Krakow, Poland*

2-oxoglutarate dehydrogenase complex (2-OGDH), that is composed of 3 catalytical subunits (E1,E2,E3), catalyzes the oxidative decarboxylation of 2-oxoglutarate to succinyl-CoA. As a key enzyme of tricarboxylic acid cycle the complex exerts a control on overall oxidative/energy cell metabolism.

5-fluorouracil (5-FU) is widely used in the treatment of different types of cancer. It has been demonstrated that 5-FU has the potential to cause a wide spectrum of cardiotoxicity, ranging from asymptomatic electrocardiographic changes to cardiomyopathy and subsequent cardiac failure. We hypothesized that 5-FU modifies 2-OGDH activity and affects heart metabolism.

The present study was aimed at the investigation of the in vivo effect of 5-FU on 2- OGDH activity and mRNA level for E1 subunit. Wistar male rats were administered with 4 doses of 5-FU, 150 mg/kg b.wt. each (study group) or 0.3% methylcellulose (control group). 2- OGDH activity was assayed spectrophotometrically. The mRNA level was determined by quantitative real-time PCR.

We found that in rats treated with 5-FU, activity of 2-OGDH was 29% higher, while the mRNA level for E1 subunit was 50% lower than in the control group. The differences were statistically significant ($p < 0.05$).

In conclusion, 5-FU has a stimulatory effect on 2-OGDH in the rat heart. Alteration in the complex activity did not correspond with change in E1 mRNA level. Therefore, it is possible that 5-FU affects 2-OGDH via factors regulating the complex activity at post-transcriptional level. It is conceivable that stimulation of 2-OGDH by 5-FU may lead to changes in energy metabolism and in heart functions.

* Corresponding author, e-mail: justyna.bielen@uj.edu.pl

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Alginate hydrogels in 3D cell cultures

Justyna Bożek[1*], Sylwia Wójcik[1*], Beata Kamińska[1], Joanna Tomala[1], Ewa Pocheć[2], Elżbieta Szostak[1]

[1]*Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland*

[2]*Department of Glycoconjugate Biochemistry, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

The oldest and most common way of culturing cells is the 2D model. The new approach focuses on recreating the *in vivo* environment in 3D models. hey allow researchers to perform more complex experiments and obtain more accurate and reliable data. Three-dimensional cell culture models can be used in areas such as drug discovery, cancer research or studying the biochemistry of the cells. One of the techniques that is currently being developed is the scaffold method, using various nontoxic materials.

Sodium alginate is a natural polymer derived from brown algae that together with calcium ions creates a natural hydrogel, a porous material characterized by the high water retention. The important advantage of hydrogels is their ability to mimic extracellular matrix (ECM) structure, which provides similar physical, mechanical, and biological properties to those in natural cell environment and allows cells to communicate in three dimensions.

The aim of our work was to optimize the process of producing unmodified and modified alginate spheres that create 3D environment. As part of the research, the influence of the alginate matrix on the biological characteristics of the cutaneous primary human melanoma cell line WM-115 was assessed. The conducted research is a starting point for the verification of the research question "Do alginate dressings based on extracts of *Piptoporus betulinus* exhibit an anti-cancer activity in an *in vitro* model of skin melanoma".

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* Corresponding author, e-mail: justyna98.bozek@student.uj.edu.pl; sylwia.weronika.wojcik@student.uj.edu.pl

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EFFECT OF ETHYLENE AND THIDIAZURON APPLICATION ON CALLUS MORPHOLOGY AND REGENERATIVE EFFICIENCY IN *HYPERICUM PERFORATUM* L. TISSUE CULTURE

ALEKSANDRA BRANKIEWICZ*, MONIKA TULEJA

Department of Plant Cytology and Embryology, Institute of Botany, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Plant growth regulators (PGR's) are responsible for the developmental physiology and morphogenesis *in vivo* and *in vitro* in plants. Ethylene is an inductor of adventitious roots formation and shoots elongation inhibitor. It promotes also the plant tissues senescence. Thidiazuron (TDZ), a common synthetic cytokinin, is an advantageous PGR in *Hypericum perforatum* tissue culture. St. John's wort, a valuable herb used in pharmacy, is the aim of interest for plant biotechnology. Callus was induced from leaf blades and the parts of stems on standard ½ MS medium supplemented by 0,45 M TDZ. After 2-3 weeks of culture the first symptoms of the indirect organogenesis were visible. Obtained cultures were divided into two experimental systems – the first with TDZ and the second were transferred on medium without TDZ. The long-term tissue with the symptoms of necrosis was put into several media with ethylene inhibitors for improving the quality of the callus. The role of ethylene was tested by ethylene signaling inhibitor (silver nitrate – $AgNO_3$) and inhibitor of ethylene biosynthesis (aminoethoxyvinylglycine – AVG) application on long-term *H. perforatum* callus culture. The absence of ethylene in the cultures provided to decrease of regeneration efficiency (the average number of regenerants/explant), while $AgNO_3$ application caused in regeneration potential increment, similar to the effect of TDZ. The indirect asynchronous organogenesis was characteristic for all experimental setups. Deprivation of ethylene synthesis (AVG presence) inhibited the adventitious roots formation, while the presence of $AgNO_3$ and TDZ inducted ryzogenesis. During this experiments the noticeable effect of plant origin was revealed.

* Corresponding author, e-mail: aleksandra.brankiewicz@student.uj.edu.pl

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Role of itaconic acid and pyruvate kinase isozyme M2 in the activity of neutrophils and their ability to form Neutrophil Extracellular Traps (NETs)

Gabriela Burczyk*, Iwona Cichoń, Elżbieta Kołaczkowska

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Immunometabolism is a new field of biology which studies the multilevel interactions between the immune and metabolic systems. These include impact of leukocyte's own metabolism on their effector functions. As studies on immunometabolism of neutrophils are limited, herein we aimed to study the role of a "broken Krebs cycle" metabolite – itaconic acid, and pyruvate kinase isozyme M2 (PKM2), a critical enzyme of the glycolytic pathway. Previous studies on itaconic acid and PKM2 have shown their bactericidal and anti-inflammatory abilities in macrophages/lymphocytes. The main goal of this study was to determine the effect of itaconic acid and pyruvate kinase isozyme M2 on inflammatory responses of neutrophils and formation of neutrophil extracellular traps (NETs). The backbone of NETs is composed of extracellular DNA, to which proteins are attached, including enzymes (e.g. cathepsin G, neutrophil elastase) from the neutrophil granules. In the study, bone marrow derived neutrophils isolated from C57BL/6J mice were treated with various concentrations of 4-octyl itaconate (4-OI) or TEPP-46/shikonin (inhibitors of PKM2) in the presence or absence of lipopolysaccharide (LPS). NETs were detected by immunocytochemistry and the cell viability/cytotoxicity were tested by PrestoBlue, NBT, MTT and CV. The microscopic analysis clearly showed that formation of NETs by LPS stimulated cells, but pre-treated with 4-OI or TEPP-46/shikonin was decreased. We conclude that pre-exposure of cells to both tested metabolites/enzymes has an immunosuppressive impact on neutrophils and can down-regulate their cellular functions.

