ACTA BIOLOGICA CRACOVIENSIA

SERIES BOTANICA

Vol. 65 suppl. 1 2023

Polish Academy of Sciences – Cracow Branch
Jagiellonian University, Cracow

ACTA BIOLOGICA CRACOVIENSIA
Series Botanica

The Official Publication of the Biological Commission of the Polish Academy of Sciences – Cracow Branch and the Jagiellonian University, Cracow

DEVOTED TO PLANT ANATOMY, MORPHOLOGY, CYTOLOGY, GENETICS, KARYOLOGY, EMBRYOLOGY, TISSUE CULTURE, PHYSIOLOGY, BIOCHEMISTRY, BIOSYSTEMATICS, MOLECULAR PHYLOGENETICS AND PHYLOGEOGRAPHY, PHYTOCHEMISTRY

ESTABLISHED 1958

Polish Academy of Sciences provided partial funding for the publication of this volume

ACTA BIOLOGICA CRACOVIENSIA Series Botanica is published twice a year by the Polish Academy of Sciences – Cracow Branch, ul. św. Jana 28, 31-018 Cracow, Poland and the Jagiellonian University, ul. Gołębia 24, 31-007 Cracow, Poland

Computer typesetting: LogoScript Sp. z o.o., ul. Dembowskiego 4/54, 02–784 Warszawa, e-mail: logoscript@logoscript.pl, www.logoscript.pl

Managing editor: Monika Tuleja

ACTA BIOLOGICA CRACOVIENSIA Series Botanica on the Internet
The home page of *Acta Biologica Cracoviensia Series Botanica* can be found at https://abcbot.pl

The Acta Biologica Cracoviensia Series Botanica is participating in CrossCheck System's users in order to ensure that the content published is original and trustworthy.

Indexed in *AGRICOLA (National Agricultural Library) AGRIS, AGRO, CABI databases (Agroforestry Abstracts, Botanical Pesticides, CAB Abstracts, Crop Physiology Abstracts, Crop Science Database, Dairy Science Abstracts, Field Crop Abstracts, Forest Products Abstracts, Forestry Abstracts, Grasslands and Forage Abstracts, Horticultural Science Abstracts, Maize Abstracts, Ornamental Horticulture, Plant Breeding Abstracts, Plant Genetic Resources Abstracts, Plant Growth Regulator Abstracts, Potato Abstracts, Rice Abstracts, Seed Abstracts, Soils and Fertilizers Abstracts, Soybean Abstracts, Sugar Industry Abstracts, Wheat, Barley and Triticale Abstracts), CNPIEC, EBSCO Discovery Service, Elsevier – SCOPUS, Foodline Science, FSTA - Food Science & Technology Abstracts, Google Scholar, Index Copernicus, J-Gate, Microsoft Academic Search, Naviga (Softweco), Polish Scientific Journals Contents, Primo Central (ExLibris), Summon (Serials Solutions/ProQuest), TDOne (TDNet), Thomson Reuters - Biological Abstracts, Thomson Reuters - BIOSIS Previews, Thomson Reuters - Current Contents/Agriculture, Biology, and Environmental Sciences, Thomson Reuters - Journal Citation Reports/Science Edition, Thomson Reuters - Science Citation Index Expanded, WorldCat (OCLC)*

ACTA BIOLOGICA CRACOVIENSIA
Series Botanica

Section name:
Molecular biology; cytometry; biotechnology

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:
Plant physiology - photosynthesis and respiration; biotic and abiotic stresses; inter- and intracellular signalling; plant movements; phytohormones in plant growth and development

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

ADVISORY EDITORIAL BOARD

XXX International Symposium Molecular and Physiological Aspects of Regulatory Processes in the Organism

October 14th 2023, Krakow, Poland

Organizers

The Biological Committee of the Krakow Division
of the Polish Academy of Sciences, Krakow, Poland

Institute of Zoology and Biomedical Research,
Jagiellonian University in Krakow, Poland

Scientific Committee

Elżbieta Kołaczkowska	Laboratory of Experimental Hematology, Jagiellonian University in Krakow, Poland
Paweł Grzmil	Department of Genetics and Evolution, Jagiellonian University in Krakow, Poland
Anna Błasiak	Department of Neurophysiology and Chronobiology, Jagiellonian University in Krakow, Poland
Joanna Gdula-Argasińska	Department of Radioligands, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
Małgorzata Kotula-Balak	Department of Animal Anatomy and Preclinical Sciences, University Centre of Veterinary Medicine JU-UA, University of Agriculture in Krakow, Poland
Małgorzata Lenartowicz	Department of Genetics and Evolution, Jagiellonian University in Krakow, Poland
Marian Lewandowski	Department of Neurophysiology and Chronobiology, Jagiellonian University in Krakow, Poland
Krystyna Pierzchała-Koziec	Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Poland
Ewa Pocheć	Department of Glycoconjugate Biochemistry, Jagiellonian University in Krakow, Poland
Marzena Popielarska-Konieczna	Department of Plant Cytology and Embryology, Institute of Botany, the Jagiellonian University in Krakow, Poland
Małgorzata Opydo	Laboratory of Experimental Hematology, Jagiellonian University in Krakow, Poland
Małgorzata Przybyło	Department of Glycoconjugate Biochemistry, Jagiellonian University in Krakow, Poland
Anna Ptak	Laboratory of Physiology and Toxicology of Reproduction, Jagiellonian University in Krakow, Poland
Agnieszka Rak	Laboratory of Physiology and Toxicology of Reproduction, Jagiellonian University in Krakow, Poland
Kinga Sałat	Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
Andrzej Sechman	Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Poland
Zbigniew Tabarowski	Laboratory of Experimental Hematology, Jagiellonian University in Krakow, Poland

Organizing Committee

Elżbieta Kołaczkowska	President of the Biological Committee of the Cracow Division of the Polish Academy of Sciences
Ewa Pocheć	Vice-President of the Biological Committee of the Cracow Division of the Polish Academy of Sciences
Paweł Grzmil	Director of the Institute of Zoology and Biomedical Research of the Jagiellonian University in Krakow, Poland
Małgorzata Opydo	Symposium Secretary, Laboratory of Experimental Hematology, Jagiellonian University in Krakow, Poland
Zbigniew Arent	University Centre of Veterinary Medicine UAK, University of Agriculture in Krakow, Poland
Anna Błasiak	Department of Neurophysiology and Chronobiology, Jagiellonian University in Krakow, Poland
Magdalena Chadzińska	Department of Evolutionary Immunology, Jagiellonian University, Krakow, Poland
Joanna Gdula-Argasińska	Department of Radioligands, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
Małgorzata Kotula-Balak	Department of Animal Anatomy and Preclinical Sciences, University Centre of Veterinary Medicine JU-UA, University of Agriculture in Krakow, Poland
Małgorzata Lenartowicz	Department of Genetics and Evolution, Jagiellonian University in Krakow, Poland
Tadeusz Librowski	Department of Radioligands, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
Joanna Majerczak	Chair of Exercise Physiology and Muscle Bioenergetics, Faculty of Health Sciences, Jagiellonian University Medical College, Krakow, Poland
Agnieszka Mazur-Biały	Department of Biomechanics and Kinesiology, Jagiellonian University Medical College, Krakow, Poland
Marzena Popielarska-Konieczna	Department of Plant Cytology and Embryology, Institute of Botany, the Jagiellonian University in Krakow, Poland
Małgorzata Przybyło	Department of Glycoconjugate Biochemistry, Jagiellonian University in Krakow, Poland
Anna Ptak	Laboratory of Physiology and Toxicology of Reproduction, Jagiellonian University in Krakow, Poland
Agnieszka Rak	Laboratory of Physiology and Toxicology of Reproduction, Jagiellonian University in Krakow, Poland
Kinga Sałat	Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
Krystyna Żuwała	Department of Comparative Anatomy, Jagiellonian University in Krakow, Poland

CONTENTS

Lectures/Invited Speakers 11

Oral Presentations 15

Poster Presentations 21

Lectures/Invited Speakers

PL ISSN 1898-0295

The molecular GPS of cells: Understanding the primary cilium

Sigrid Hoyer-Fender

Johann-Friedrich-Blumenbach-Institute of Zoology and Anthropology – Developmental Biology, Georg-August-Universität Göttingen, Göttingen, Germany

e-mail: shoyer@gwdg.de

The primary cilium, a microtubule-based organelle that protrudes from the surface of most mammalian cells, has gained increasing attention for its multifaceted role as a molecular guidance system for cell development. Once considered a vestigial remnant, the primary cilium is now recognized as a key player in controlling essential cellular processes. It acts as a sensory hub, detecting various extracellular cues, including mechanical forces and chemical signals, and translating these external inputs into intracellular responses, thereby guiding cell fate, behaviour, and homeostasis. The critical role of primary cilia in development and tissue homeostasis is underscored by a spectrum of human diseases associated with cilia dysfunction, termed ciliopathies. Understanding the role of the primary cilium and the underlying molecular processes holds the potential to discover novel therapeutic targets and provide insight into a wide range of cellular and developmental disorders.

This talk aims to provide a comprehensive understanding of the intricate structure of the primary cilium, its development and functions as a navigational cell center. We address the molecular mechanisms underlying the formation and maintenance of the primary cilium. We examine how centrioles transition into ciliary basal bodies, the initial steps in cilium biogenesis, and the intricate intraflagellar transport (IFT) system responsible for cilium assembly and maintenance. We also discuss the key molecular players involved in cilia formation, focusing on the outer dense fibre protein 2 (ODF2) and its role in ciliogenesis and beyond. Finally, we address the question of the regulation of *Odf2* expression and the transcription factors involved.

PL ISSN 1898-0295

Protein S-palmitoylation as regulator of LPS-induced pro-inflammatory response of macrophages

Katarzyna Kwiatkowska

Laboratory of Molecular Membrane Biology, Nencki Institute of Experimental Biology PAS, Ludwika Pasteura 3, 02-093 Warsaw, Poland

e-mail: k.kwiatkowska@nencki.edu.pl

Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria that induces strong pro-inflammatory reactions of mammals. Inflammation facilitates combating bacterial infections, however, when exaggerated, it can lead to potentially fatal sepsis while prolonged low-grade endotoxemia triggered by, e.g., gut-derived LPS has been linked with the development of several diseases plaguing modern societies. The pro-inflammatory reaction to LPS is triggered upon its sequential binding to the CD14 protein and the TLR4/MD2 receptor complex in the plasma membrane of immune cells. Subsequently, CD14 governs endocytosis of LPS-activated TLR4, allowing its signaling to be continued. As a result, numerous cytokines are produced, and activation of inflammasome, a cytoplasmic multi-protein signaling complex, can follow. Data on the contribution of lipids of the plasma membrane and endomembranes to the LPS-induced signaling pathways will be presented. These include sphingolipids, phosphorylated derivatives of phosphatidylinositol and also lipid moieties linked to proteins. Among the latter, palmitic acid is attached posttranslationally to cysteine by *S*-acyltransferases of the zDHHC family. This modification is reversible and affects protein localization, stability and activity. Proteomic analysis performed with the use of so-called click chemistry followed by mass spectrometry revealed that stimulation of Raw264 macrophage-like cells with LPS induces global changes of the level of palmitoylated proteins. Among them, several enzymes of the phosphatidylinositol cycle were discovered, including type II phosphatidylinositol 4-kinase-β (PI4KIIβ) and diacylglycerol kinase-ε (DGKε). Down-regulation of PI4KIIβ or DGKε led to inhibition of the TLR4 signaling, thus indicating that novel lipid kinases regulating LPS-induced responses have been identified.

PL ISSN 1898-0295

New photoswitchable compounds with physiological activity

Marta Stolarek[1,2], Kamil Kamiński[2], Marta Kaczor-Kamińska[3], Piotr Bonarek[4], Aleksandra Pycior[2], Małgorzata Opydo[5], Mateusz Brela[2], Andrzej Mogielnicki[6], Maria Nowakowska[2], Elżbieta Kołaczkowska[5], Krzysztof Szczubiałka[2,*]

[1]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, S. Lojasiewicza 11, 30-348 Krakow, Poland*

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

[3]*Faculty of Medicine, Jagiellonian University Medical College, Kopernika 7, 31-034 Krakow, Poland*

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

[5]*Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[6]*Department of Pharmacodynamics, Medical University of Bialystok, Mickiewicza 2C, 15-089 Białystok, Poland*

**e-mail: k.szczubialka@uj.edu.pl*

Selective, efficient, and safe control over the biological activity of the low-molecular-weight- and macromolecular compounds may be achieved via the photopharmacological approach. One of its variants is based on the use of photoactive compounds able to undergo reversible photoisomerization (photoswitches, PSs). The PSs can be attached to their targets, such as drugs, proteins, ion channels, enzymes, etc., with covalent or noncovalent bonds. The photoisomerization of the PS results in a change in its geometry and size, which is expected to induce a significant alteration in the biological activity of the target molecule. Of particular interest as PSs are the derivatives of arylazopyrazoles (AAPs) which may be considered as azobenzene analogs with one phenyl ring replaced by a pyrazolyl ring. Therefore, a novel AAP-based compound was designed, synthesized and used as a PS in our studies. Its *cis* photoisomer is characterized by an exceptionally long half-life in the aqueous media at 37°C (tens of hours) enabling photoswitching of the biological properties of the compounds substituted with this PS. Two types of photoswitchable systems were obtained, i.e., low-molecular-weight- and unfractionated heparins (Enoxaparin and UFH, respectively) and an analog of cisplatin (Figure 1), a mainstay anticancer drug. The significant changes in the biological properties of both systems resulting from their photoswitching with near-UV/visible light were studied.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from National Science Centre (grant UMO-2021/41/B/NZ7/02484).

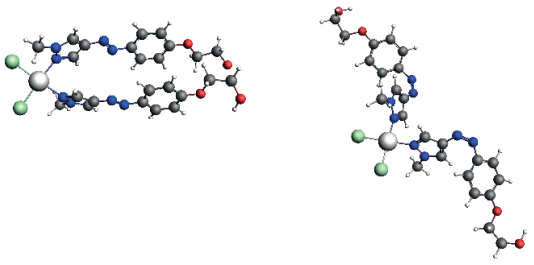

Figure 1. The spatial structure of *trans* (left) and *cis* (right) isomers of the photoswitchable cisplatin analog.

PL ISSN 1898-0295

Oral Presentations

PL ISSN 1898-0295

LIPOPOLYSACCHARIDE FROM *PORPHYROMONAS GINGIVALIS* IS A WEAK ACTIVATOR OF GINGIVAL FIBROBLAST INFLAMMATORY RESPONSES

ELWIRA NIEBOGA[1,2,*], AURELIUSZ SCHUSTER[1,2], ALEKSANDER M. GRABIEC[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, S. Lojasiewicza 11, 30-348 Kraków, Poland*

**e-mail: elwira.nieboga@doctoral.uj.edu.pl*

Gingival fibroblasts (GF) play an important role in the progression of inflammation through producing many pro-inflammatory mediators. Periodontitis is a chronic inflammatory disease of the periodontium caused by microbial imbalance and *Porphyromonas gingivalis* plays a central role in disease development. GF stimulation with *P. gingivalis* lipopolysaccharide (*Pg*-LPS) is commonly used to study host-pathogen interactions, but the biological relevance of this model remains controversial.

Here, we show that *Pg*-LPS caused a very weak transcriptional induction and production of many inflammatory mediators, including interleukin (IL)-6 and IL-8, in GFs. In contrast, stimulation with *Escherichia coli*-derived LPS or infection with live *P. gingivalis* caused very strong induction and production of these cytokines. Next, we demonstrated that expression of *TLR4, CD14,* and *LY96* (molecules responsible for LPS recognition) was significantly lower in GFs compared to MDMs. Supplementation of GFs with exogenous CD14 caused a dose-dependent increase in IL-6 and IL-8 production in GFs stimulated with *Pg*-LPS. However, it was still much lower compared to GF activation induced by *Ec*-LPS. Finally, we proved that *P. gingivalis* activates GF mainly through Toll-like receptor-2 (TLR-2). Polymyxin-B, which blocks the LPS-TLR4 interaction, or a TLR4-blocking antibody had no effect on GF inflammatory response upon infection with live *P. gingivalis*. Additionally, silencing of TLR4 in GFs had no effect on their response to *P. gingivalis,* while silencing TLR2 caused a decrease in IL-6 and IL-8 production,

Collectively, we show that *Pg*-LPS is a very weak activator of GFs and plays a negligible role in GF activation by *P. gingivalis*.

ACKNOWLEDGEMENTS

The study was supported by an NCN grant to AMG (2019/35/B/NZ5/01823).

PL ISSN 1898-0295

The Balbiani body in Tettigoniidae

Małgorzata Sekuła[1,*], Magda Sochaczewska[1,2], Patrycja Maciak[2,3], Wacław Tworzydło[1], Szczepan M. Biliński[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

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

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

Balbiani body (Bb) is a specialized group of organelles specific to the female germline cells, typically consisting of multiple mitochondria, characteristic accumulations of the nuage material, Golgi complexes and elements of endoplasmic reticulum. This study describes morphology and ultrastructure of the Bb in six bush cricket species belonging to four disparate subfamilies of the Tettigoniidae family. We revealed notable variations in the morphology of Bbs among closely related species. In three species: *Meconema meridionale*, *Pholidoptera griseoaptera* and *Tettigonia cantans* the Bb has a compact form, attains the shape of a hollow hemisphere, covering only a part of the surface of the oocyte nucleus (referred to as germinal vesicle). In *Conocephalus fuscus*, *Leptophyes albovittata* and *Phaneroptera falcata* the Bb is less distinct, has more dispersed form and encompasses the entire or almost entire surface of the germinal vesicle. Apart from those clearly visible differences, the Bbs in the oocytes of all studied six bush crickets species are composed of the same organelle set and most importantly are characterized by a direct contact between mitochondria and nuage material.

ACKNOWLEDGEMENTS

The study was supported by PRELUDIUM 20 grant (2021/41/N/NZ3/00360) from National Science Centre.

PL ISSN 1898-0295

THE ROLE OF FAS *N*-GLYCOSYLATION IN NTHY-ORI 3-1 CELL DEATH – *IN VITRO* MODEL OF THYROCYTE APOPTOSIS IN HASHIMOTO'S THYROIDITIS

SARA TRZOS[1,2,*], NATALIA WOJTOWICZ[1], MAŁGORZATA OPYDO[3], PAWEŁ LINK-LENCZOWSKI[4], 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]*Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

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

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

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

In Hashimoto's thyroiditis Th1 cells secrete pro-inflammatory cytokines, which activate Tc cells to induce apoptosis of thyrocytes via the Fas/FasL pathway. Fas is a membrane receptor, belonging to the TNF family of death receptors. It contains two *N*-glycosylation sites located close to the ligand binding site. The importance of Fas *N*-glycosylation in apoptosis is still uncleared. Therefore, the aim of our study was to evaluate the role of Fas *N*-glycosylation in apoptosis of the human Nthy-ori 3-1 thyrocyte line.

Fas expression was stimulated with interferon gamma (INFγ, 40 ng/ml, 48h), and apoptosis of thyrocytes was induced by human recombinant FasL (40 ng/ml, 18 h). Kifunensin (5 μM, 48 h) and swainsonine (2,5 μg/ml, 48 h), alkaloids that inhibit processing of oligomannose structures, were used to block the synthesis of complex-type *N*-glycans, which were previously identified in the Fas molecule. Thyrocyte viability in the presence of the wide range of concentrations of *N*-glycosylation inhibitors was determined by alamarBlue. The effectiveness of the inhibitors was confirmed by MALDI mass spectrometry and lectin blotting. Apoptosis of thyrocytes was analysed with annexin V/propidium iodide assay, and assessment of caspase 3 and 7 activity by flow cytometry.

The cytometric data obtained in both assays showed an induction of thyrocyte apoptosis via Fas/FasL pathway, but we did not observe the statistically significant changes in the percent of apoptotic cells in the presence of *N*-glycosylation inhibitors under the selected experimental conditions. This result needs to be verified using other methods.

ACKNOWLEDGEMENTS

The study was supported by the BioS Priority Research Area under the "Initiative of Excellence – Research University" program at Jagiellonian University, grant No. U1U/P03/NO/03.28 (EP). The participation in the Symposium was supported by the Jagiellonian University grant No. N18/DBS/000007.

PL ISSN 1898-0295

Caesarean section piglets as a suitable animal model for the study of iron metabolism in premature neonates

Xiuying Wang[1,*], Jarosław Woliński[2], Paweł Lipiński[1], Rafał Mazgaj[1], Magdalena Ogłuszka[3], Zuzanna Kopeć[1], Beata Żelazowska[1], Rafał R. Starzyński[1,*]

[1]*Institute of Genetics and Animal Biotechnology PAS, Department of Molecular Biology, Laboratory of Iron Molecular Biology, Jastrzębiec, Postępu 36a, 05-552 Magdalenka, Poland*

[2]*Kielanowski Institute of Animal Physiology and Nutrition PAS, Department of Animal Physiology, Laboratory of Large Animal Model, Instytucka 3, 05-110 Jabłonna, Poland*

[3]*Institute of Genetics and Animal Biotechnology PAS, Department of Genomics and Biodiversity, Jastrzębiec, Postępu 36a, 05-552 Magdalenka, Poland*

**e-mail: x.wang@igbzpan.pl*

Children aged 0 to 5 years are most at risk of anemia due to their high need for iron during rapid growth. Iron stores in the fetal liver are formed as a result of intensive iron transfer from the mother during the third trimester of pregnancy. Therefore, shortening pregnancy may cause insufficient iron stores in the liver, which is the main source of iron in newborns. Our knowledge of the regulation of iron homeostasis in premature infants is insufficient, and therefore animal models reflecting iron metabolism in premature infants are greatly needed. Therefore, the main goal of our research was to create and describe/validate an animal model based on preterm domestic pigs. To develop this animal model, we used preterm piglets obtained after cesarean section in sows on day 109 of gestation, which corresponds to the last trimester of late pregnancy in humans. The second aim was to compare iron metabolism in premature and full-term piglets. Preterm piglets showed reduced body weight, RBC indices, plasma iron levels, and total body iron content compared to full-term piglets. Interestingly, premature piglets had higher plasma and liver ferritin levels and non-heme iron content in the liver and spleen. The expression of hepatic hepcidin and BMP6 mRNA was also increased in premature piglets. In summary, the porcine model of prematurity is a convenient animal model for studying anemia in human newborns after preterm birth, suitable for both basic and applied research.

ACKNOWLEDGEMENTS

The study was carried out under the National Science Center grant no. 2020/39/B/NZ5/02469.

NOVEL SALICYLAMIDE DERIVATIVE AND ITS INFLUENCE ON LONG-TERM EMOTIONAL AND RECOGNITION MEMORY IN MICE

ELŻBIETA ŻMUDZKA[1,*], KINGA SAŁACIAK[2], AGATA SIWEK[3], JOLANTA JAŚKOWSKA[4], JACEK SAPA[2], KAROLINA PYTKA[2]

[1]*Department of Social Pharmacy, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland*

[2]*Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland*

[3]*Department of Pharmacobiology, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland*

[4]*Department of Organic Chemistry and Technology, Faculty of Chemical and Engineering and Technology, Cracow University of Technology, Warszawska 24, 31-155 Krakow, Poland*

**e-mail: ela.zmudzka@uj.edu.pl*

Cognitive impairments may manifest the presence of dementia or Alzheimer's disease but may also accompany other psychiatric disorders, such as: schizophrenia or depression. Difficulties in learning and memory processes significantly affect patient's daily life and functioning. Moreover, available therapies are very limited and not sufficiently effective. Therefore, there is a need to investigate novel compounds as potential anti-amnesic and procognitive drugs.

The aim of this study was to assess the influence of novel salicylamide derivative, JJGW08 on the long-term emotional and recognition memory in mice.

Step-through passive avoidance and object recognition tests were performed using male CD-1 mice. In the passive avoidance test the latency to entered dark compartment was measured. Mice which remembered the task stayed in the light chamber. In the object recognition test the time of novel object exploration was measured. Moreover, the ability of the compound to reverse cognitive disturbances induced by MK-801 was evaluated in both tests.

Studied compound at the doses 0.15-2.5 mg/kg increased latency time in retention trial in the passive avoidance task as well as reversed MK-801-induced cognitive disturbances at the doses 0.3 and 2.5 mg/kg in this test. Moreover, JJGW08 increased the time of novel object exploration at the doses 0.15-0.625 mg/kg and reversed memory impairments induced by MK-801 administration at the dose 0.15 mg/kg.

The obtained results indicated that JJGW08 possess potential procognitive and anti-amnesic activity in the long-term emotional and recognition memory in mice. Extended studies are desirable to fully understand the influence of the compound on cognitive functioning.

Poster Presentations

PL ISSN 1898-0295

AGE-DEPENDENT EFFECT OF VITAMIN D_3 ON THE OVARIAN FOLLICULOGENESIS AND SECRETION OF ESTRADIOL AND AMH - AN *IN VITRO* RESEARCH USING A RABBIT MODEL

PAULA AJERSCH[1,*], KINGA KAMIŃSKA[1], SYLWIA PAŁKA[2], MICHAŁ KMIECIK[2], MAŁGORZATA GRZESIAK[1]

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

[2]*Department of Genetics, Animal Breeding and Ethology, University of Agriculture in Kraków, Al. Adama Mickiewicza 24/28, 30-059 Kraków, Poland*

**e-mail: paula.ajersch@student.uj.edu.pl*

Folliculogenesis is a process that involves the growth and development of ovarian follicles. Ovarian reserve decreases with age that is related to depletion of the pool of follicles able to maturation. Therefore, the undisturbed course of folliculogenesis determines reproductive success in females. Recent studies revealed that vitamin D_3 is a factor influencing follicle development and steroidogenesis, notably anti-Mullerian hormone (AMH) and estradiol (E_2) release. Noteworthy, the level of vitamin D_3 also decreases with age. Therefore, the aim of the study was to examine whether vitamin D_3 *in vitro* influences ovarian morphology, as well as secretion of hormones, such as AMH and E_2, by ovarian explants harvested from immature, one-, two- and three-year-old rabbits. Ovarian slices from each age group (n = 4/group) were cultured on inserts (12 h, 37°C, 5% CO_2) in control medium or with addition of vitamin D_3 (100 ng/ml). Next, medium was collected for AMH and E_2 assessment using ELISA kits, whereas tissue fragments were fixed in formalin for hematoxylin and eosin staining. Both AMH and E_2 release into the medium was increased following vitamin D_3 treatment in one-, two- and three-year-old groups. As for the group of immature rabbits, decreased secretion of these hormones was observed. The analysis of ovarian morphology revealed that the addition of vitamin D_3 led to greater number of follicles at different stages of development. Since AMH level indicates the degree of ovarian aging, obtained findings suggest the beneficial effect of vitamin D_3 on depletion of ovarian reserve.