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* Corresponding author, e-mail: gabriela.burczyk@doctoral.uj.edu.pl

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INVESTIGATING THE INTERPLAY BETWEEN NEUTROPHILS, PLATELETS AND NEUTROPHIL EXTRACELLULAR TRAPS (NETS) IN PATHOLOGIES INVOLVING OBESITY AND SYSTEMIC INFLAMMATION USING "INTRAVITAL MICROSCOPY"

IWONA CICHON*, WERONIKA ORTMANN, ELŻBIETA KOLACZKOWSKA

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Obesity manifests itself with low-grade chronic inflammation which shapes immune responses during infection. Albeit obese individuals are at risk of higher mortality due to comorbidities, they are better protected from systemic inflammation/sepsis, a phenomenon described as an obesity paradox. Excessive fat is associated with neutrophil infiltration into adipose tissue and elevated neutrophil markers, e.g. neutrophil elastase (NE). One of the mechanism utilized by neutrophils to fight invading pathogens is release of mesh-like DNA/protein complexes, named neutrophil extracellular traps (NETs). Importantly, NETs can be trigger also by interactions with activated platelets, acknowledging importance of other immune players in inflammatory milieu. Thus, we aimed to compare differences between mice on high-fat diet (HFD) and lean (ND) individuals focusing on neutrophils and NETs release. We further put under scrutiny interactions of neutrophils with platelets. Two models of obesity were used – fat diet induced obesity (C57BL/6J) and leptin mutants (ob/ob mice). Endotoxemia was induced by intraperitoneal inoculation of lipopolysaccharide (LPS). NETs were visualized in the liver vasculature of living mice using spinning disk confocal microscopy (intravital microscopy, IVM). The obtained results indicate that neutrophils from obese mice produce substantially less NETs in comparison to lean controls. IVM also revealed impaired platelet-neutrophil interactions in obese mice. Importantly, upon selective platelet transfer from lean to obese mice, the NET formation was partially restored. We conclude, that platelet-neutrophil-NET interactions are important factors behind the obesity paradox in sepsis and thus may determine survival during this systemic inflammation.

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* Corresponding author, e-mail: iwona.cichon@doctoral.uj.edu.pl

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UP-REGULATED ST6GAL1 IN SERA OF GRAVES' DISEASE PATIENTS NORMALIZES UNDER IMMUNOSUPPRESSIVE TREATMENT

MAGDALENA DOŁĘGA[1*], SARA TRZOS[1], GRZEGORZ SOKOŁOWSKI[2], EWA POCHEĆ[1]

[1]*Department of Glycoconjugate Biochemistry, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Chair and Department of Endocrinology, Jagiellonian University Medical College, Jakubowskiego 2, 30-688 Krakow, Poland*

Sialylation of serum proteins, among other immunoglobulin class G (IgG), regulates significantly their half-life and activity. The attachment of sialic acid (Sia) by 2,6 bonds enables the Fc fragment of IgG to change its conformation from open to closed, which increases the plasticity of antibody and redirects its activity towards anti-inflammatory. Recently, it has been demonstrated that serum proteins are α2,6-sialylated in blood independently on classical intracellular glycosylation pathway.

The higher mRNA level and activity of sialyltransferase ST6Gal1, associated with the elevated Sia content in thyroid gangliosides, was shown in Graves' disease (GD) compared to control thyroid tissue. So far no data on serum concentration of ST6Gal1 in thyroid autoimmunity have been presented. The aim of the study was to determine the ST6Gal1 level in the serum samples from GD patients before (n=35) and after (n=29) thyroid-stimulating hormone (TSH) normalization as the results of immunosuppressive therapy, and the sex- and age-matched healthy donors (n=24, control group). The concentration of TSH and anti-TSHR IgG differed significantly between GD and healthy donors. STGal1 level was analyzed using human ST6GAL1 ELISA assay. The non-parametric statistical Kruskal-Wallis test was applied between the control and both GD groups ($p<0.05$). Our study showed the statistically significant increase of ST6Gal1 serum level in the non-treated patients versus healthy controls and the normalization of this sialyltransferase in the patients as the result of immunosuppressive therapy. The statistical analysis did not show the correlation between ST6Gal1 serum level and TSH or anti-TSHR concentrations.

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* Corresponding author, e-mail: magdalena.dolega@student.uj.edu.pl

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ANTINOCICEPTIVE ACTIVITY OF NEW 1H-ISOINDOLE-1,3(2H)-DIONES

ANNA DZIUBINA[1*], DOMINIKA SZKATUŁA[2]

[1]*Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-668 Krakow, Poland*

[2]*Department of Chemistry of Drugs, Faculty of Pharmacy with Division of Laboratory Diagnostics, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland*

As indicated by literature data, isoindole-1,3-diones (phthalimides) represent a promising direction for drug research. They have broad-spectrum biological activities such as antibacterial, anticancer, anti-inflammatory, anticonvulsant, antipsychotic.

In view of broad activities of phthalimides we herein present the antinociceptive and antiedematous properties of the new synthesized N- arylpiperazine-alkyl derivatives of phthalimide in various experimental models of pain. Four compounds (F1-F4) differing in the type of pharmacophore in the phenyl group of the 2-hydroxy-3- (4- aryl-1-piperazinyl) propyl on the imide nitrogen atom (R, F1-F3) and the 4-benzhydryl analog (F4) were selected for *in vitro* study. Based on *in vitro* studies, the most potent compounds, was selected for *in vivo* study and its safety *in vitro* was evaluated. In *in vivo* study the writhing test, the hot plate test, the formalin test and the carrageenan model test were performed.

Compounds F1- F4 significantly attenuated pain of peripheral origin, more strongly than the reference compound aspirin, and to a lesser extent (F1-F3) pain of central/supraspinal origin. Compound F1 was the most potent in reducing edema and hyperalgesia. None of the compounds tested, at the highest analgesic dose, impaired motor coordination, implying no neurotoxic effects.

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* Corresponding author, e-mail: anna.dziubina@uj.edu.pl

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IMPACT OF ATORVASTATIN ON MURF-1 AND MAFBX GENE EXPRESSION IN SKELETAL MUSCLE OF RATS FED LOW PROTEIN DIET

ANNA GAWEDZKA*, MALGORZATA KNAPIK-CZAJKA, JAGODA DRAG, JUSTYNA BIELEN, MALGORZATA BELCZYK

Department of Biochemical Analytics, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

Background: Insufficient dietary protein intake results in adaptive changes in muscle protein turnover manifesting as reduction in protein breakdown. The main components of muscle intracellular ubiquitin-proteasome system (UPS) involved in protein breakdown are E3 ubiquitine ligases encoded by muscle RING finger 1 (MuRF-1) and muscle atrophy F-box (MAFbx) genes.

Myotoxicity is the most common side effect caused by statins which are commonly used lipid-lowering drugs. We hypothesized that statins stimulate expression of MuRF-1 and MAFbx genes and disturb muscle adaptation to reduced dietary protein intake. The aim of this study was to evaluate the effect of atorvastatin on MuRF-1 and MAFbx genes expression in rats fed low protein diet.

Material and methods: Male Wistar rats were randomized into 3 groups: standard diet-fed controls (ST), low protein diet-fed controls (LP) and low protein diet-fed rats treated with atorvastatin at 50 mg/kg b.wt/day (LP+A). ST and LP groups received 0,2% methylcellulose (vehicle). Atorvastatin and vehicle were administered orally by gavage once daily for 14 days. The levels of mRNA for MuRF-1 and MAFbx genes in gastrocnemius muscle were measured using RT-QPCR.

Results: The level of mRNA for MAFbx in LP+A group was significantly higher as compared to ST group ($p<0.05$), but did not differ from LP group ($p>0.05$). There was no difference in MuRF-1 mRNA level between groups ($p>0.05$).

Conclusion: Atorvastatin-induced increase in MAFbx mRNA level in the skeletal muscle of rats fed low-protein diet may lead to and excessive protein breakdown. Therefore, it may disturb muscle adaptation to low protein diet.