PL ISSN 1898-0295

A SYNTHETIC ΔM4 ANTICANCER PEPTIDE LEADS TO CELL DEATH OR CAUSES DRUG RESISTANCE IN THE LONG-TERM TREATMENT OF HUMAN LUNG CANCER CELL LINE *IN VITRO*, DEPENDING ON THE CONCENTRATION

ALEKSANDRA BRANKIEWICZ[1,*], MARCELA MANRIQUE-MORENO[2], IBETH GUEVARA-LORA[1]

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

[2]*Chemistry Institute, Faculty of Exact and Natural Sciences, University of Antioquia, Medellin, Colombia*

**e-mail: aleksandra.brankiewicz@doctoral.uj.edu.pl*

Anticancer peptides (ACP) are a group of molecules with cationic residues that can interrupt the continuity of the cancer cell membrane or cause cytotoxic effects by disrupting intercellular metabolic pathways. In this study, we focused on ΔM4, a synthetic derivative of cecropin-D-like peptide with proven antimicrobial properties, designed as the ACP. The aim of our research was to determine the response of A549 cells to the ΔM4 peptide depending on the concentration.

Firstly, we proved that the IC_{50} for the cells cultured in medium with reduced FBS content (1%) was three times lower than the IC_{50} for cells cultured in medium supplemented with 10% FBS. Furthermore, the decline in cell viability under ΔM4 treatment also persisted after 48 hours. Subsequently, we discovered that ΔM4 in concentrations of 2.5 and 5 μM causes strong oxidative stress lasting up to 6 hours in the A549 cell line. A higher level of NRF2 and COX2 proteins was demonstrated after incubation with ΔM4 concentrations of 1.25 – 5 μM. Nevertheless, the *NFE2L2* gene expression did not change. We also observed a decrease in *BAX* and an increase in *BCL2* gene expression. Finally, we noted early stages of apoptosis at concentrations of 2.5, 5 μM, and higher, observing increased externalization of phosphatidylserine in the cell membrane.

In summary, we confirmed a significant environmental and concentration impact on ΔM4's mechanism of action, and we suppose that low doses may cause resistance in surviving cells. The newly designed ΔM4 shows some potential for use as an ACP, but we need more research to understand its exact bioactivity.

PL ISSN 1898-0295

ITACONIC ACID AS A POTENTIAL THERAPEUTIC ANTI-INFLAMMATORY AGENT

GABRIELA BURCZYK[1,2,*], ELZBIETA KOLACZKOWSKA[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

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

The systemic overproduction of inflammatory mediators and neutrophil extracellular traps (NETs), as well as continuous activation of innate immune responses might cause long term side effects leading to organ damage. Endogenous mechanisms limiting inflammation are the subject of ongoing research, and recently their scope has been expanded to include molecules/metabolites related to immunometabolic processes. The latter category also includes itaconic acid. The aim of the current study was to verify anti-inflammatory role of 4-octyl itaconate (4-OI), a derivative of itaconic acid, as a potential inhibitor of neutrophil extracellular traps (NETs). NETs are beneficial during early inflammation as they trap pathogens but when not timely removed they cause collateral damage. Firstly, various parameters of mice bone marrow derived neutrophils were investigated *ex vivo*. The cells were treated with 4-OI or inhibitors of various metabolic pathways in the presence or absence of lipopolysaccharide (LPS). In the second approach, mice were injected intraperitoneally with 4-OI and then endotoxemia was induced by LPS. NETs were visualized in the liver vasculature of living mice using intravital microscopy (IVM). In *ex vivo* studies 4-OI dramatically inhibited NET formation *via* inhibition of hypoxia-inducible factor-1α (Hif-1α) and induction of anti-inflammatory nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase (HO-1) axis. The IVM confirmed that 4-OI reduces NET formation also *in vivo* during systemic inflammation without affecting neutrophil numbers. Our results indicate the possible therapeutic potential of itaconic acid as an anti-inflammatory agent selectively inhibiting NETs.

ACKNOWLEDGEMENTS

The study was supported by National Science Centre of Poland, OPUS 22, 2021/43/B/NZ6/00782.

PL ISSN 1898-0295

STUDIES ON THE MITOCHONDRIAL GENOME OF HOLOPARASITIC PLANT *PHELIPANCHE RAMOSA* (OROBANCHACEAE)

ANNA BURDA-URYGA[1,2,*], DAGMARA KWOLEK[2], MAREK SZKLARCZYK[3], RENATA PIWOWARCZYK[4], GRZEGORZ GÓRALSKI[2]

[1]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, St. Łojasiewicza St 11, PL30348, Kraków, Poland*

[2]*Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University, Gronostajowa St 9, PL30387, Kraków, Poland*

[3]*Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture, Al. 29 Listopada St 54, PL31425, Kraków, Poland*

[4]*Center for Research and Conservation of Biodiversity, Department of Environmental Biology, Institute of Biology, Jan Kochanowski University, Uniwersytecka St 7, PL25406, Kielce, Poland*

**e-mail: anna.burda@doctoral.uj.edu.pl*

Mitochondria are organelles possessing its own genome and playing crucial roles in cell and organism functioning. The most widely known of ATP production, they are also important in regulation processes of metabolism and development (including e.g. apoptosis). Mitochondrial functioning may be modified by changes in their genomes, as mutations and horizontal gene transfer (HGT). The latter is known as one of the phenomena significantly influencing evolution not only of uninuclear but also multicellular organisms including plants. Plant mitochondria have some features that may facilitate integration of foreign DNA. Parasitic plants, due to their close relationships with their hosts, are regarded as potentially good recipients (but also donors) of genes. These reasons inspired us to study the mitochondrial genome of plant holoparasite from the Orobanchaceae family - *Phelipanche ramosa* - to find traces of HGT but also other changes that may be related to its parasitic style of life. It's worth noting that mitochondrial genomes of plants are generally much bigger, more complex and variable than those in animal cells.

The key step to achieve our goal was sequencing and assembling the mitochondrial genome and transcriptome. Later we checked the presence of genes in mtDNA, their potential activity and we searched for HGT cases. We present our preliminary results of the studies: the complex architecture of the mitogenome, mitochondrial genes found and their expression.

ACKNOWLEDGEMENTS

The study was supported by the program "Research Support Module - Faculty of Biology" at the Jagiellonian University in Kraków, Poland (U1U/W18/NO/28.04).

PL ISSN 1898-0295

CALCIUM MODULATES THE RESPONSE FOR HYPOXIA STRESS IN TOMATO

MAŁGORZATA CZERNICKA[1], KINGA KĘSKA-IZWORSKA[1], MICHAŁ DZIURKA[2], ANNA KOŁTON[3], MARZENA POPIELARSKA-KONIECZNA[4,*]

[1]*Department of Plant Biology and Biotechnology, University of Agriculture in Krakow, 29 Listopada 54, 31-425 Krakow, Poland*

[2]*Department of Developmental Biology, The F. Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland*

[3]*Department of Botany, Physiology and Plant Protection, University of Agriculture in Krakow, 29 Listopada 54, 31-425 Krakow, Poland*

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

**e-mail: m.popielarska-konieczna@uj.edu.pl*

Waterlogged soil (an effect of intensive rainfall after drought) caused hypoxia stress that severely limits the yielding of tomato. Under oxygen deprivation (hypoxia) plants shift their metabolism inhibiting aerobic respiration, increasing alcohol fermentation and enhancing ethylene synthesis, that caused the lowest productivity. Identification of factors which enhance the tolerance to hypoxia could help to solve this problem.

The effect of exogenous calcium has been investigated on two cultivars of tomato, 'Faworyt' and 'Krakus', under hydroponic cultivation. The expression levels of *adh gene*, a key in alcohol fermentation, as well as of *acs* and *aco5* genes involved in ethylene biosynthesis pathways were up-regulated under hypoxia (Hyp) in contrast to control conditions. The double calcium supplementation (Hyp+2Ca) caused the decreasing of the expression of all of analyzed genes for both of cultivars. The higher concentration of 1-aminocyclopropane-1-carboxylic acid (ACC), precursor of ethylene, was detected for Hyp in roots, stem and leaves in both cultivars. The concentration of ACC significantly decreased under Hyp+2Ca condition. Additionally, the auxins concentration decreased after Hyp+2Ca in comparison with Hyp and was at similar level like in control. The modification at histological level concerning the enlarged intercellular spaces were detected for both Hyp and Hyp+2Ca in stem and root in Krakus rather than Faworyt, and observations were correlated with the analysis of porosity. The enriched supplementation with calcium seems to be beneficial for hypoxia tolerance regardless of tomatoes cultivars.

PL ISSN 1898-0295

CANDIDATE FACTORS INVOLVED IN MULTICELLULAR ORGANIZATION OF PROTUBERANCES FOLLOWED BY SHOOT FORMATION

MAŁGORZATA CZERNICKA[1], KINGA KĘSKA-IZWORSKA[1], MAŁGORZATA KOZIERADZKA-KISZKURNO[2], EMILIA WILMOWICZ[3], JOANNA MALEC[4,5], JAN KANTY TROŚCIŃSKI[4], MARZENA POPIELARSKA-KONIECZNA[4,*]

[1]*Department of Plant Biology and Biotechnology, University of Agriculture in Kraków, 29 Listopada 54, 31-425 Krakow Poland*

[2]*Department of Experimental Plant Biology and Biotechnology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk Poland*

[3]*Department of Plant Physiology and Biotechnology, Nicolaus Copernicus University in Toruń, Lwowska 1, 87-100 Toruń, Poland*

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

[5]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, St. Łojasiewicza St 11, PL30348, Kraków, Poland*

**e-mail: m.popielarska-konieczna@uj.edu.pl*

Organogenic callus is growing mass of unorganized or less differentiated and pluripotent plant parenchyma cells. Research on the shoot meristem arising from a disorganized callus tissue is crucial for basic research and applications in biotechnology. Protuberances are ball shaped, distinct structures detected within long-term callus in kiwifruit. The most important trait is that only on these specific structures the adventitious shoots are formed. Contrary to other region of callus, protuberances show also the regular orientation of cells, the presence of starch granules in chloroplasts and the unique transcriptomic data like up-regulation of genes involved in carbohydrate metabolism and secondary metabolite biosynthesis. For instance, the higher expression of *PHENYLALANINE AMMONIA-LYASE* (*PAL*) gene (LogFC=2.7) correlates with the higher concentration of this protein in protuberances. PAL enzyme – responsible for production of precursors of important secondary metabolites, like lignin or flavonoids – is regarded as a regulation point between primary and secondary metabolism in plant. The protuberances show also up-regulation (LogFC=9.1) of *RAPTOR1* gene, called "master regulator", which is crucial for enhancing cell expansion and controlling the multicellular growth. The specific plant *PROTODERMAL FACTOR2* (*PDF2*) (LogFC=7.6) is related to the organization of shoot epidermal cells. This homeobox-leucine zipper protein, together with the *RAPTOR1* gene, can be considered as some of the many regulator factors that have impact on the protuberances, specific kind of callus and transitional step before organogenesis and adventitious shoot formation.

ACKNOWLEDGEMENTS

The study was supported by NCN grant No. 2012/07/B/NZ9/01325 (MPK).

PL ISSN 1898-0295

The role of neutrophil extracellular trap (NET) creation in psoriasis – expression and serum concentration of peptidylarginine deiminase 4 (PAD – 4)

Joanna Czerwińska[1,*], Agnieszka Owczarczyk-Saczonek[1]

[1]*Department of Dermatology, Sexually Transmitted Diseases and Clinical Immunology, School of Medicine, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Oczapowskiego 2, 10-719 Olsztyn, Poland*

**e-mail: joanna.czerwinska@uwm.edu.pl*

Neutrophil extracellular trap (NET) formation plays a significant role in immune surveillance, but its overproduction may contribute to the development of autoimmune diseases such as psoriasis. The initial stage of the NET creation is the chromatin decondensation occuring as a result of histone citrullination, which is a consequence of activation of peptidyl arginine deiminase 4 (PAD-4). This enzyme seems to be essential for activation of NET. However, data on PAD-4 in NETs is still inconclusive and knowledge concerning PAD-4 in pathogenesis of psoriasis is limited. The aim of the study was to investigate the enzyme localization and its expression in the lesional and non-lesional skin and determine PAD-4 level in the serum and observe the response of this factor to systemic (anti-17a, anti-TNFα and methotrexate) therapies used in psoriatic patients. Results showed increased protein expression in lesional skin and higher level of PAD-4 in serum of pre-treatment patients, correlated with disease severity (evaluated by PASI and BSA). All of the applied therapies led to a decrease PAD-4 expression in skin and concentration in serum after 12 weeks. The most significant changes were noted with anti-TNFα therapy. The obtained data suggest the participation of PAD-4 in the activation of neutrophils to produce NETs in psoriasis, which may provide new opportunities of therapies with PAD inhibitors. However, further exploration of the molecular mechanism is needed.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Higher Education [project No. 61.610.006-110].

PL ISSN 1898-0295

SEARCHING FOR A UNIQUE AND SPECIFIC METHOD TO DETECT EXTRACELLULAR TRAPS (ETS) USING THYMIDINE ANALOGS AND BIOORTHOGONAL REACTIONS

DOMINIKA DRAB[1,2,*], ELZBIETA KOLACZKOWSKA[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

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

Extracellular trap (ET) formation is a mechanism shared by various leukocytes and it has been extensively studied in neutrophils but other innate immune cells, such as monocytes and macrophages, are also capable of releasing extracellular DNA (extDNA) decorated with nuclear and granular proteins. However, research has not yet been able to develop a method of specifically labelling DNA of a given population of cells, including monocytes/macrophages. However, bioorthogonal chemistry can be used to label biological molecules in a living organism by introducing a chemically modified molecule that binds to the biological target and can be detected with a fluorescently labeled compound. Therefore, the aim of our research was to attempt to establish a method for the detection of ETs using the thymidine analog VdU (5-Vinyl-2'-deoxyuridine) incorporated into DNA of proliferating cells for which fluorescently labeled tetrazine is used in a bioorthogonal chemistry reaction. Studies were performed *ex vivo* on isolated neutrophils from mice injected with VdU. After LPS stimulation of isolated neutrophils, extDNA was successfully detected using fluorescent tetrazine, which also colocalized with another extDNA dye, Sytox Green. It was also tested whether the detection of ETs would be specific *in vivo* using intravital microscopy. However, detection of VdU incorporated into extDNA released by neutrophils was non-specific. Although bioorthogonal chemistry represents a promising tool in biomedical research, it remains challenging for *in vivo* applications and requires further refinement to specifically label biomolecules *in vivo*.