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* Corresponding author, e-mail: anna.gawedzka@uj.edu.pl

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THE ROLE OF GROWTH FACTORS ON THE GASTROINTESTINAL TRACT DEVELOPMENT IN CHICKENS

KLAUDIA JASZCZA*, KRYSTYNA PIERZCHAŁA-KOZIEC

Department of Animal Physiology and Endocrinology, University of Agriculture in Kraków, Adama Mickiewicza 24/28, 30-059 Krakow, Poland

After hatching significant changes occur in the anatomy, physiology, and roles of the birds digestive system. Many studies were devoted to discover gastrointestinal hormones, one of which is ghrelin. Ghrelin is involved in the regulation of appetite and stimulates growth hormone release. Insulin-like growth factor-1 (IGF-1) is the main regulator of growth and metabolism in chicken. The question arises as to how the growth and development of GI tract depend on the ghrelin and IGF-1 interaction shortly after chicken hatching. Thus, the aim of the study was to investigate the changes of ghrelin and IGF-1 concentrations in the crop, stomach, and intestine of newly hatched chickens. The *in vitro* experiment was carried out on newly hatched chickens (n=24), three hours after hatching. Sections of tissues were stained with hematoxylin and eosin (H&E), then evaluated with a light microscope. Fragments of tissues were homogenized and the supernatant was stored at 80°C until ghrelin and IGF-1 levels were measured by RIA and ELISA methods, respectively. The highest concentration of ghrelin was found in the stomach followed by crop and intestine. The highest concentration of IGF-1 was in the crop followed by stomach and intestine. The microscopic evaluation clearly showed diversified cells in each part of the digestive system. The tissues variability was correlated with different ghrelin and IGF-1 concentrations in tested GI fragments. The obtained results suggest that ghrelin and IGF-1 might be stimulators of growth and development as well as modulators of GI tissues functions in growing hens.

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* Corresponding author, e-mail: klaudia.jaszcza@urk.edu.pl

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IN VITRO EFFECT OF PCB118, PCB153, 4-OH-PCB107 AND 3-OH-PCB153 ON MRNA EXPRESSION OF THYROID HORMONE TRANSPORTERS (*OATP1C1, MCT8, MCT10, LAT1*) IN THE CHICKEN THYROID GLAND AND LIVER

KINGA KOWALIK*, DOROTA KATARZYŃSKA-BANASIK, ANDRZEJ SECHMAN

Department of Animal Physiology & Endocrinology, University of Agriculture in Krakow, Adama Mickiewicza 24/28, 30-059 Krakow, Poland

To assess the effect of PCB118 and PCB153 and their hydroxylated metabolites (4-OH-PCB107 and 3-OH-PCB153, respectively) on mRNA expression of thyroid hormone (TH) transporters (*MCT8, MCT10, OATP1C1, LAT1*), chicken thyroid and liver explants were incubated for 6h at 39°C in control medium or in medium sumplemented with TSH (250 mU/ml; the thyroid) or DEX (100 nM; the liver), PCB118, 4-OH-PCB107, PCB153, 3-OH-PCB153 (at doses of 0.5×10^{-8} M) or TSH/DEX together with each PCB and OH-PCB. The gene expression was determined by qPCR; results obtained were statistically evaluated by means of one-way analysis of variance (ANOVA). Differences between means were analysed by post-hoc Tukey's test at $p<0.05$. The results of this experiment showed mRNA expression of all tested TH transporters in the chicken thyroid and liver, except for *OATP1C1* mRNA in liver explants. Furthermore, PCB-dependent effects on *OATP1C1, MCT8,* and *MCT10* mRNA expression were found in the thyroid explants and *MCT10* and *LAT1* in the liver ones. In conclusion, both PCB118 and PCB153 and their OH-PCBs affect TH transport across the thyrocyte and hepatocyte membrane. In addition, the effects of PCBs and OH-PCBs depend mainly on the type of PCB congener. Further studies are necessary to explain a mechanism of PCB and OH-PCB action in the avian thyroid gland and liver.

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* Corresponding author, e-mail: kinga.kowalik@urk.edu.pl

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A MIXTURE OF PERSISTENT ORGANIC POLLUTANTS DETECTED IN HUMAN FOLLICULAR FLUID INDUCES THE TRANSITION FROM AEROBIC GLYCOLYSIS TO MITOCHONDRIAL RESPIRATION IN HUMAN GRANULOSA HGrC1 CELLS

KINGA KRAWCZYK*, WERONIKA MARYNOWICZ, JUSTYNA GOGOLA-MRUK, ANNA PTAK

Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Endocrine-disrupting chemicals (EDCs), such as perfluorooctanoate, perfluorooctane sulfonate, 2,2-dichlorodiphenyldichloroethylene, polychlorinated biphenyl 153, and hexachlorobenzene are persistent organic pollutants that are found in human follicular fluid (FF). These compounds may affect endocrine function, disrupt steroid secretion by granulosa cells. Steroidogenesis, especially mitochondrial cholesterol import, is exquisitely sensitive to ATP supply and demand. Moreover, some data suggest that there are relationships between steroid hormone synthesis and glycolysis. Whether the mixture of POPs found in FF may disrupt the mitochondrial function of the granulosa cells in the ovary remains an open question. Considering that humans are exposed to multiple pollutants simultaneously, we elucidated the effects of a mixture of endocrine-disrupting chemicals (EDCs) on human granulosa HGrC1 cells glucose transporter 1 (GLUT1) expression, glycolysis rate, mitochondrial activity, and ATP synthesis. We showed that the EDC mixture increases ATP synthesis. However did not affect the expression of glucose transporter 1 (GLUT1), hexokinase 2 (HK2), which catalyzes the first step of glycolysis and lactate dehydrogenase A (LDHA), which converts the glycolytic product pyruvate to lactate. Our data proves that the EDC mixture does not change the rate of glycolysis but its shift from aerobic glycolysis to mitochondrial oxidative phosphorylation, which results in increased ATP synthesis.

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* Corresponding author, e-mail: kinga.krawczyk@doctoral.uj.edu.pl

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A MIXTURE OF PERSISTENT ORGANIC POLLUTANTS DETECTED IN HUMAN FOLLICULAR FLUID INCREASES ACTIVITY OF MITOCHONDRIA AND STEROID SECRETION IN HUMAN GRANULOSA HGrC1 CELLS

Kinga Krawczyk[1*], Weronika Marynowicz[1], Justyna Gogola-Mruk[1], Wacław Tworzydło[2], Anna Ptak[1]

[1]*Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Department of Developmental Biology and Invertebrate Morphology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

Disruption of granulosa cells (GCs), the main functional cells in the ovary, is associated with impaired female fertility. Epidemiological studies demonstrated that women have detectable levels of organic pollutants (e.g., perfluorooctanoate, perfluorooctane sulfonate, 2,2-dichlorodiphenyldichloroethylene, polychlorinated biphenyl 153, and hexachlorobenzene) in their follicular fluid (FF), and these compounds may directly affect the function of GCs. Considering that humans are exposed to multiple pollutants simultaneously, we elucidated the effects of a mixture of persistent organic pollutants (POPs) on human granulosa HGrC1 cells. Importantly, there is a connection between mitochondrial dysfunction and the structural disruption and function of the ovaries. Mitochondria are the central sites of lipid synthesis and thus are essential for the biosynthesis of steroid hormones by ovarian GCs. As already know, mitochondrial fusion is also needed for the proper steroidogenesis. The mixture of POPs increased mitochondrial network formation and activity of mitochondria, which resulted in a direct increase in progesterone (P4) secretion by upregulating 3 -hydroxysteroid dehydrogenase (3 HSD) expression, without effects on estradiol (E2). Taken together, the mixture of POPs, present in FF, can alter the functions of human GCs by disrupting steroidogenesis. Further, the imbalance between P4 and E2 secretion can lead to ovarian pathologies and disruption of female reproductive health. Interestingly, this study highlights the POPs mixture elicits effects by targeting mitochondria and increases mitochondrial network formation, mitochondrial activity, and expression of 3 HSD, which is associated with the inner mitochondrial membrane.

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* Corresponding author, e-mail: kinga.krawczyk@doctoral.uj.edu.pl

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PELLINO3 IS ENGAGED IN STAT1 DEPENDENT IL-6 EXPRESSION DURING INFLUENZA B VIRUS INFECTION

ANNA KULA*, EDYTA MAKUCH, JAKUB SIEDNIENKO

Bioengineering Research Group, Life Sciences & Biotechnology Center, Łukasiewicz Research Network – PORT Polish Center for Technology Development, ul. Stabłowicka 147, 54-066 Wroclaw, Poland

Influenza virus infection activates various intracellular signaling pathways. This activation is mediated by selected transcription factors and results in cytokines expression. In this study we found that in human lung epithelial cell line A549 activation of Stat1 transcription factor is observed after IBV infection and this process is regulated byE3 ubiquitin ligase Pellino3. Stat proteins can be activated either in canonical or non-canonical pathways.

IFN dependent Jak/Tyk phosphorylation is involved in first type while latter may engage PI3K/Akt, MAPK or NF B pathways. Our research demonstrates that excessive phosphorylation of Stat1 in *Peli3*$^{-/-}$ A549 cells is mediated by non-canonical pathway and requires p38 MAP kinase activity. Furthermore, our study shows that IL-6 expression is upregulated in *Peli3*$^{-/-}$ A549 cells after IBV infection and IL-6 overexpression also requires p38 MAP kinase activity. Finally, our observations indicate that Pellino3 attenuates p38 MAP kinase dependent phosphorylation of Stat1 which inhibits the production of IL-6 after IBV infection.

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* Corresponding author, e-mail: anna.kula@port.lukasiewicz.gov.pl

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ASSESSMENT OF THE ANTI-INFLAMMATORY EFFECT OF ZINC DERIVATIVES OF XANTHYLOXYALKANOIC ACIDS IN INFLAMED ENDOTHELIAL CELLS

ANNA LIPKOWSKA, JULIA ADAMSKA, ANNA ZAJĄC-GRABIEC, KATARZYNA SROCZYŃSKA, JOANNA GDULA-ARGASIŃSKA*, TADEUSZ LIBROWSKI

Department of Radioligands, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-668 Kraków, Poland

One of the main causes of damage to the endothelium of blood vessels is oxidative stress causing inflammation. This leads to serious complications, which can be atherosclerosis, hypertension, or diabetes. Xanthones have high antioxidant, anti-inflammatory, and anti-cancer activity. Their most popular source is mangosteen (*Garcinia mangostana* Linn.). Zinc is an essential element involved in many processes of the immune response. It plays a key role in reducing oxidative stress and inflammation.

The aim of this study was to evaluate the effect of zinc derivatives of xanthyloxyalkanoic acids (compounds MH-106 and MH-109) on the proinflammatory proteins level cyclooxygenase-2 (COX-2), TLR4 receptor and prostaglandin E2 synthase (cPGES) in lipopolysaccharide (LPS) activated endothelial cells.

We observed a statistically significant decrease in the level of COX-2 cPGES and TLR4 receptor in HUVEC cells after activation with LPS and incubation with MH-106 and MH-109 compound, which suggestes the anti-inflammatory effect of zinc derivatives of xanthyloxyalkanoic acids. It is necessary to conduct further studies in in vitro and in vivo models on the anti-inflammatory properties of zinc derivatives of xanthyloxyalkanoic acids.

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* Corresponding author, e-mail: joanna.gdula-argasinska@uj.edu.pl

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THE EFFECTS OF CONDITION MEDIA OBTAINED FROM GEL-DERIVED BIOACTIVE GLASS-PLGA COMPOSITES ON HUMAN BMSC MIGRATION RATE

KRZYSZTOF ŁUKOWICZ[1*], BARBARA ZAGRAJCZUK[2], KATARZYNA CHOLEWA-KOWALSKA[2], ANNA M. OSYCZKA[1]

[1]*Deptartment. Biology and Cell Imaging, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Department of Glass Technology and Amorphous Coatings, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Adama Mickiewicza 30, 30-059 Krakow, Poland*

We studied a range of PLGA polymer-based composites enriched with sol-gel bioactive glasses (SBG) from the $CaO–SiO_2/\pm P_2O_5$ systems. SBGs were incorporated at 50% w/w to PLGA matrix and structured into thin films suitable for cell culture. Given that such films display surface activity (i.e. bioactivity) and exchange ions with the physiological fluids, they were soaked for 3 days in a growth medium for BMSC culture to obtain materials-derived "condition media". Human bone marrow-derived stromal cells (hBMSC) were then cultured in the presence of such "condition media" (CM) for up 3 days. Treating hBMSC with CM resulted in the activation of the extracellular signal-regulated kinase (ERK1/2) in all cultures, but the highest ERK activity was observed in cells subjected to CM from the scaffolds enriched with SBG of the highest CaO/SiO_2 ratio without P_2O_5. These cells also displayed increased gene expressions of transcription factor c-Fos (c-Fos), osteopontin (OPN), matrix metalloproteinase – 2 (MMP2) and C-X-C chemokine receptor type 4 (CXCR4) and CM from these high calcium scaffolds increased the rate of hBMSC migration that had been assessed by Boyden chamber assay. Our results indicate that the ions released from the bioactive scaffolds may affect hBMSC migration rate, depending on SBG chemical composition and the absence/presence of P_2O_5. Such scaffolds evaluation approach can be thus helpful to design scaffold of specific chemical composition that act through the stimulation of human stromal cells to colonize them and thus accelerating the process of tissue regeneration.

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* Corresponding author, e-mail: krzysztof.lukowicz@doctoral.uj.edu

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TARGETING ACUTE MYELOID LEUKEMIA CELLS WITH ANTI-CD123 MONOCLONAL ANTIBODY

ANNA MLYCZYŃSKA, MICHAŁ SANTOCKI, MAŁGORZATA OPYDO-CHANEK*

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

The interleukin-3 receptor alpha chain (IL-3R), more commonly referred to as CD123, is widely overexpressed in various hematological malignancies, including acute myeloid leukemia (AML). Importantly, CD123 is expressed at both the level of leukemic stem cells and more differentiated leukemic blasts, which makes CD123 an attractive therapeutic target. Recently, agents that effectively target neoplastic cells expressing this molecule have been developed. Among of them, an anti-IL-3R neutralizing monoclonal antibody, 7G3, was designed to inhibit IL-3-dependent cell signaling and proliferation. In the present study, the *in vitro* effects of 7G3 on a panel of leukemia cell lines that represent various subtypes of AML, were evaluated. CD123 expression on leukemic cells was surveyed using flow cytometry. AML cells were incubated with 7G3 either alone or with 10 ng/ml of IL-3 for 24 h or 48 h. The AML cell viability and proliferation were studied using PrestoBlue and BrdU assays, respectively. The obtained results showed that 7G3 as a single agent was able to slightly decrease cell viability what was dependent on its concentration and AML cell line used. Moreover, the data has demonstrated that 7G3 may act as inhibitor of IL-3 activity. Additional studies on the use of anti-CD123 antibody in AML targeted therapy are warranted.

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* Corresponding author, e-mail: malgorzata.opydo-chanek@uj.edu.pl

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VISFATIN EXPRESSION AND REGULATION BY DIFFERENT HORMONES IN PORCINE OVARIAN FOLLICLES DURING THE ESTROUS CYCLE

EWA MLYCZYŃSKA[1*], PATRYCJA KUROWSKA[1], KAROLINA PICH[1], MARTA KIEŻUN[2], KAMIL DOBRZYŃ[2], EWA ZAOBIDNA[2], EDYTA RYTELEWSKA[2], GRZEGORZ KOPIJ[2], NINA SMOLIŃSKA[2], TADEUSZ KAMIŃSKI[2], AGNIESZKA RAK[1]

[1] *Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2] *Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn-Kortowo, Poland*

Visfatin (NAMPT, nicotinamide phosphoribosyltransferase) is one of adipokines with a significant role in regulation of metabolism, angiogenesis, inflammation. The aim of the study was to determine visfatin mRNA and protein expression in the porcine ovarian follicles as well as direct effect of several hormones on visfatin level in granulosa cells (Gc). Ovarian follicles were obtained from mature pigs on days 2-3, 10-12, 14-16 of the estrous cycle; expression of the visfatin gene was measured by qRT-PCR, the protein level using Western blot, while visfatin concentrations in the follicular fluid by ELISA assay. Next, *in vitro* effect of estradiol (E2, 1, 10, 100 nM), progesterone (P4, 10, 100, 1000 nM), insulin (10 ng/ml) and prostaglandins PGE2 and PGF2 (100, 250, 500 ng/ml) was measurement on visfatin protein in Gc. Differences between groups were analyzed by one-way ANOVA followed by Tukey's post hoc test. We observed the highest expression of visfatin in ovarian follicles on days 14-16 at the gene level, while on days 2-3 at the protein; the levels of visfatin in the follicular fluid was unchanged. Moreover, we noted that E2 and P4 significantly stimulated visfatin expression, while insulin and both prostaglandins had inhibitory effects. In conclusion, our study indicates visfatin as a new potential regulator of ovarian physiology, like follicles development in pigs.