ACKNOWLEDGEMENTS

The study was funded by the National Science Centre of Poland (grant 2021/43/B/NZ6/00782).

EXPLORING THE ANTIBACTERIAL AND ANTI-INFLAMMATORY POTENTIAL OF BROAD-SPECTRUM HISTONE ACETYLATION DISRUPTION IN MACROPHAGES

DOMINIKA M. DRAPAŁA*, ALEKSANDER M. GRABIEC

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

**e-mail: dominika.drapala@doctoral.uj.edu.pl*

Histone deacetylase (HDAC) inhibitors have shown promise against hematological neoplasms, but their anti-inflammatory effects are a recent revelation. In conditions like periodontitis, characterized by chronic inflammation leading to tissue damage and tooth loss, reducing inflammation is crucial. While HDAC inhibition is well-studied, it is also essential to understand the roles of histone acetyltransferases (HAT) and bromodomain-containing BET proteins, which mediate acetylation signaling, in inflammatory processes. This research aims to assess the therapeutic potential of broadly targeting the histone acetylation system in macrophages infected with *Porphyromonas gingivalis*, a major periodontitis pathogen.

Monocyte-derived macrophages were cultured in the presence of GM-CSF to promote an M1-like inflammatory phenotype. After 7 days, the cells were exposed to a panel of inhibitors: JQ1 (BET inhibitor), ITF-2357 (Pan-HDAC inhibitor), and C646 (HAT inhibitor) for 1 or 24 hour(s). Subsequently, macrophages were infected with *P. gingivalis* at MOI 20. Disrupting the histone acetylation system altered macrophage inflammatory activation by modulating the production of IL-6, CCL2, and CCL5 without compromising cell viability. Analysis of p65, p38, and ERK phosphorylation showed no disruption of MAP kinase and NF-κB pathway activation, supporting a direct effect of the inhibitors on the chromatin. Moreover, these inhibitors significantly impacted the phagocytosis of fluorescently-labelled *P. gingivalis*, resulting in a substantial reduction in bacterial load within macrophage phagolysosomes. This reduction in bacterial load was accompanied by an enhancement of macrophage bactericidal capacity. These results show that tested inhibitors targeting histone acetylation can reduce excessive inflammation in periodontitis, with the HAT inhibitor as the most promising.

ACKNOWLEDGEMENTS

This work was supported by the NCN OPUS grant to AMG (2021/43/B/NZ5/03165).

PL ISSN 1898-0295

COMPLETE GENOME SEQUENCE OF *LEPTOSPIRA INTERROGANS* SEROVAR HARDJO: INSIGHTS INTO ITS GENETIC DIVERSITY AND POTENTIAL VIRULENCE TRAITS

KLAUDIA DUBNIEWICZ[1,*], LAURA PARDYAK[2], IGOR JASIELCZUK[2], ARTUR GURGUL[2], ZBIGNIEW J. ARENT[1,2]

[1] *University Centre of Veterinary Medicine, University of Agriculture in Krakow, Al. Mickiewicza 21, 31-120 Kraków, Poland*

[2] *Center of Experimental and Innovative Medicine, University of Agriculture in Krakow, Al. Mickiewicza 21, 31-120 Kraków, Poland*

**e-mail: klaudia.dubniewicz@urk.edu.pl*

Leptospirosis is a neglected disease of global significance caused by pathogenic species of *Leptospira*. These pathogenic bacteria are responsible for major human and animal diseases with important economic and public health impacts. Leptospira interrogans serovar Hardjo colonizes cattle and sheep kidneys and reproductive tract and may occasionally infect humans and other mammals.

The main objectives of this study were to sequence and assembly selected *Leptospira interrogans serovar Hardjo* genomes and to examine their phylogeny to capture differences among the different genomes and clades. Techniques used to examine these differences included inter alia an in-depth study of areas of possible horizontal transfer into the *L. interrogans serovar Hardjo* genomes, and a comparison of known genes and pseudogenes present in other *Leptospira* genomes that correspond to them. The study was based on 4 European *Leptospira interrogans* serovar Hardjo strains. The bacteria were isolated from different hosts, namely cattle in Belgium, horse in Italy, and dog with wallaby in the United Kingdom. The annotated genomes had similar number of detected genes (3,945 on average, with slightly higher number in hybrid assemblies), of which on average 96% were protein coding genes. Pan-genome analysis revealed genomic diversity and an open pan-genome state. Genomic plasticity analysis exhibited abundant mobile genetic elements including genome islands. Phylogenetic analysis showed differences between European isolates and the publicly available genomic sequences of South American isolates.

ACKNOWLEDGEMENTS

Financial support: Grant 2019/33/B/NZ9/02159

PL ISSN 1898-0295

Effect of SRR504734, a selective glycine transporter type 1 inhibitor, on seizure thresholds in mice

Nikola Gapińska[1,2,*], Piotr Wlaź[1], Marcin Jakubiec[3], Michał Abram[3], Krzysztof Kamiński[3], Katarzyna Ciepiela[3,4], Elżbieta Wyska[5], Katarzyna Socała[1]

[1]*Department of Animal Physiology and Pharmacology, Faculty of Biology and Biotechnology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland*

[2]*Doctoral School of Quantitative and Natural Sciences, Maria Curie-Skłodowska University, Weteranów 18, 20-400 Lublin, Poland*

[3]*Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Cracow, Poland*

[4]*Selvita S.A., Bobrzyńskiego 14, PL 30-348 Cracow, Poland*

[5]*Department of Pharmacokinetics and Physial Pharmacy, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland*

**e-mail: nikola.gapinska98@gmail.com*

Glycine transporter type 1 (GlyT1) is a homeostatic regulator of glycine levels in the mammalian central nervous system (CNS). Glycine plays a dual role in the CNS. It is a co-agonist of excitatory NMDA receptors, but it also acts as an inhibitory neurotransmitter by binding to the strychnine-sensitive glycine receptor. In recent years, GlyT1 become the subject of many studies, especially as a potential therapeutic target for schizophrenia. Moreover, it appears that GlyT1, by regulating extracellular glycine concentrations, may modulate the processes of excitation/inhibition balance in hippocampal networks and thereby influence seizures susceptibility. However, data on the role of GlyT1 in seizures are very limited.

The aim of the study was to investigate the effect of SSR504734 – a selective GlyT1 inhibitor – on seizure thresholds in three acute seizure tests in mice. In a pharmacokinetic study, the highest serum concentration of SSR504734 was reached at 15 min after intraperitoneal injection, while the highest concentration in the brain was observed 30 min after administration. In the in the intravenous pentylenetetrazole seizure threshold test, 30 mg/kg slightly increased the seizure threshold for the first myoclonic twitch, but it did not affect the thresholds for generalized clonus and tonic seizures. SSR504734 also did not affect the current intensity necessary to induce psychomotor seizures in the 6 Hz-induced seizure threshold test. In a subsequent experiment, SSR504734 (30 and 50 mg/kg) significantly increased the threshold for the tonic hindlimb extension in the maximal electroshock-induced seizure threshold test. The obtained results suggest that inhibition of GlyT1 mainly suppress tonic seizures.

ACKNOWLEDGEMENTS

Research project financed by the National Science Center UMO-2021/41/B/NZ7/00328.

PL ISSN 1898-0295

EFFECTS OF DOCOSAHEXAENOIC ACID AND LOVASTATIN ON THE LIPID METABOLISM-RELATED GENES AND PROTEINS IN INFLAMED MURINE ADIPOCYTES

JOANNA GDULA-ARGASIŃSKA[1,*], ANNA WIKAR[1], AGATA PIETRZYCKA[1], JOANNA GÓRKA-DYNYSIEWICZ[2], LILLA PAWLIK-SOBECKA [3], TADEUSZ LIBROWSKI[1]

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

[2] *Department of Pharmaceutical Biochemistry, Wroclaw Medical University, Borowska 211 A, 50-556 Wroclaw, Poland*

[3] *Department of Basic Medical Sciences, Wroclaw Medical University, Chałubińskiego 4, 50-368 Wroclaw, Poland*

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

Adipose tissue is an active endocrine organ that secretes adipokines, which play a role in pathologies related to obesity, such as insulin resistance and type 2 diabetes. Saturated free fatty acids, such as lauric acid and palmitic acid, released from altered adipocytes can activate signaling and the expression of pro-inflammatory genes. Unlike saturated fatty acids, docosahexaenoic acid and its derivatives have anti-inflammatory properties. By inhibiting HMG-Co-A reductase, statins reduce intermediate products of the cholesterol synthesis pathway, isoprenoids.

This study aimed to evaluate the effect of docosahexaenoic acid (DHA) and lovastatin (LOVA) on the level of genes and proteins related to lipid metabolism in inflamed murine adipocytes.

Using an immunodetection technique showed a significant decrease in the pro-inflammatory proteins TLR4, cPGES, and COX-2 levels in inflamed mouse adipocytes supplemented with DHA and LOVA. It was also shown that there was a significant decrease in the expression of pro-inflammatory genes Ptgs2 and Pla2g4a, as well as an increase of Pparg expression in 3T3-L1 cells after supplementation with DHA and LOVA and activation with lipopolysaccharide. The obtained results suggest synergistic anti-inflammatory effects of DHA and LOVA in adipocytes. Thus, increased n-3 polyunsaturated fatty acids intake and lovastatin may represent a promising strategy to reduce the pro-inflammatory signaling in inflamed adipocytes.

ACKNOWLEDGEMENTS

Funding: This work was supported by Jagiellonian University Medical College's statutory activity N42/DBS/000347.

PL ISSN 1898-0295

FUNCTIONING OF INTERNAL PROMOTERS RORE OF *CLOCK* GENE IN PRESENCE OF NATURAL AND CHEMICAL (CARBON MONOXIDE) LIGHT SIGNALS

PRZEMYSŁAW GILUN[1,*], MAGDALENA KOZIOROWSKA-GILUN[2], BARBARA WĄSOWSKA[1], KRZYSZTOF FLISIKOWSKI[3], TATIANA FLISIKOWSKA[3]

[1]*Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn, Tuwima 10, 10-748 Olsztyn, Poland*

[2]*University of Warmia and Mazury in Olsztyn, Faculty of Animal Bioengineering, Dept. Animal Biochemistry and Biotechnology, Oczapowskiego 5, 10-719 Olsztyn, Poland*

[3]*Livestock Chair of Biotechnology Technical University of Munich, Freising, Germany*

**e-mail: p.gilun@pan.olsztyn.pl*

The aim of this study was to investigate whether internal promoters RORE 1, 2, 3, are functional in three introns (2, 7, 15, respectively) of the *CLOCK* gene from a pig hypothalamus and can be regulated by epigenetic manner. An experiment was conducted on three groups of immature female pigs: 1) the normal group without any factors in natural conditions, 2) the control group of animals housed in constant darkness with 72-hour infusion of blank plasma into angular vein, 3) experimental group with conditions as before, with 72-hour infusion of plasma with dissolved CO. Obtained results showed that the *CLOCK* gene has an oscillatory rhythm of expression in 12 hours intervals in every investigated groups, but the oscillatory *CLOCK* expression was reverse pattern in the experimental group. Further analysis of RNA expression in the area of examined introns with internal promoters RORE showed an oscillatory expression pattern. The RORE1 and RORE 2 patterns were the same as the general pattern of the *CLOCK* gene expression, but RORE 3 was reverse oscillation in the normal group but in two of the patterns was the same as before. Additionally, we examined the methylation pattern of CpG islands near the promoter sequence only in RORE2 because only this sequence was located between CpG islands. Obtained results showed that natural light in the normal group and chemical as a CO increased CpG methylation in the experimental group compared to that in the control group without a light signal and significantly lower methylation level.

ACKNOWLEDGEMENTS

The study was supported by KNOW consortium.

PL ISSN 1898-0295

Thyroid hormone receptors (THRα and THRβ) determines T3 action in ovarian granulosa cell tumor

Justyna Gogola-Mruk*, Izabela Kumor, Gabriela Wojtaszek, 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

**e-mail: justyna.gogola@uj.edu.pl*

Epidemiological studies indicate that thyroid dysfunction doubles the risk of ovarian cancer. In addition, ovarian cancer patients with a history of thyroid disease have a worse prognosis compared to patients without such history. However, both the level of expression levels of thyroid hormone receptors α and β (THRα and THRβ) and their ratio in ovarian granulosa cell tumors, which are rare cancers, have not been determined.

Here we demonstrated expression of THRα and THRβ in human granulosa cell line (HGrC1), juvenile granulosa tumor cell line (COV434) and adult granulosa tumor cell line (KGN). The expression of gene and protein was analyzed by qRT-PCR and Western Blot respectively. We show that the expression of both THRα and THRβ on the gene and protein level was significantly higher in ovarian granulosa tumor cell lines (COV434 and KGN) in comparison to normal granulosa cell (HGrC1). Moreover the highest expression of THRα and THRβ was observed in juvenile granulosa cell tumor (COV434). Furthermore thyroid hormone T3 in doses 1, 10, 100 nM increased proliferation of granulosa cells (HGrC1) and ovarian granulosa cell tumor (COV434 cell line).