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[*] Corresponding author, e-mail: ewa.mlyczynska@uj.edu.pl

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SIMILAR BUT DIFFERENT – GENOME SEQUENCES OF TWO RELATED *PROTEUS MIRABILIS* ISOLATES

SYLWIA NAWROT*, ILONA PACAK*, DAWID GMITER, WIESŁAW KACA

Division of Microbiology and Parasitology, Institute of Biology, Jan Kochanowski University, Uniwersytecka 7, 25-406 Kielce, Poland

Proteus mirabilis is a Gram-negative bacteria known from its swarming motility – ability to migrate over solid surfaces. It is responsible for up to 40% of Catheter Associated Urinary Tract Infections (CAUTIs), where swarming motility play an important role in catheter colonization. Therefore, this feature is considered as a virulence factor.

Presented study aims at comparison of genome sequences of two *P. mirabilis* isolates showing remarkably differences in swarming ability. It is suggested that one isolate originated from the other.

Bioinformatics analysis was carried out using the Unicycler, Mauve, fastANI algorithm, CSI Phylogeny, RAST server. Due to the conducted analyses information about basic parameters, structural organization and phylogeny of studied genome sequences was obtained. Analysis allowed as well to annotate genomes and to assign genes into the functional subsystems. The virulome of both genomes was investigated using local version of the BLAST+.

The performed analysis revealed high level of isolates genomic relatedness despite phenotypic differences in swarming ability. Studied genomes share >99% of ANI similarity and shown conservation of virulence genes. Further, obtained genome sequences will serve for better understanding the regulation of swarming motility.

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* Corresponding author, e-mail: sylwianawrot18@gmail.com; ilona.pacak@gmail.com

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RELEASE OF EXTRACELLULAR VESICLES (EVs) AND NEUTROPHIL EXTRACELLULAR TRAPs (NETs) DURING SYSTEMIC INFLAMMATION IN MICE

WERONIKA ORTMANN[1*], IWONA CICHON[1], MICHAL SANTOCKI[1], MONIKA BAJ-KRZYWORZEKA[2], ELŻBIETA KOLACZKOWSKA[1]

[1]*Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Department of Clinical Immunology, Jagiellonian University Medical College, Wielicka 265, 30-663 Krakow, Poland*

Extracellular vesicles (EVs) are structures secreted by various cells including neutrophils. EVs transport bioactive molecules, thus they are involved in multiple biological processes occurring during homeostasis and pathological states. Neutrophils are also known for neutrophil extracellular trap (NET) formation which display antimicrobial properties. Interestingly, EVs can induce NETs, and also can be associated with these structures. The aim of the study was to investigate the secretion of extracellular vesicles and to correlate it with NETs in healthy and endotoxemic – lipopolysaccharide-stimulated mice. As EVs are found in numerous body compartments, herein they were quantitatively estimated in blood (plasma) and inflammatory exudate (peritoneal fluid) by Nanoparticle Tracking Analysis (NTA). The results indicate low amounts of EVs in plasma and inflammatory exudate of healthy mice, and their abundant secretion in endotoxemic animals in a time-dependent manner. In order to investigate secreted neutrophil-derived EVs and ejected NETs in vivo, intravital microscopy (IVM) was used to image vasculature (liver sinusoids and vessels of cremaster muscle) of healthy and endotoxemic mice. The in vivo studies indicate that the number of neutrophil-derived EVs increases over time during systemic inflammation in both imaging vascular beds, and reveals positive correlations between ejected NETs and numbers of neutrophils infiltrating the liver. However, no NETs were detected in vasculature of the cremaster muscle. We conclude that secretion of EVs and NETs are highly dynamic processes occurring in different tissue/body compartments during systemic inflammation and their release occurs in parallel.

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* Corresponding author, e-mail: weronika.ortmann@doctoral.uj.edu.pl

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COMPARISON OF CYTOTOXIC EFFECT OF *HYPERICUM PERFORATUM* L. EXTRACTS ON MELANOMA CELLS CULTURED IN NORMOXIA AND HYPOXIA

SOPHIE OSTROWSKA-PATON*[1], MAGDALENA DOŁĘGA*[2], ALEKSANDRA BRANKIEWICZ*[3], KAROLINA KRÓLIK[2], ELŻBIETA SZOSTAK[4], MONIKA TULEJA[3], AGNIESZKA ŁOBODA[1], EWA POCHEĆ[2]

[1]*Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland*

[2]*Department of Glycoconjugate Biochemistry, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[3]*Department of Plant Cytology and Embryology, Faculty of Biology, Institute of Botany, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[4]*Phase Transition Research Team, Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland*

*These authors contributed equally to the work

Hypericum perforatum L. is a medicinal plant rich in secondary metabolites with cytotoxic properties. The aim of the study was to compare the cytotoxic effect of ethanolic extracts derived from *H. perforatum* on melanoma cells cultured in normoxia and hypoxia conditions.

Melanoma cell lines established from the same patient: WM115 from a primary tumour (RGP/VGP phenotype), and WM266-4 from lymph node metastasis, and the metastatic cells with *N*-acetylglucosaminyl-transferase III and V overexpression were used in the study. The extracts of *H. perforatum* were prepared from (1) the wild individuals, and (2) *in vitro* cultured plants from the same population, and (3) a commercially available *Tinctura Hyperici*. The cells, treated with the different volumes of *H. perforatum* extracts, were maintained in 10% FBS RPMI1640 and antibiotics in standard human cell culture conditions and oxygen levels (normoxia) and hypoxia (0.5% O_2). Cell viability was measured with MTT colorimetric assay.

The cells in normoxia showed the decreased viability with the increased extracts' concentrations, however, in hypoxia, all cell lines showed more persistent viability after stimulation with the examined extracts. Importantly though, the viability dropped for all lines in hypoxia at the highest concentration for the commercial extract, which may be a critical point of its cytotoxic effect. Interestingly, the viability of the primary tumour cells and the metastatic cells showed the reversed response to the increased doses of all tested extracts; the number of living WM115 cells was fallen while WM266-4 cells was increased in the presence of the rising extract concentrations. The results showed a difference in cell response to *H. perforatum* the extracts dependent on oxygen levels.

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* Corresponding author, e-mail: sophie.ostrowska-paton@student.uj.edu.pl

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INTERACTIONS OF GHS-R AND OPIOIDS RECEPTORS IN THE LAMB HYPOTHALAMUS

RADOSŁAW PIWOWAR, KLAUDIA JASZCZA, KRYSTYNA PIERZCHAŁA-KOZIEC*

Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Adama Mickiewicza 24/28, 30-059 Kraków, Poland

At the level of hypothalamus opioids (act through mi, delta and kappa receptors) and ghrelin (binds to GHS-R receptors) are involved into the control of satiety, stress axis and somatotropin axis activity. Hexarelin is a synthetic six-amino-acid compound belonged to the growth hormone releasing peptides (GHRP), capable of secreting GH through GHS-R receptor in animals and man.

The aim of study was to estimate whether hexarelin affects the activity of Met-enkephalin and ghrelin in the hypothalamus by stimulation/inhibition of opioid and ghrelin receptors.