In summary, T3 increases the cell proliferation of ovarian granulosa cell tumor, expressing THRα and THRβ, contributes to the progression of these cancers.

ACKNOWLEDGEMENTS

The work was supported by the programme “Excellence Initiative-Research University” at the Jagiellonian University in Kraków, Poland.

PL ISSN 1898-0295

PHYSIOLOGICAL AND PHYTOCHEMICAL ASPECTS OF THE SALT STRESS RESPONSE OF THE MODEL DIOECIOUS SPECIES *RUMEX THYRSIFLORUS* FINGERH

KACPER GOZDUR[1,*], AGNIESZKA SZOPA[2], HALINA ŚLESAK[1]

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

[2] *Chair and Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Kraków, Poland*

**e-mail: kacpergozdur@alumni.uj.edu.pl*

The experiment aimed to determine whether the response of *Rumex thyrsiflorus* (thyrse sorrel) to salt stress differs according to sex. It was also investigated whether the appropriate sodium chloride (NaCl) concentration in the culture medium can increase the production of phenolic compounds in callus suspension and hydroponic cultures of male and female regenerants.

Regenerated plants were obtained *in vitro* through indirect organogenesis on a Murashige&Skoog (MS) medium with 0,5 mg/l thidiazuron, using hypocotyls isolated from 11-day-old seedlings. Suspension culture was obtained from callus formed on a MS+0.4 mg/l 6-benzylaminopurine +1 mg/l 2,4-dichlorophenoxyacetic acid. The sex of the plants and callus was determined by molecular analysis based on genetic sex markers. It was revealed that the response to salt stress depended on sex and NaCl concentration. A concentration of 0.5% (w/v) NaCl in Hoagland's nutrient solution caused a decrease in morphometric parameters, relative water content, photosynthetic pigment content and photosynthetic activity based on chlorophyll fluorescence parameters and an increase in proline accumulation among regenerants. They also contained more phenolic compounds determined with HPLC-DAD method, in comparison to callus tissue. The optimal NaCl content to increase the biosynthesis of phenolic compounds in callus tissue was 0.75% for females and 0.25% for males.

These results have significant implications for the study of the resistance of dioecious plants to environmental stress in the context of climate change. Additionally, they suggest potential applications of NaCl as an elicitor to increase the production of pharmaceutically valuable compounds and highlight the importance of dioecy in phytochemistry.

ACKNOWLEDGEMENTS

The study was supported by the program BioS Priority Research Area – structural and translational biology "Excellence Initiative – Research University", edition 2021 at the Jagiellonian University in Kraków.

PL ISSN 1898-0295

Serum fatty acids profile in patients with nonalcoholic steatohepatitis (NASH)

Joanna Górka-Dynysiewicz[1], Lilla Pawlik-Sobecka [2], Monika Pazgan-Simon[3] , Tadeusz Librowski[4], Joanna Gdula-Argasińska[4,*]

[1]*Department of Pharmaceutical Biochemistry, Wroclaw Medical University, Borowska 211 A, 50-556 Wroclaw, Poland*

[2]*Department of Basic Medical Sciences, Wroclaw Medical University, Chałubińskiego 4, 50-368 Wroclaw, Poland*

[3]*Department of Infectious Diseases. J. Gromkowski Regional Specialistic Hospital, Koszarowa 5, 51-149 Wroclaw, Poland*

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

e-mail: joanna.gdula-argasinska@uj.edu.pl

Nonalcoholic fatty liver disease (NAFLD) is when excess fat builds up in the liver. Two types of NAFLD are nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). The inflammation and liver damage of NASH can cause fibrosis, or scarring, of the liver. NASH may lead to cirrhosis, in which the liver is scarred and permanently damaged. Cirrhosis can lead to liver cancer. The study aimed to determine the fatty acid (FA) profile in the serum of patients with NASH and healthy volunteers using gas chromatography with flame ionization detection.

Significant differences were observed in serum fatty acid profile in patients with NASH compared to the control group. Saturated (SFAs) and monounsaturated fatty acids (MUFAs) differ significantly in patients with NASH. The level of n-6 fatty acids was higher, and the level of n-3 FA was lower in the serum of NASH patients compared to the data received for healthy people. Circulating fatty acids, detected in serum, may be used as diagnostic markers of nonalcoholic fatty liver disease. Further studies are necessary for searching non-invasive diagnostic tools for differentiating fatty liver from NASH and determining the presence and extent of fibrosis.

PL ISSN 1898-0295

Diverse modulatory impact of red blood cell lysate on lipopolysaccharide-stimulated neutrophils in 2D and 3D culture models

Daniel Gucwa[1,*], Anna Such[1,2], Elżbieta Kołaczkowska[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

**e-mail: dan.gucwa@student.uj.edu.pl*

In numerous cases, symptoms of hematological diseases lie in uncontrolled, premature breakdown of red blood cells (RBCs) which might impact leukocyte functioning or fate. Thus the aim of this study was to determine impact of whole blood hemolysate on the viability and immune activity of murine bone marrow-derived neutrophils. Neutrophils were treated with various concentrations of the hemolysate (originating from $0{,}25\cdot10^6$; $2{,}5\cdot10^6$ or $25\cdot10^6$ RBCs) obtained from whole blood of C57Bl/6J mice by freezing an isolated fraction of erythrocytes (-80°C). Some neutrophils were additionally stimulated with lipopolysaccharide (LPS). Cell viability, phagocytic activity and neutrophil extracellular traps (NETs) formation were evaluated. Some experiments (cell viability) were additionally performed in 2D vs 3D (Puramatrix scaffolds) cell culture models. We report that the addition of hemolysate, regardless of the number of lysed erythrocytes, coincided with a lower mortality rate of neutrophils. Interestingly, this effect was observed in 2D culture conditions but not in the 3D model. Moreover, independently on the hemolysate concentration or presence/absence of LPS no differences were detected in regard to the cells phagocytic activity. However, preliminary studies on NET formation indicate that hemolysate at each tested concentration dramatically decreased formation of NETs by LPS stimulated cells in 2D culture models. Evaluation of NETs in 3D conditions turned out to be challenging. We conclude that depending on the local milieu, hemolysis may not only inhibit the negative effects of LPS on neutrophil viability but also diminish its NET formation properties.

ACKNOWLEDGEMENTS

The study was supported by Jagiellonian University grant nr. N18/DBS/000017.

PL ISSN 1898-0295

LOCALIZATION OF COPPER IN THE SPERMS OF MALE MICE WITH MUTATION IN THE *ATP7B* GENE – A MOUSE MODEL OF WILSON'S DISEASE

SYLWIA HERMAN[1,*], MATEUSZ OGÓREK[1], DARIA KRZYSZTOFIK[2], MONIKA JAKUBOWSKA[2], MAŁGORZATA LENARTOWICZ[1]

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

[2]*Research Group of Molecular Mechanisms of Disease, Malopolska Centre of Biotechnology, Jagiellonian University, Gronostajowa 7A, 30-387 Krakow Krakow, Poland*

**e-mail: sylwia.herman@doctoral.uj.edu.pl*

Copper is an essential element for many biological processes in all types of cells. This element is also important during the spermatogenesis. The localization of copper in sperms seems related to the localization of copper-dependent proteins. The main proteins responsible for copper homeostasis in male gonads are Atp7a and Atp7b Cu-transporting ATPases. Lack of activity of ATP7B/Atp7b proteins caused by mutations in the *ATP7B/Atp7b* genes leads to Wilson's disease in humans and *tx-J* mutation in mice. In the previous studies, we have discovered that sperms of mentioned mice mutants have worse mobility than sperms of wild-type individuals. It is known that both, the excess and the deficit of copper can impair sperm mobility.

For movement sperms need energy, and a Cu-dependent protein called cytochrome c oxidase is crucial for cellular respiration and energy production. Thus, there was an assumption that a significant amount of copper can be observed in the sperm midpiece because it is a part of the sperm where the mitochondria are present and the energy is released.

In the present study, we investigated copper localization and estimative copper amount in the sperms of 3-month-old wild-type mice and *tx-J* mutants. Our results are pioneering because it is the first time we can observe copper localization in male gametes. It may help us explain the copper metabolism in the cells necessary for fertilization.

ACKNOWLEDGEMENTS

The research has been supported by a grant from the Faculty of Biology under the Strategic Programme Excellence Initiative at Jagiellonian University; grant no: U1U/W18/NO/28.37.

PL ISSN 1898-0295

SELECTION OF MITOCHONDRIA IN THE FEMALE GERMLINE OF NON-MODEL INSECT SPECIES

MARIUSZ K. JAGLARZ

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

e-mail: m.jaglarz@uj.edu.pl

During animal reproduction, oocytes pass to the next generation not only their nuclear genome, but also mitochondria with their separate set of genes. It is well established that the mitochondrial genome is prone to mutations, which may have deleterious effect on progeny. Mitochondrial inheritance has been studied in several model species of both invertebrates and vertebrates. Most of these studies has implicated the Balbiani body (Bb), a conspicuous assemblage of mitochondria and other organelles occurring in oocytes of some species, in selection and elimination of dysfunctional mitochondria ensuring transfer of only mutation-free mitochondria into the next generation. However, it is not clear how widespread this mechanism is. Here, I demonstrate how mitochondria are selected and transferred into the oocytes in non-model insects representing the Gyrinidae family. In these beetles, the follicular stage oocytes do not contain Bb. However, staining of ovaries with mitochondrial fluorescent markers revealed that mitochondria assemblages, resembling Bb, form very early during syncytial stage of oogenesis in the perinuclear region of the oogonial cells (cystocytes). In contrast to the *Drosophila* model system, Bb is formed in each cystocyte of eight-cell germline cysts. The JC-1 fluorescence assay revealed high membrane potential only in the Bb-associated mitochondria. Ultrastructural analysis and computer assisted 3D-reconstruction showed that mitochondria form a complex network in Bb. In contrast, isolated mitochondria are surrounded by cisternae of the endoplasmic reticulum and eliminated by mitophagy. Based on the obtained results, a model of the mitochondria selection and transfer within the ovarian cysts of Gyrinidae is proposed.

ACKNOWLEDGEMENTS

This research was supported by a mini grant U1U/W18/NO/02.34.

PL ISSN 1898-0295

The effect of sea buckthorn (*Hippophae rhamnoides L.*) and metformin on histological changes in the liver of Zucker Diabetic Fatty (ZDF) rats

Klaudia Jaszcza[1,*], Rudolf Dupák[2], Peter Massányi[2], Anna Kalafová[2], Marcela Capcarová[2]

[1]*Department of Animal Physiology and Endocrinology, University of Agriculture, Al. Mickiewicza 24/28, 30-059 Kraków, Poland*

[2]*Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic*

**e-mail: Klaudia.Jaszcza@urk.edu.pl*

Many scientific studies have proven that sea buckthorn (*Hippophae rhamnoides* L.) has many beneficial health properties. This plant contains bioactive substances that have antioxidant properties and reduce oxidative stress, which is involved in the development of diabetes.

The aim of the experiment was to examine the effects of sea buckthorn and metformin on histological changes in the liver of Zucker diabetic fatty (ZDF) rats, which represent an animal model of type 2 Diabetes mellitus. Particular concentrations were applied (250 and 500 mg.kg^{-1} body weight of sea buckthorn, and 150 mg.kg^{-1} body weight of metformin) by gastric gavage. The histological method used in the experiment was Toluidine blue staining.

The experimental results indicated the appearance of lipid droplets in diabetic control ZDF rats in the liver lobules, which was not noticeable in the control group (healthy, homogenous dominant). Administration of both substances led to a reduction in the number of lipid droplets in the liver.

The results represent an interest in sea buckthorn and its potential use in the treatment of Diabetes mellitus as well as other experimental studies.

ACKNOWLEDGEMENTS

This study was supported by APVV grant no 19/0243 and VEGA grant 1/0304/23.

PL ISSN 1898-0295

INHIBITION OF HUMAN BRAIN CANCER CELLS 1321N1 AND T98G BY CHANGES IN THE EXPRESSION OF ENZYMES INVOLVED IN L-CYSTEINE METABOLISM

HALINA JURKOWSKA[1,*], MARIA WRÓBEL[1], LESZEK RYDZ[1], EWA JASEK-GAJDA[2]

[1]*Jagiellonian University, Medical College, Faculty of Medicine, Chair of Medical Biochemistry, Kopernika 7 St., 31-034 Kraków, Poland*

[2]*Jagiellonian University, Medical College, Faculty of Medicine, Department of Histology, Kopernika 7 St., 31-034 Kraków, Poland*

**e-mail: halina.jurkowska@uj.edu.pl*

Oxidative metabolism of L-cysteine in the cells depends on the activity of cysteine dioxygenase 1 (CDO1) and leads to formation of hypotaurine, taurine or sulfate. Desulfuration pathway of L-cysteine in the cells is the source of H_2S and sulfane sulfur-containing compounds; these compounds are produced in the reactions which are catalyzed by enzymes: cystathionine β-synthetase (CBS), 3-mercaptopyruvate sulfurtransferase (MPST), thiosulfate sulfurtransferase (TST), and γ-cystathionase (CTH). Both CBS and CDO1 are enzymes dependent on iron ions.

The studies were performed in human brain cancer cells differing in the degree of malignancy, such as 1321N1 (astrocytoma, grade II), and T98G (glioblastoma, grade IV). The cells were culture in the presence of thiosulfate (a sulfane sulfur donor), 4-hydroxybenzyl isothiocyanate (HBITC, a natural donor of H_2S obtained from white mustard), and aminooxyacetic acid (AOAA, inhibitor of cystathionine β-synthase).

We found that thiosulfate, HBITC, and AOAA resulted in inhibition of 1321N1 and T98G cell proliferation. Interestingly, we have shown that in T98G cells, the expression of both CBS and CDO1 was higher than in 1321N1 cells. Moreover, we observed that also the expression of transferrin receptor 1 was higher in T98G cells compared to 1321N1 cells. In cells cultured in the presence of AOAA, the expression of CBS was reduced, which was simultaneously associated with a decrease in the intracellular level of Fe^{2+} ions and a decrease in the production of reactive oxygen species.

ACKNOWLEDGEMENTS

This study was supported by the Polish Ministry of Science and Higher Education, grant no. N41/DBS/000942 of the Jagiellonian University Medical College.

PL ISSN 1898-0295

ORALLY GIVEN HEME IRON TO PREGNANT SOWS IS INEFFICIENT TO PREVENT IRON DEFICIENCY ANEMIA IN NEWBORN PIGLETS

ZUZANNA KOPEĆ*, RAFAŁ MAZGAJ, RAFAŁ R. STARZYŃSKI, PAWEŁ LIPIŃSKI

Department of Molecular Biology, Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Jastrzębiec, Postępu 36a, 05-552 Magdalenka, Poland

**e-mail: r.kopeć@igbzpan.pl*

Inadequate iron supply during pregnancy is responsible for iron deficiency anemia (IDA) in mothers and neonates. Previous studies on pigs have proven that oral inorganic iron supplementation does not significantly improve maternal and neonatal iron status. Considering that heme iron is an efficient source of organic iron in the diet, the aim of this study was to check the impact of orally given bovine hemoglobin to pregnant sows, on their RBC/iron status and that of their progeny. PLW pregnant sows at day 80 of pregnancy were randomized into following groups (6 sows/group): non-supplemented, supplemented with $FeSO_4$ (60 mg Fe/day), supplemented with heme iron (the same dose). Supplementation continued until farrowing. 2 piglets from each sow were selected for analyses.

Results of our study show that iron supplements do not ameliorate neither iron homeostasis nor hematological status of pregnant sows at the end of pregnancy. At day 114, RBC indices were similar in sows from all groups, and showed no difference compared to day 80. Similarly, plasma parameters did not differ between sows from 3 experimental groups at the end of pregnancy. Exceptionally, iron plasma levels were decreased in iron-supplemented sows (although remained still above the borderline of ID) at 114 compared to day 80 of pregnancy. Neonatal iron status of piglets from iron supplemented sows was not improved compared with the progeny born to non-supplemented sows.

Our results show that iron from supplements given to pregnant sows is neither used for the improvement of their RBC/iron status nor is efficiently transferred across the placenta to fetuses.

ACKNOWLEDGEMENTS

The study was supported by NCN grant no. 2019/33/B/NZ9/02566.

PL ISSN 1898-0295

TiLV INFECTION-INDUCED CHANGES IN THE EXPRESSION OF GLYCOSYLTRANSFERASES AND GLYCOSIDASES IN ZEBRAFISH LARVAE

Karolina Kot[1], Miriam Mojżesz[2], Maria Zawisza[2,3], Mikołaj Adamek[4], Krzysztof Rakus[2], 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 Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[3] *Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, S. Lojasiewicza 11, 30-348 Krakow, Poland*

[4]*Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany*

**e-mail: ewa.pochec@uj.edu.pl*

Glycosylation, as the most important post-translational modifications, is essential in interactions between viruses and host cells. Pathogens use oligosaccharides as a tool to regulate their virulence and infectivity. Due to the constantly growing threat from viruses, analysis of glycan alterations induced by viral infections is of particular importance. The aim of our research was to assess an impact of Tilapia Lake Virus (TiLV) infection on the expression of genes encoding glycosyltransferases and glycosidases in zebrafish (*Danio rerio*) larvae from ABxTL line. Zebrafish larvae at 2 days post fertilization (dpf) were infected with TiLV or mock-infected by microinjection into the duct of Cuvier. Expression of genes encoding fucosyltransferases (*fut8*, *fut9b*), sialotransferases (*st6galnac5a*, *st6gal1*, *st3gal4*, *st8sia7*), neuraminidases (*neu1*, *neu4)* and other enzymes related to sialic acid synthesis (*cmah*, *cmas1*) as well as viral load was studied at 24- and 48-hours post infection (hpi) by Real-time qPCR. Statistical analysis was performed using one- and two-way ANOVA ($p<0.05$). Viral load analysis demonstrated increase in the TiLV copy number during infection. The up-regulation of the expression of *fut8*, *fut9b*, *cmah*, *cmas1*, *neu1*, *st6gal1*, and *st8sia7* was observed in TiLV-infected individuals in comparison to mock-infected larvae. There was also a significant difference in the expression of *cmah*, *neu1*, *st6gal1*, *st3gal4* and *st8sia7* in infected larvae between time points.

The obtained results showed that the TiLV significantly affects the expression of genes encoding enzymes involved in the glycosylation process. Further research on the protein level is required to determine the significance of these changes and their origin.

ACKNOWLEDGEMENTS

The participation in the Symposium was supported by the Jagiellonian University grant (N18/DBS/000007).

PL ISSN 1898-0295

ANTIFUNGAL POTENTIAL OF *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS RHAMNOSUS* EXTRACELLULAR VESICLES: IMPLICATIONS FOR *CANDIDA ALBICANS* BIOFILM INHIBITION

KATARZYNA KOWALIK*, KAMILA KULIG, MAGDALENA SUROWIEC, MARIA RAPALA-KOZIK, JUSTYNA KARKOWSKA-KULETA

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

**e-mail: katarzyna.kowalik@doctoral.uj.edu.pl*

Extracellular vesicles (EVs) are considered important carriers of bioactive molecules, facilitating long-distance communication between cells. These structures derived from probiotic bacteria such as *Lactobacillus plantarum* and *Lactobacillus rhamnosus* may exhibit some antimicrobial properties, making them promising candidates for various therapeutic applications.

This study focuses on the verification of the antifungal potential of bacterial EVs and their inhibitory effect on formation of biofilm by the opportunistic pathogen, yeast-like fungus *Candida albicans*. *Lactobacillus* EVs were obtained by sequential centrifugation of bacterial cultures grown on solid substrates. The analysis of three consecutive stages of fungal biofilm formation included assessment of biofilm thickness with a measurement of optical density at 600 nm and estimation of metabolic activity of biofilm cells. After treatment with bacterial EVs, there were no differences in the production of fungal biofilm biomass compared to untreated biofilms, although a noticeable reduction in the metabolic activity of biofilm cells was detected. Further examination of the impact of EVs on biofilm was performed using RT-PCR analysis of gene expression, including several key biofilm regulators and adhesins, and decreased expression of particular genes was observed in the case of *L. plantarum* EVs.

Since biofilm formation is a critical virulence mechanism, often associated with persistent fungal infections resistant to conventional antifungal treatment, understanding the detailed mechanisms by which probiotic-derived EVs influence gene expression and thwart biofilm formation holds significant promise for the development of innovative antifungal therapies.

ACKNOWLEDGEMENTS

This work was supported by the National Science Centre of Poland (grant no.2021/43/D/NZ6/01464 to JKK).

PL ISSN 1898-0295

Effect of CO as a chemical light signal on the level of HIF1a mRNA expression in the pig hypothalamus

Magdalena Koziorowska-Gilun[1], Paulina Zając[1,2],
Anna Dziekońska[1], Przemysław Gilun[3]

[1]*Department of Animal Biochemistry and Biotechnology, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-718 Olsztyn, Poland*

[2]*Department of Physiology and Toxicology of Reproduction, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, Tuwima 10, 10-748, Olsztyn, Poland*

[3]*Department of Local Physiological Regulations, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, Tuwima 10, 10-748, Olsztyn, Poland*

**e-mail: magda.koziorowska@uwm.edu.pl*

Carbon monoxide (CO) functions as the chemical signal of light, and its presence is closely related to seasonal changes. It blocks the transcription factors: BMAL1: CLOCK / NPAS2 by attaching to their domains containing heme groups. Because HIF - 1 α has sequences which are recognized by the above-mentioned transcription factors in its promoter, we assumed that CO influenced the level of HIF - 1α mRNA expression in the pig hypothalamus. The research material consisted of hypothalamic tissues (preoptic, dorsal and abdominal area) obtained from three groups of pigs: **normal -** no experimental procedures; **control** - animals kept in the dark plus autologous plasma infusion with physiological CO concentration; **experimental -** animals kept in the dark plus infusion of autologous plasma with increased concentration of CO. HIF-1 α mRNA expression levels were determined by Real-Time PCR. A higher mRNA expression level of the transcription factor HIF-1 was demonstrated during the day regardless of the experimental group and the part of the hypothalamus studied. After CO administration, a similar relationship of the effect of the light signal was noted in the mRNA expression levels. This indicates that CO may be a humoral light signal that affects the transcription factor HIF-1α levels.

ACKNOWLEDGEMENTS

This study was supported by National Leading Science Centre (KNOW), Scientific Consortium „Healthy Animal – Safe food" 05-1/KNOW2/2015 and funds from the University of Warmia and Mazury in Poland as part of statutory activity (No. 11.610.003-300).

PL ISSN 1898-0295

THE ALTERATIONS OF GLYCOSYLATION IN LPS-STIMULATED RAW 264.7 MACROPHAGES

ELENA KUSTOLAINEN[1,*], SARA TRZOS[1,2], PAWEŁ LINK-LENCZOWSKI[3], AGNIESZKA MAZUR-BIAŁY[4], EWA POCHEĆ[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

[3]*Department of Medical Physiology, Institute of Physiotherapy, Jagiellonian University Collegium Medicum, Michałowskiego 12, 31-126 Krakow, Poland*

[4]*Department of Biomechanics and Kinesiology, Faculty of Health Science, Jagiellonian University, Skawińska 8, 31-066 Krakow, Poland*

**e-mail: elena.kustolainen@student.uj.edu.pl*

Macrophages as one of the innate immune cells, are the first line to be bordered by different pathogenic invaders. Macrophages play an important role not only in phagocytosis and elimination of pathogenic agents, but the engagement of their PRR receptors can transduce signals to alarm the presence of pathogens and to recognize specific microbial components. Despite the increasing number of publications in the field of glycoimmunology, there are missing links, among others in terms of the glycan patterns of macrophages activated by different kinds of pathogens. Our study was aimed to appreciate the possible influence of LPS stimulation on RAW 264.7 cell line glycosylation.

RAW 264.7 cells were treated with lipopolysaccharide (LPS, 10 μg/ml), and cultured for 4 hours for gene expression analysis and for 24 hours for *N*-glycan analysis. Real-time PCR with SYBR Green was used for the estimation of the expression of genes encoding the selected glycosyltransferases and one glycosidase. For qualitative and quantitative determination of *N*-glycan structures released from macrophage proteins, MALDI-ToF-MS analysis was applied.

We found that the expression of *A4galt1*, *St3gal1*, *St8sia6,* and *Man1a* genes was markedly increased. We identified 7 common *N*-glycans (paucimannosidic, oligomannose, hybrid and fucosylated complex-type) in LPS-treated and control probes that have up-regulated quantities after stimulation. The significantly altered glycosylation in LPS-stimulated macrophages can suggest that the specific glycans are important to the elimination of Gram-negative bacteria. Further experiments are necessary to assess the importance of this remodeling of macrophages glycan profile.

ACKNOWLEDGEMENTS

The participation in the Symposium was supported by the Jagiellonian University grant (N18/DBS/000007).

PL ISSN 1898-0295

THE EFFECT OF PREANALYTICAL PHASE ON THE OSMOTIC FRAGILITY AND THE MORPHOLOGY OF ERYTHROCYTES IN CATTLE (*BOS TAURUS*)

MARTA MACHURA*, PIOTR ANTOS

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

**e-mail: mmachuramko@gmail.com*

The erythrocyte osmotic fragility reflects their ability to withstand hypotonicity. The proper biconcave shape promotes high osmotic resistance of normal RBCs. The determination of erythrocyte osmotic fragility has greatest usefulness in the diagnosis of hereditary spherocytosis in which spherically-shaped RBCs are produced. Spherocytes are particularly susceptible to osmotic lysis while being exposed to hypotonic solution.

Hematological results are often influenced by a number of preanalytical variables including anticoagulant type, the storage temperature and the time interval of sample analysis after collection. The aim of the study was to determine the effect of the preanalytical phase on the osmotic fragility and the morphology of bovine erythrocytes. The blood was collected from the tail vein from Holstein-Friesian cows (n = 20) into tubes with EDTA or lithium heparin. The erythrocyte resistance to hemolysis was measured by osmotic fragility test (OFT) and blood smears were prepared and stained by MGG method immediately after blood collection and 24, 48 and 72 hours after storage at 4°C or room temperature.

The results showed that bovine erythrocyte osmotic fragility remains stable during 24 hours storage at 4°C regardless of the anticoagulant used. It suggests the possibility of performing OFT the day after blood collection when storing blood at 4°C. It was also found that bovine erythrocytes stored at room temperature have reduced osmotic resistance. However, heparin better preserves osmotic resistance of bovine erythrocytes at room temperature. The blood smear examination showed that reduced osmotic resistance is associated with the formation of echinocytes during sample storage.

PL ISSN 1898-0295

CAN ZEBRAFISH HELP US TO STUDY AND UNDERSTAND HOW ORAL PATHOGENS ARE IMPLICATED IN NEUROINFLAMMATION?