Experiment was carried out on the 3-months old female lambs divided into: control, injected i.v. with 1mg/kg b.w. of Met-enkephalin, injected with 10 μg/kg b.w. of hexarelin, injected with Met-enkephalin and hexarelin. Sixty min after the injection hypothalamus was taken out and directed to measurements of Met-enkephalin and ghrelin concentrations and to opioid receptors binding with tritiated agonists.

Met-enkephalin significantly decreased the endogenous peptide concentration, hexarelin increased Met-enkephalin concentration. Combination of both agonists did not change the opioid concentration. Met-enkephalin increased ghrelin hypothalamus concentration. Hexarelin decreased the ghrelin level in the hypothalamus and it was potentiated by injection of both agonists. Met-enkephalin and hexarelin significantly increased binding of tritiated agonists to mi and delta receptors. Agonist binding to the kappa receptors was decreased by exogenous Met-enkephalin and increased by hexarelin.

Conclusion: Lamb hypothalamus areas abundant in opioids and GHS-R receptors are sensitive to peripheral injection of the enkephalin and hexarelin what supports the hypothesis of their close interaction in this crucial brain structure.

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* Corresponding author, e-mail: krystyna.koziec@urk.edu.pl

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DIFFERENTIAL CATECHOLAMINES RESPONSE TO ACUTE AND PROLONGED RESTRAINT STRESS IN RATS

ADRIAN RYŚ, KLAUDIA JASZCZA, KRYSTYNA PIERZCHAŁA-KOZIEC

Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Adama Mickiewicza 24/28, 30-059 Kraków, Poland

Catecholamines, mainly adrenaline and noradrenaline, are synthetized by specific neurons in the brain and adrenal medulla serving as the main neurotransmitters activating HPA axis during acute and prolonged stress. Opioids modulate catecholamines response and stimulate process of adaptation to stressful situation.

The aims of the experiments were to compare the effects of acute (experiment I, single restraint) and prolonged emotional stress (experiment II, restraint by 7 consecutive days) on the interaction of opioid and catecholamine systems.

Experiments were carried out on male rats divided into groups in each series: control; stressed by 30 min of restraint; injected with naltrexone 5 mg/kg b.w. and injected with naltrexone and stressed. Blood for catecholamines estimation was taken before stress, and at 0, 5, 30 and 33 minutes after start of restraint.

Plasma catecholamines levels in control and naltrexone treated rats were not changed during the experiments. The profile of catecholamine responses to stress were similar in the first and second experiment but with different magnitude of the ratio acute: prolonged restraint. The highest levels of both catecholamines were observed after 5 min of restraint, followed by gradual decrease to the end of stress. The response of adrenaline was stronger in the acute stress, in contrast prolonged stress potentiated the reaction of noradrenaline. Naltrexone decreased acute catecholamines response and potentiated the effect of prolonged stress.

It may be suggested that opioids are the crucial neurotransmitters modulating the process of different catecholamines adaptation to prolonged stress in rats.

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* Corresponding author, e-mail: krystyna.koziec@urk.edu.pl

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THE ROLES OF LONG NONCODING RNAs IN RNA EDITING IN CANCEROUS CELLS

KLAUDIA SAMOROWSKA*, MICHAŁ SZCZEŚNIAK

Institute of Human Biology and Evolution, Faculty of Biology, Adam Mickiewicz University in Poznan, Uniwersytetu Poznańskiego 6, 61-614 Poznan, Poland

Long noncoding RNAs (lncRNAs) are transcripts over 200 nucleotides long that do not encode proteins. They have a lot of regulatory roles, especially in cancer cells, where levels of their expression are changed compared to normal cells. The roles of lncRNAs are associated with their cellular localization: those located in the nucleus can be involved in the process of RNA editing or alternative splicing, among other things. Natural antisense transcripts (NATs) represent a large group of lncRNAs - they are transcribed from a DNA strand opposite to another gene, typically a protein coding one. Owing to this genomic context, NATs possess a natural potential to come into direct RNA:RNA interactions with transcripts of the sense gene, thus they may be involved in initiation of the RNA editing process – the deamination of adenosine to inosine (A>I), which requires formation of perfect RNA duplexes. Such duplexes are recognized by ADAR enzymes that catalyze the A>I deamination events.

Our goal is to focus on the NATs initiating the process of RNA editing by ADAR enzymes in breast cancer ER+, which accounts for c.a. 80% of breast cancers worldwide. Definitely most studies are focusing on changes in the genetic material that appears in the genomic DNA, however RNA editing may lead to similar functional consequences, such as affecting the protein coding potential or altering patterns of splicing.. Our *in silico* analyses led to discovery of NATs that are potentially associated with RNA editing in breast cancer cells. Experimental tests, such as knock down with LNA gapmeRs, migration assay and proliferation assay, will be performed to learn more about the biological roles played by particular lncRNAs. These steps will be done with MCF-7 and T-47D cell lines that are typically applied in studies on ER+ breast cancer. Selected tests will also be done using samples from patients, for example Sanger sequencing of mRNAs and corresponding DNA sequences aimed to verify whether the studied transcripts are indeed edited in cancer cells and how their editing status compares to non-cancerous samples.

* Corresponding author, e-mail: klaudia.samorowska@amu.edu.pl

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THE IMPORTANCE OF MACROPHAGES IN REMOVING EXTRACELLULAR PROTEINS DURING RESOLUTION OF SYSTEMIC INFLAMMATION

MICHAL SANTOCKI*, ELŻBIETA KOLACZKOWSKA

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

During systemic inflammation (e.g., sepsis), various mediators, antibacterial proteins and enzymes are released by activated leukocytes. Majority of them affects target cells directly by binding with their surface receptors, some act on infecting pathogens, while some are deposited along vascular endothelium or in tissues, such as the liver. The systemic overconcentration of inflammatory mediators might cause long term side effects leading to endothelium and/or organ damage if inappropriately removed by phagocytes.

In this study we followed resolution of lipopolysaccharide-induced sepsis in C57BL/6J mice, concentrating on the involvement of liver macrophages (Kupffer cells) in removal of extracellular proteins deposited in liver vasculature. By depleting mice of Kupffer cells (KC) with clodronate liposomes, an agent designed to specifically remove selected populations of macrophages based on the route of its administration, we measured the deposition of neutrophil elastase (NE) alongside the liver sinusoids of septic mice with the use of confocal spinning-disk intravital imaging.

At first, we verified two routes of liposomes administration, intraperitoneal and intravenous, and confronted the efficacy of KC depletion. Next, we verified if the KC depletion affected number of other cells presented in the liver, such as neutrophils. Then, we compared the amount of NE in liver sinusoids in KC-depleted and control animals at 4H and 24H after induction of sepsis. With this approach we managed to emphasize the importance of KCs in extracellular protein removal during sepsis and established the basis for further research on phagocyte capabilities during systemic inflammation.

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* Corresponding author, e-mail: michal.santocki@doctoral.uj.edu.pl

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MITOCHONDRIAL DYNAMICS IN FEMALE GERMLINE CELLS: THE ROLE OF DRP1, LYSOSOMES AND CYTOSKELETON

MALGORZATA SEKULA*, WACLAW TWORZYDLO, SZCZEPAN M. BILINSKI

Department of Developmental Biology and Invertebrate Morphology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Mitochondria are highly dynamic organelles that constantly undergo fusion and fission (processes termed collectively mitochondrial dynamics) and translocate along cytoskeletal elements. Mitochondrial fusion leads to the formation of highly interconnected mitochondrial networks, whereas fission results in disassembly of those networks into individual bean-shaped organelles. A major regulator of mitochondrial fission is a highly conserved dynamin-related protein 1 (Drp1), which belongs to a large superfamily of dynamins that participate in the regulation of membrane structure. Interestingly, recent studies have shown that mitochondrial fission, in addition to Drp1, may also involve microfilaments and elements of rough endoplasmic reticulum or (alternatively) lysosomes, depending on the quality of a dividing mitochondrion. In this study, we analysed distribution of Drp1, cytoskeletal elements and lysosomes in developing oocytes of a bush cricket, *Meconema meridionale*. We demonstrate that the Drp1 distribution pattern changes during the oogenesis. Initially, this protein is present in the entire cytoplasm and then concentrates in well-defined spots that presumably represent constriction points of mitochondria. Our analyses revealed additionally that lysosomes are present only in the cytoplasm of early (previtellogenic) oocytes. At the same stage, microtubules are concentrated around the germinal vesicle (oocyte nucleus) where they form a distinct ring-like zone. These results are discussed in the context of current ideas on the participation of mitochondrial dynamics in the elimination of dysfunctional mitochondria from female germline cells, and transmission of only "healthy" mitochondria to the next generation.