MAGDALENA MARCINKOWSKA[1,2,*], MAGDALENA WIDZIOLEK[1], ANNA MIESZKOWSKA[1], MARIA ZAWISZA[1,2], ALEKSANDRA DOMAGALSKA[1], TOMASZ PRAJSNAR[1], KRZYSZTOF RAKUS[1], JAN POTEMPA[3,4], MAGDALENA CHADZINSKA[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

[3]*Department of Microbiology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland*

[4]*Department of Oral Immunology and Infectious Diseases, University of Louisville School of Dentistry, Louisville, KY, USA*

**e-mail: magdalena.maria.marcinkowska@doctoral.uj.edu.pl*

There are numerous evidence showing a correlation between periodontitis (PD) and various diseases, including neurodegenerative diseases. *Porphyromonas gingivalis* (*Pg*) is a keystone oral pathogen in the development of PD. Importantly, *Pg*-derived DNA and gingipains - the main virulence factors of this pathogen, were found in the brains of Alzheimer's patients. However, the mechanism of bacterial dissemination to the brain remains unclear.

We established zebrafish as an infection model to study *Pg* infection. This organism serves as a great alternative infection model due to the 70 % genetic similarity with humans, while providing the added advantage of tissue transparency and numerous transgenic lines e.g. with fluorescently labelled specific cell types. This allows for visualisation and tracking of bacterial interactions with the host cells in real-time upon infection.

Here, using zebrafish larvae infected systemically with the wild-type *Pg* or with its gingipain-deficient mutant strain, we studied the significance of gingipains in (i) *Pg* pathogenic potential, (ii) bacterial survival, and (iii) the onset of systemic inflammation.

Next, we verify whether systemic infection with *Pg* can induce neuroinflammation. We demonstrated the (i) presence of *Pg* in the brains of infected larvae. We also showed (ii) gingipain-dependent activation of microglia and (iii) increased gene expression of pro-inflammatory mediators in the heads of systemically infected larvae.

Our results indicate that zebrafish serves as a great tool to study host-*Pg* interactions, especially in the context of immune response and neuroinflammation, and in the future it can pave the way for discovering new therapeutics.

ACKNOWLEDGEMENTS

This work was supported by the Polish National Science Centre (grant no. 2021/43/B/NZ6/00733).

PL ISSN 1898-0295

Innate immune memory in gingival fibroblasts: effects of porphyromonas gingivalis infection

Mariia Melnykova*, Katarzyna B. Łagosz-Ćwik,
Guillem Ylla, Aleksander M. Grabiec

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

**e-mail: mariia.melnykova@doctoral.uj.edu.pl*

Periodontal disease (PD), characterized by chronic inflammation stemming from microbial dysbiosis, leads to degradation of gingival and bone tissues. Gingival fibroblasts (GFs), beyond their structural role, contribute to PD pathogenesis by exhibiting distinctive attributes of "non-classical" innate immune response cells. The anaerobic bacterium *Porphyromonas gingivalis* (PG) stands as a keystone pathogen in PD development.

This study seeks to determine whether GFs develop innate immune memory in response to PG infection. To this end, we established a chronic infection model where GFs underwent 24-hour exposure to live PG or its virulence factor (FimA), followed by 24-hour incubation in medium containing high concentrations of antibiotics to eliminate the remaining bacteria, and were cultured for a week. GFs were subsequently stimulated with tumor necrosis factor α (TNFα). We observed a significant increase in interleukin-6 (IL-6) production, both at the protein and mRNA levels, in pre-infected cells compared to the control group. Furthermore, pre-infection with live PG resulted in higher IL-6 production compared to that induced by treatment with heat-inactivated PG or FimA. To elucidate the mechanism underlying GF memory of previous infections, an analysis of global DNA methylation status was conducted, revealing no statistically significant differences in the methylation levels between pre-infected and control GFs. However, a significant increase in TNFα-induced phosphorylation of the NF-κB transcription factor in GFs pre-infected with PG was noted.

In summary, pre-infected GFs exhibit an augmented production of IL-6, suggesting that they develop a form of "memory" following bacterial challenge, which is likely driven by non-epigenetic mechanisms.

PL ISSN 1898-0295

GFP-TAGGED EXPRESSION OF TETRASPANIN CD63 IN THE BRAIN OF *DROSOPHILA MELANOGASTER*

MONIKA MITKA*, JOLANTA GÓRSKA-ANDRZEJAK

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

**e-mail: m.mitka@student.uj.edu.pl*

Exosomes have been widely recognised as communication vesicles that transfer bioactive proteins and RNAs between cells under normal and pathophysiological conditions.

In this study, we used a genetic and imaging approach to examine to what extent this mode of intercellular communication can occur in the brain of the fruit fly, *Drosophila melanogaster*, and to check whether its intensity reveals daily changes.

We used transgenic flies with the targeted expression of GFP-tagged tetraspanin CD63, a mammalian transmembrane exosome marker previously used to mark fly exosomes, and examined its expression in neurons and different types of glial cells at different times of day/night cycle (LD 12:12; 12 h of light and 12 h of darkness). We found the expression of GFP-CD63 at a high level in the different types of glial cells (in the form of GFP-marked puncta), the photoreceptor cells of the compound eye, and also the cells of the circadian network. Additionally, GFP-CD63 expression in glial cells revealed daily changes in LD12:12. It was the strongest at the beginning of the day.

Our study on GFP-tagged tetraspanin CD63 gives insight into the cellular network of exosomal trafficking in the brain of *D. melanogaster*, showing that glia cells and photoreceptor cells may be particularly inclined to use this kind of communication. In the case of glia cells, it appears to be controlled by the circadian clock.

ACKNOWLEDGEMENTS

This work was supported by the Department of Cell Biology and Imaging of the Institute of Zoology and Biomedical Research (Grant number N18/DBS/000015).

PL ISSN 1898-0295

Inhibition of PI3K/AKT signaling pathway potentiates cytotoxic activity of Gemtuzumab ozogamycin against acute myeloid leukemia cells

Aleksandra Mosurek, Małgorzata Opydo*

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

**e-mail: malgorzata.opydo@uj.edu.pl*

Acute myeloid leukemia (AML) is a highly heterogeneous hematopoietic malignancy characterized by excessive proliferation and accumulation of immature myeloid blasts in the bone marrow. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) is one of the signaling pathways proven to be hyperactivated in AML patient blasts and to contribute to enhanced survival and drug resistance of these cells. Hence, this pathway is an attractive target for the development of novel antileukemia strategies. In this study, we assessed the *in vitro* effects of PI3K inhibitor, LY294002 (LY), on gemtuzumab ozogamycin (GO)-mediated cytotoxicity in human AML cell lines. GO belongs to the antibody-drug conjugates and consists of a humanized murine anti-CD33 IgG4 antibody and a cytotoxic derivative of calicheamicin. Although GO has been shown to exert high anti-leukemic activity, not all CD-33 positive AML cells were found to be sensitive to its treatment. Therefore, in the present study we determined the changes in cell viability and apoptosis induction in GO-sensitive MV4-11 and GO-resistant HL-60 AML cell lines exposed to a combination of PI3K inhibitor, LY294002, and GO. The impact of this compounds on AML cell viability was assessed using Prestoblue test. The induction of apoptosis was investigated using flow cytometry annexin V/propidium iodide assay. It was found that after treatment of leukemia cells with the combination of the tested compounds, a decrease in the cell viability and an increase of apoptosis in both GO-resistant and GO-sensitive AML cells, were found. The results of the present study indicated the synergistic action of PI3K inhibitor and GO in leukemia cells, and thus the significant role of the PI3K/AKT pathway in the anti-leukemic activity of the tested antibody-drug conjugate. The obtained results require further detailed studies on the mechanisms responsible for the observed effects.

ACKNOWLEDGEMENTS

The study was supported by the grant from Polpharma Scientific Foundation (1027.0641.33.2020).

PL ISSN 1898-0295

THE INFLUENCE OF SELECTED STRESS FACTORS ON *OMPR* GENE EXPRESSION IN *PROTEUS MIRABILIS* HI4320

KLAUDIA MUSIAŁ*, DAWID GMITER

Department of Microbiology, Institute of Biology, Jan Kochanowski University in Kielce, Uniwersytecka 7 25-406 Kielce, Poland

**e-mail: musial.klaudia0805@gmail.com*

OmpR and EnvZ are part of two component regulation system that plays a role in mediating signal transduction in response to environmental osmotic pressure in Gram-negative bacteria. This stress-sensing system is important for pathogens to detect and respond to changing environment during course of infection. *Proteus mirabilis* is known as frequent cause of urinary tract infections, especially important during catheter-associated urinary tract infections. These bacteria cause serious problems with wellbeing management of catheterized patients at hospitals worldwide. It was shown that OmpR does not affect swarming motility in *P. mirabilis*, however the importance of the gene in the physiology of this bacterium has not yet been fully known.

This study aimed to indicate the effect of stress factors on *ompR* gene expression in *P. mirabilis* reference strain HI4320.

Total RNA isolation was performed on *P. mirabilis* at 4h of culture in LB medium without and with different environment stress factors: H_2O_2, high salt concentration and polymyxin B. Then synthesis of cDNA was performed using 2 µg of cDNA. The expression of the *ompR* gene was monitored using RT-qPCR methods. Changes in *ompR* expression was calculated in comparison to reference gene *rpoA* using the ΔΔCt method.

The obtained results will felicitate understanding the role of the *ompR* as a stress sensor in *P. mirabilis*.

ACKNOWLEDGEMENTS

The presented study was supported by NCN grant No. 2019/33/N/NZ6/02406 (D.G.).

PL ISSN 1898-0295

ASSESSMENT OF THE OCCURRENCE AND CONSERVATISM OF TWO-COMPONENT REGULATORY SYSTEMS IN *PROTEUS MIRABILIS*

SYLWIA NAWROT*, ILONA PACAK*, DAWID GMITER

Department of Microbiology, Institute of Biology, Jan Kochanowski University in Kielce, Uniwersytecka 7 25-406 Kielce, Poland

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

Two-component regulatory systems (TCS) are systems that enable bacteria to adapt to a changing environment. These systems receive signals from the environment, which leads to the regulation of the cellular response by changing the expression of specific genes. TCS are potential targets that could be blocked to deregulate the pathogenetic mechanisms of several bacteria.

In the genome of the *Proteus mirabilis* reference strain HI4320, 16 TCS were identified, but their role is not yet fully understood.

The aim of the study was to assess the occurrence and genetic conservatism of proteins that are elements of TCS encoded by genes located in the genomic sequences of *P. mirabilis*.

The source material included 50 complete *P. mirabilis* genomes downloaded using NCBI's publicly available online database. Proteins were then identified using the BLAST+ program based on created databases containing protein sequences derived from the *P. mirabilis* HI4320 reference genome.

The presented research results provide a more complete understanding of the role and function of two-component regulatory systems in the physiology of *P. mirabilis* bacteria.

ACKNOWLEDGEMENTS

The presented study was supported by NCN grant No. 2019/33/N/NZ6/02406 (D.G.).

PL ISSN 1898-0295

Illuminating the dopaminergic system: electrophysiological recordings from the VTA/SNc

Karolina Nowalińska*, Magdalena Walczak, Martyna Marzec, Tomasz Błasiak

Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University in Kraków, Gronostajowa 9, 30-387 Kraków, Poland

**e-mail: kar.nowalinska@student.uj.edu.pl*

There exists a neural pathway that suggests light may influence the activity of the neurons within ventral tegmental area (VTA) and substantia nigra compact part (SNc) – the primary sources of dopamine in the mammalian brain. The involved polysynaptic pathway originates from M4-type retinal ganglion cells that project to the ventral lateral geniculate nucleus/intergeniculate leaflet (vLGN/IGL). From there, the innervation extends to the lateral habenula (LHb), and subsequently from LHb to the VTA/SNc. Given the previously reported effects of light stimuli on LHb neurons activity, we aimed to investigate whether dopaminergic neurons within the VTA/SNc region exhibit responses to the light stimuli. To address this question, we conducted *in vivo* electrophysiological recordings of putative dopaminergic neurons in albino rats under urethane anesthesia. During the recordings light stimuli were delivered to the animals' eyes. Our study also accounted for spontaneous changes in the brain state occurring under the urethane anesthesia. We observed the existence of subpopulation of neurons within the VTA/SNc that exhibit suppression of electrical activity in response to the light stimuli. Furthermore, for a subset of these neurons, the responses to light pulses were dependent on the brain state and/or whether the stimulated eye was ipsilateral or contralateral to the recording site. These findings warrant further research to explore the following inquiries: Do the light-induced responses correlate with alterations in dopamine release along the ascending dopaminergic pathways? Could these changes influence motivation and motor control? Additionally, could these findings have implications for animals' decision-making regarding body orientation and movement direction?

PL ISSN 1898-0295

EXPRESSION OF THE URIDINE MONOPHOSPHATE KINASE GENE IN SPRING WHEAT SEEDLINGS DURING DEHYDRATION AND REHYDRATION

AGNIESZKA OSTROWSKA*, TOMASZ HURA

Department of Ecophysiology, The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, ul. Niezapominajek 21, 30-239 Kraków, Poland

**e-mail: a.ostrowska@ifr-pan.edu.pl*

Spring droughts pose a serious challenge to young spring wheat seedlings, as their root system is incompletely developed. Also, a shorter growing season than of winter cereals requires more rapid course of repair processes after the soil drought ceases to limit yield loss. Uridine monophosphate kinase (UMPK) catalyzes the ATP-driven conversion of UMP to a corresponding uridine diphosphate (UDP), which is the first step in pyrimidine metabolism. UDP plays an important role in the synthesis of sucrose and cellulose that are involved in plant growth. The *UMPK* transcript level was measured on the last day of dehydration (4^{th} day) and after 24 h of rehydration, using a q-PCR method. The seedlings were exposed to dehydration at the heterotrophic and autotrophic stages of growth.