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* Corresponding author, e-mail: malgorzata.sekula@doctoral.uj.edu.pl

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INFLUENCE OF GAMMA-LINOLENIC ACID AND LOVASTATIN ON THE LEVEL OF PRO-INFLAMMATORY PROTEINS IN ENDOTHELIAL CELLS

KATARZYNA SROCZYŃSKA, KAROLINA WOLNIK, ANNA ZAJĄC-GRABIEC, TADEUSZ LIBROWSKI, JOANNA GDULA-ARGASIŃSKA*

Department of Radioligands, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-668 Kraków, Poland

The molecular mechanism of gamma-linolenic acid (GLA) action in inflammation has not yet been well documented. Lovastatin is a lipid-lowering drug, and recent reports indicate that it also has anti-inflammatory properties.

The aim of this study was to determine the effect of GLA and lovastatin on the level of cyclooxygenases (COX-1 and COX2), phospholipase A2 (cPLA2) and toll-like receptor 4 (TLR4) in endothelial cells, activated with lipopolysaccharide (LPS).

HUVEC cells were incubated with 50 μmol GLA and/or with 5 μmol lovastatin for 24 hours and activated with 10 ng/ml LPS. The level of COX-1, COX-2, cPLA2, and TLR4 proteins were determined by the western blot technique.

Significantly higher levels of COX-1, COX-2, cPGES, and TLR4 were observed in HUVEC cells after LPS activation. Supplementation of endothelial cells with GLA and lovastatin resulted in a significant reduction in the level of pro-inflammatory proteins. The obtained results suggest a synergistic anti-inflammatory effect of the tested compounds.

In order to understand the molecular mechanisms of the synergistic and protective action of GLA and lovastatin, further research seems advisable

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* Corresponding author, e-mail: joanna.gdula-argasinska@uj.edu.pl

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EFFECT OF STRESS ON GLUTAMIC ACID DECARBOXYLASE IN VIVO RELEASE FROM RABBIT ADRENAL GLAND

IZABELA SZPRĘGIEL[1*], DANUTA WROŃSKA[1], BOGDAN KANIA[2]

[1]*Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Adama Mickiewicza 24/28, 30-059 Krakow, Poland*

[2] *University Centre of Veterinary Medicine JU-AU, University of Agriculture in Krakow, Adama Mickiewicza 24/28, 30-059 Krakow, Poland*

Glutamic acid decarboxylase (GAD) is a key enzyme that catalyses the formation of -aminobutyric acid (GABA), the most important inhibitory neurotransmitter, from glutamic acid (Glu), which is the major neuromodulator in the central nervous system and is involved in most processes such as learning and memory, and in the mechanisms underlying aggressive animal behaviour. Hence, it can be assumed that if the GAD executes the synthesis of GABA from Glu, it is important in the stress response, and thus also in triggering the emotional states of the body that accompany stress.

The aim of the study was to investigate the concentration of the GAD in the adrenal gland of the European rabbit under altered homeostatic conditions caused by stress and variable availability of Glu.

The experiment was carried out on 12 weeks old, 24 Popielno White female rabbits divided into groups:

Gr. 1 – Control

Gr. 2 – Stress

Gr. 3 – GLU (G1626, Sigma Aldrich, 30 M/kg b.w.)

Gr. 4 - Stress + GLU

Gr. 5 – GLU antagonist (LY-367385, Sigma Aldrich, 30 M/kg b.w.)

Gr. 6 - Stress + GLU antagonist

The stress factor will be induced by hanging the rabbit for 30 minutes. The method of suspension will be to prevent contact between the fore and hind legs with the ground. All of the above-mentioned compounds were administered intraperitoneally (*i.p.*) in a volume of 2.0-2.5 ml, 0.9% NaCl. After 30 min animals from all groups were killed and the adrenal glands obtained from them were frozen and on the day of the analysis were homogenized. The GAD concentration in the analyzed homogenates was determined using the ready Rabbit (GAD) ELISA Kit, 201-09-0310 (SunRed; China). The results were converted for 1 mg of tissue.

The conducted experiment clearly showed changes in the concentration of the GAD in the adrenal gland of the rabbit during stress and variable availability of glutamic acid in these structures.

The increased concentration of GAD in individual experimental groups was determined to a greater extent by the influence of a specific glutamic acid antagonist than by the action of glutamic acid alone or the stress factor. Moreover, an additive effect of glutamic acid in stressed rabbits on GAD concentration was observed. The results of the experiment suggest that a relatively rapid change in GAD activity at the adrenal level may contribute to local-modulating the activity of the adrenal glands, which in turn may have an impact on stress responsiveness.

* Corresponding author, e-mail: i.szpregiel@gmail.com

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PXT1 AND BAG6 INTERPLAY IN THE CONTROL OF SPERM QUALITY IN MOUSE

IGOR TOMCZYK[1], BERNADETA PAWLICKA[1], KATARZYNA ROŻEK[2], AUER AGNETA[3], PAWEŁ GRZMIL[1*]

[1]*Department of Genetics and Evolution, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2] *Department of Taxonomy, Fitogeography and Paleobotany, Instytute of Botany, Jagiellonian University, Gronostajowa 3, 30-387 Krakow, Poland*

[3]*Institute of Human Genetics, University Medical Centre Göttingen, Germany*

During spermatogenesis two opposite processes act to assure efficient gametes production. Proliferation is required to provide adequate number of sperm but the elimination of damaged germ cells, usually by apoptosis, guaranties the quality of produced sperm. The balance of both processes guarantee efficient production of high quality gametes. *Pxt1* is a male germ cell specific gene. Its overexpression leads to massive apoptosis of transiently transfected cells. In transgenic mouse model the overexpression of PXT1 initiated apoptosis of male germ cells, mainly primary spermatocytes, resulting in male infertility. We have demonstrated that PXT1 interacts with BAG6 protein and that this interaction protects cells from apoptosis. In the *Bag6* knockout mice massive degeneration of male germ cells was also observed and this phenotype reflected that observed in *Pxt1*-overexpressing males. We have demonstrated that in *Bag6* knockout mice the expression of *Pxt1* is significantly increased. Therefore, we stress the hypothesis, that the activity of PXT1 is related with elimination of defective germ cells. To verify this hypothesis we generated *Pxt1* knockout line. One of important sperm quality parameters is DNA fragmentation index (DFI). Using the SCSA assay, we have analyzed the proportion of sperm with DNA breaks in *Pxt1* knockout and control animals. Significant increase in sperm with DNA breaks was noticed in mutants. Human genome contains an orthologous *PXT1* gene that might be a good candidate for mutation screening in patients with idiopathic fertility disruption.

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* Corresponding author, e-mail: pawel.grzmil@uj.edu.pl

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NOGGIN PROMOTES OSTEOGENESIS IN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS THROUGH THE ACTIVATION OF SRC, AKT AND ERK1/2 INTRACELLULAR SIGNALING

KAROLINA TRUCHAN*, ANNA MARIA OSYCZKA

Department of Biology and Cell Imaging, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Noggin is broadly recognized for its inhibitory effects on bone morphogenetic protein (BMP) signaling, due to binding BMPs at the extracellular environment and preventing them from binding to the BMP receptors at the cell membrane surface. The other actions of Noggin are still poorly elucidated, although some studies suggested that Noggin itself binds to BMP receptors and induces osteogenesis in osteoblastic cells. We have recently determined that Noggin increased an early indicator of osteogenic progression - ALP activity in human adipose-derived stem cell line immortalized by hTERT (ASC52telo) and other adult human mesenchymal stem cell lines (MSCs) and thus we sought to explore the potential role of Noggin in osteogenesis of ASC52telo cells. This cell line provides a useful model to explore the mechanisms of osteogenesis in human MSCs, which are now extensively studied for several tissue engineering and clinical applications.

Our results indicate that constant treatment of ASC52telo cells with Noggin led to significant mRNA expression of Runx2, Osterix, osteonectin, vascular endothelial growth factor at 7-day culture and osteocalcin, osteoprotegerin, osteopontin, bone sialoprotein at 21-day culture. At 30-day culture, Noggin significantly increased extracellular matrix mineralization. Notably, we found that Noggin was effective when the cells were simultaneously treated with dexamethasone. The analyses of the potential signaling pathway of Noggin showed that Noggin can signal via Src/Akt/ERK activation, which probably leads to TAZ proteins stabilization and favors osteogenic differentiation.

The results of this study indicate a positive role of Noggin in the osteogenesis of human adipose-derived MSCs and they may prove important for other types of human MSCs, especially if they are prompted to osteogenesis. Although there is now apparent that Noggin can activate intracellular signaling in adipose-derived MSCs and does not signal through BMP receptors, as it was recently suggested. Thus, further studies are required to establish the Noggin-related putative receptors.

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* Corresponding author, e-mail: karolina.truchan@uj.edu.pl

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DOES IMMUNOSUPPRESSIVE THERAPY AFFECT IgG *N*-GLYCAN STRUCTURES IN GRAVES' DISEASE?

SARA TRZOS[1*], PAWEŁ LINK-LENCZOWSKI[2], MARTA ZĄBCZYŃSKA[1], GRZEGORZ SOKOŁOWSKI[3], EWA POCHEĆ[1]

[1]*Department of Glycoconjugate Biochemistry, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Department of Medical Physiology, Faculty of Health Sciences, Jagiellonian University Medical College, Michałowskiego 12, 31-126 Krakow, Poland*

[3]*Chair and Department of Endocrinology, Jagiellonian University Medical College, Jakubowskiego 2, 30-688 Krakow, Poland*

N-glycosylation is a key post-translational modification that not only modulates the effector functions of immunoglobulin G (IgG), but also serves as an indicator of the ongoing inflammatory process. Rebuilding of IgG *N*-glycans can induce pathological changes, including autoimmunity. One of the most common autoimmune disorders is Graves' disease (GD) characterized by the presence of IgGs directed against thyrotropin receptor (TSHR), which show the pro-inflammatory activity. According to current knowledge, oligosaccharide structures on IgGs in GD are depleted in fucose and sialic acid compared to healthy subjects. So far, no data on the effect of immunosuppression applied in GD therapy on changes in IgG *N*-glycosylation have been presented. Therefore, the aim of this study was to analyze IgG glycome in patients with GD before (n=42) and after (n=30) treatment with immunosuppressive drugs in comparison to healthy donors (control group; n=42). IgG was isolated from human serum by affinity chromatography with protein G. IgG *N*-glycans were released by *N*-glycosidase F digestion, fluorescently labeled with 2-aminobenzamide (2-AB) and analyzed by ultraperformance liquid chromatography-mass spectrometry (UPLC-MS). The obtained chromatograms were integrated into 25 peaks, to which glycans were assigned on the basis of glucose unit values and mass-to-charge ratios (*m*/*z*) of the detected ions using Waters UNIFI Scientific Information System. The statistical analysis was performed using the Kruskal-Wallis test ($p<0.05$). The study showed the statistically significant increase of *N*-glycans with bisected *N*-acetylglucosamine and core fucose as well as the reduction of sialylated glycans on IgG from the patients during immunosuppressive therapy compared to these donors before the treatment implementation.

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* Corresponding author, e-mail: sara.trzos@doctoral.uj.edu.pl

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TARGETING MCL-1 BY SELECTIVE INHIBITOR S63845 EFFICIENTLY INDUCES APOPTOSIS IN ACUTE MYELOID LEUKEMIA CELLS

MAŁGORZATA WOJTASZEK, ANNA MLYCZYŃSKA, MAŁGORZATA OPYDO-CHANEK*

Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

Acute myeloid leukemia (AML) is a malignancy of the hematopoietic system in which production of blood cells is disturbed. In spite of the progress in research on the biology of leukemia, and introduction of new treatment regimes, AML is still in general an incurable disease. Therefore, there is a clear unmet need to develop a new effective treatment. It has been shown in previous studies that increased expression of anti-apoptotic MCL-1 protein is associated with resistance to chemotherapy, decreased rates of complete remission, and abbreviated survival of AML patients. Disturbances in the expression of MCL-1 protein cause leukemia cells less sensitive to pro-apoptotic signals. S63845, a specific small-molecule inhibitor of MCL-1, has recently bees synthetized. S63845 binds to the hydrophobic groove of the MCL-1 protein blocking its anti-apoptotic effect. The aim of this study was to determine the cytotoxic activity of S63845 against AML cells. The experiments were performed *in vitro* on two AML cell lines, KG-1 and ML-1. Leukemia cells were exposed to S63845 at various concentrations for 24 and 48 hours. The PrestoBlue viability test, FSC/SSC cytometric analysis, the annexin V/propidium iodide assay and the Western blot technique were used to assess the effects of inhibitor on leukemia cells. S63845 caused a concentration-dependent decrease in the viability of KG-1 and ML-1 cells and increase in the percentage of cells undergoing apoptosis. Moreover, the expression of the MCL-1 protein, which was originally high in AML cells, decreased after the S63845 treatment. The obtained results identified S63845 as a promising antileukemic agent and encouraged further research on the mechanisms of its anti-cancer action.

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* Corresponding author, e-mail: malgorzata.opydo-chanek@uj.edu.pl

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EXPRESSION OF PRO-INFLAMMATORY PROTEINS IN INTESTINE EPITHELIAL CACO-2 CELLS SUPPLEMENTED WITH EICOSAPENTAENOIC ACID AND ACTIVATED WITH LIPOPOLYSACCHARIDE

ANNA ZAJĄC-GRABIEC, KATARZYNA SROCZYŃSKA, MAŁGORZATA REISS, TADEUSZ LIBROWSKI, JOANNA GDULA-ARGASIŃSKA

Department of Radioligands, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-668 Kraków, Poland

Eicosapentaenoic acid (EPA) is a polyunsaturated fatty acid, the precursor of anti-inflammatory and pro-resolving mediators. Supplementation with EPA may have a beneficial effect on reducing the risk of a various inflammatory diseases.

The purpose of this study was to determine the effect of EPA supplementation on cyclooxygenase-2 (COX-2), prostaglandin E2 (cPEGS), aromatic hydrocarbon (AHR) protein expression and an interleukin 6 (IL-6) content in the intestinal epithelial Caco-2 cells activated with lipopolysaccharide (LPS). CaCo-2 cells were incubated with 10 mol and 25 mol of EPA for 24h and 48h, followed by LPS activation. Using Western blot technique to determine COX-2, cPGES, AHR receptor expression and using and ELISA technique to determine IL-6 concentration, it has been shown that statistically the highest expression of pro-inflammatory proteins in LPS-activated intestinal cells.

In Caco-2 cells after EPA supplementation and LPS activation, the levels of pro-inflammatory proteins have been significantly reduced, suggesting that this fatty acid exhibits anti-inflammatory and pro-resolving activity. It seems necessary to carry out further research regarding the action of eicosapentaenoic acid on intestinal epithelial cells.

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* Corresponding author, e-mail: joanna.gdula-argasinska@uj.edu.pl

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