Expression of the *UMPK* gene during drought and rehydration revealed similar changes in both tested cultivars. At both developmental stages, a greater increase in the *UMPK* transcript level was noted during rehydration than during drought. While at the heterotrophic stage the increase occurred already during drought, at the autotrophic stage it was only observed during rehydration.

PL ISSN 1898-0295

APTO-253 AND DMAPT EXERT SYNERGISTIC ANTILEUKEMIC EFFECT IN HL-60 CELLS

MONIKA A. PAPIEŻ

Department of Cytobiology, Pharmaceutical Faculty, Jagiellonian University Medical College, Medyczna 9, 30-668 Krakow, Poland

e-mail: monika.papiez@uj.edu.pl

Studies have demonstrated that the Kruppel-like factor (KLF-4) is repressed in most patients with acute myeloid leukemia (AML). Inducing KLF-4 expression in AML cells leads to their differentiation. In turn, the Nuclear Factor kappa B (NFkB) is overexpressed in approximately 40% of patients and confers resistance to treatment.

The aim of the present study was to evaluate the antileukemic effect of the combination of APTO-253 (KLF-4 activator) and dimethylaminoparthenolid (DMAPT, NFkB inhibitor) on the HL-60 leukemia cell line.

HL-60 cells were incubated with various concentrations of APTO-253 and/or DMAPT for 24 hours. KLF-4 and NFkB expression was detected by indirect immunofluorescence. To study the cell cycle, cells were fixed and incubated with RNase A and propidium iodide (PI). Viability was determined using PI staining. The analysis was performed on a flow cytometer.

DMAPT in coincubation with APTO-253 inhibited the cell cycle in the S phase at a concentration of 1 μM in comparison to DMAPT alone and controls. Treatment of the cells with DMAPT or APTO-253 did not lead to cell cycle arrest in S phase. The combination of examined compounds exerted a synergistic cytotoxic effect (Combination Index < 1). DMAPT also enhanced KLF-4 expression induced by APTO-253 but NFkB expression remained unchanged after cotreatment of the cells with DMAPT and APTO-253.

One of the mechanisms of antileukemic effect of DMAPT and APTO-253 combinations in AML cells may be the intensification of KLF-4 expression. The tested compounds are good candidates for further research on combination therapy of promyelocytic leukemia.

PL ISSN 1898-0295

Fatty acid content in serum of pediatric patients with inflammatory BOWEL disease

Lilla Pawlik-Sobecka[1], Joanna Górka-Dynysiewicz[2], Ewa Barg[1], Agata Pietrzycka[3], Tadeusz Librowski[3], Joanna Gdula-Argasińska[3,*]

[1]*Department of Basic Medical Sciences, Wroclaw Medical University, Chałubińskiego 4, 50-368 Wrocław, Poland*

[2] *Department of Pharmaceutical Biochemistry, Wroclaw Medical University, Borowska 211 A, 50-556 Wrocław, Poland*

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

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

Inflammatory bowel disease (IBD) is a group of inflammatory diseases of the digestive tract of unknown cause, the symptoms of which include changes and ulcerations in the intestinal walls and recurrent, chronic diarrhea. These diseases include ulcerative colitis, Crohn's disease, and unspecified colitis. IBD have in common the occurrence of long and exhausting diarrhea that makes normal functioning difficult. Treatment is adapted to the stage of the disease, symptoms, patient's health condition, and age. A complete cure is not possible, so therapy involves pharmacological maintenance of remission and alleviation of symptoms. Although IBD most often affects young people between 20 and 40 years of age, there is an increase in the incidence of IBD in pediatric populations.

In order to gain a deeper understanding of the role of fatty acids (FA) in the pathogenesis of IBD, we analyzed their serum content in pediatric patients with IBD and healthy controls. Serum FA content was analyzed by gas chromatography. The saturated FA index was statistically higher, and total n-3 FA was significantly decreased in the serum of IBD patients compared to the control. The n-3 FA deficit might suggest using these fatty acids as precursors of pro-resolving mediators during inflammation. There are many research methods, but none indicate an apparent cause of inflammatory bowel disease. Therefore, it is important to look for specific markers of IBD progression or remission, as well as the use of appropriate therapy, especially in young patients.

PL ISSN 1898-0295

THE INFLUENCE OF SELECTED STRESS FACTORS ON *CPXR* GENE EXPRESSION IN *PROTEUS MIRABILIS* HI4320

LEON PETRUŃKO*, DAWID GMITER

Department of Microbiology, Institute of Biology, Jan Kochanowski University in Kielce, Uniwersytecka 7 25-406 Kielce

**e-mail: leonpetrunko@gmail.com*

CpxR is a regulatory protein, a part of two-component regulatory system (CpxRA) widely present among pathogenic bacteria. CpxRA appears to play a key role in regulating the virulence potential of several bacteria. *Proteus mirabilis* is a Gram-negative bacterium, responsible for urinary tract infections. In this bacterium, CpxR regulates the expression of the *zapD* gene, which is part of the *zapABCD* operon, involved in the formation of a biofilm that is particularly difficult to eradicate and important for the virulence of *P. mirabilis*. A reduced ability to form a biofilm in mutants with a deletion of *cpxR* gene has already been demonstrated previously. However, the role of *cpxR* in response to stressors has not yet been fully elucidated.

The goal of this study was to evaluate the influence of stress-inducing factors on *cpxR* gene expression in *P. mirabilis* reference strain HI4320. The gene expression measurement was conducted based on isolated total RNA, which was transcribed to cDNA. Gene expression level was monitored using RT-qPCR reaction and ΔΔCt method, with the *rpoA* serving as housekeeping gene.

The obtained results will allow better understanding the significance of the *cpxR* in *P. mirabilis*.

ACKNOWLEDGEMENTS

The presented study was supported by NCN grant No. 2019/33/N/NZ6/02406 (D.G.).

PL ISSN 1898-0295

THE IMPACT OF EXTRACELLULAR VESICLES SECRETED BY FIBROBLASTS ON MIGRATION OF CANCER CELLS

JULIA PIEKARSKA*, AGNIESZKA PASTUŁA

Department of Genetics and Evolutionism, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland

**e-mail: j.julia.aleksandra.piekarska@student.uj.edu.pl*

Extracellular vesicles (EV) are microscopic vesicles that cells release into their surroundings. EV play an important role in the communication within the tumor microenvironment, and studies have shown their involvement in enhancing cancer cell proliferation and invasiveness. Some fibroblasts reside in close proximity to cancer cells, thus, their interactions with tumor cells are studied. In their physiological state, fibroblasts are quiescent, with reduced secretion and proliferation. They become active in wound healing, producing growth factors and cytokines, and then return to their resting state. Cancer-associated fibroblasts (CAFs) are perpetually activated and can influence the migration of cancer cells as well as their proliferation. Studies have demonstrated that even quiescent fibroblasts can impact cancer cells, exerting inhibitory effects on primary tumors while promoting their growth in more advanced stages. The aim of this study was to elucidate the impact of EV derived from fibroblasts on the migration of LNCaP cancer cell line. We hypothesized that fibroblast-derived EV promote migration of prostate cancer cells. To test this hypothesis, EV were isolated from human skin fibroblasts and incubated with metastatic LNCaP cells in the *in vitro* wound healing assay. While the differences in cancer cell migration between the control group without EV and the group exposed to EV can be observed, the statistical analysis did not yield statistically significant results. Further research would be beneficial to test our hyposthesis. Better understanding of the microenvironmental mechanisms of tumor progression can lead to identification of novel therapeutic options for cancer patients.

ACKNOWLEDGEMENTS

The work resulted from the implementation of research project No. 2020/39/D/NZ5/03552 funded by the National Science Centre.

PL ISSN 1898-0295

HEXARELIN POTENTIATED THE MET-ENKEPHALIN SYSTEM RESPONSE TO EMOTIONAL STRESS IN LAMBS

KRYSTYNA PIERZCHAŁA-KOZIEC[1,*], COLIN G. SCANES [2]

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

[2]*Center of Excellence in Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA*

**e-mail: rzkoziec@cyf-kr.edu.pl*

Stress is the leading cause of many diseases (cardiovascular, gastrointestinal, diabetes, nervous disturbances) in most developed countries. Endogenous opioid peptides (EOP), mainly Met-enkephalin, mitigate up-regulation of the hypothalamo-pituitary-adrenal axis during stress response and decrease the risk of serious diseases. Hexarelin is a synthetic analog of Met-enkephalin, mainly researched for its effect on growth hormone (GH) release in human and rodent model. The present study was conducted to assess the effects of isolation stress and/or hexarelin administration on multiple Met-enkephalin related parameters in novel animal model - three month-old lambs. Four groups were constructed: control; i.v. injected with hexarelin; stressed by isolation from the herd for 60 min, and treated with hexarelin + stress. Blood and hypothalamus were taken for cortisol and Met-enkephalin profiles, expression of proenkephalin (PENK) gene, concentration of Met-enkephalin, *in vitro* Met-enkephalin secretion, opioid receptors binding. The results showed significant effect of stress on all tested parameters; hexarelin alone decreased the cortisol level and Met-enkephalin synthesis, release and receptor binding in the hypothalamus. Hexarelin given before stress potentiated the opioid parameters responses to isolation. The obtained results showed that hexarelin interacts with Met-enkephalin modulating the stress response at the central and peripheral level in growing lambs. It may be suggested that hexarelin is important factor also during stress responses but research on its effects should be conducted simultaneously with testing opioids profile.

ACKNOWLEDGEMENTS

The study was supported by the sub.MES to the University of Agriculture in Krakow, Poland (sub. No 020002-D015, 2022).

PL ISSN 1898-0295

METFORMIN THERAPY, TRAIL AND SELECTED MARKERS OF VASCULAR ENDOTHELIAL DAMAGE IN DIABETES

AGATA PIETRZYCKA [1,*], JOANNA GDULA-ARGASIŃSKA [1], PRZEMYSŁAW MIARKA [2], KATARZYNA KRZANOWSKA [2]

[1] *Department of Radioligands, Pharmacobiology Department, Faculty of Pharmacy, Collegium Medicum of the Jagiellonian University, Medyczna 9, 30-668 Krakow, Poland*

[2] *Department of Nephrology and Transplantology, Faculty of Medicine, Collegium Medicum of the Jagiellonian University, M. Jakubowskiego 2, 30-688 Krakow, Poland*

**e-mail: agata.pietrzycka@uj.edu.pl*

The tumor necrosis factor-related apoptosis-inducing ligand-TRAIL, may play a key role in the progression of diabetic vascular complications. It can directly affect vascular endothelial smooth muscle cells, stimulate endothelial cell apoptosis, smooth muscle proliferation and migration, and inflammatory reactions. *In vitro* experiments indicate that TRAIL reduces hyperglycemia and significantly improves the peripheral response to insulin. Hyperglycemia may contribute to the pancreatic beta cells destruction by increasing the expression of membrane TRAIL receptors. Non-mitochondrial reactive oxygen species and the nuclear factor NFκB are essential for hyperglycemia-induced TRAIL regulation. It was reported that metformin has anti-inflammatory and antioxidant properties. It reduces advanced protein oxidation products (AOPPs) concentration. Metformin therapy reduces hyperglycemia-enhanced TRAIL expression and protects cells from TRAIL-mediated apoptosis.

The aim of this study was to compare TRAIL and AOPPs concentrations in plasma, in a group of diabetic patients with vascular complications treated with metformin or combined therapy of metformin and oral antidiabetic drugs (OADDs).

Possible correlations with the hyperglycemia or myocardial infarction incidence, fibrinogen and creatinine levels were also assessed.

The lowest TRAIL concentration and significantly higher AOPPs concentration were observed in patients with diabetes and vascular complications. It was found that in subjects taking pharmacotherapy with metformin or metformin combined with other OADDs, the plasma TRAIL concentration was significantly higher than in the control group.

PL ISSN 1898-0295

RECOVERY OF MOUSE PUPS FROM ANEMIA AFTER MATERNAL IRON DEFICIENCY DURING PREGNANCY: A TIME-COURSE STUDY

NATALIA PIRGA[1], WIKTORIA PŁONKA[1], MAGDALENA OGŁUSZKA[2], RAFAŁ R. STARZYŃSKI[2], XIU WANG[2], ZUZANNA KOPEĆ[2], SYLWIA HERMAN[1], MAŁGORZATA LENARTOWICZ[1,*], PAWEŁ LIPIŃSKI[2]

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

[2]*Department of Molecular Biology, Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Jastrzebiec, Postepu 36A, 05-552 Magdalenka, Poland*

**e-mail: malgorzata.lenartowicz@uj.edu.pl*

In humans, pregnancy is a physiological condition frequently associated with iron deficiency (ID). The causes of ID are high physiological iron demand and its insufficient supply due to low preconception maternal iron reserves and inadequate dietary intake. Here, using a mouse model of ID in pregnancy, we attempted to determine in a time-course study covering 11 postnatal days, molecular mechanism of postnatal recovery of mouse pups after maternal ID. Females fed low iron diet 2 weeks prior to mating and maintained on this diet throughout pregnancy manifested symptoms of ID anemia compared to healthy females given iron-replete diet. Switching of anemic mothers to a replete-iron iron diet just after delivery resulted in a progressive recovery of their progeny from ID. At postnatal day 11, RBC indices and iron plasma parameters of pups born to anemic mothers were close to those of pups born to iron-replete females. Rebuilding of RBC status in pups after gestational ID was due to an increased rate of erythropoiesis as attested by high reticulocyte count/percentage. Higher hepatic ferroportin protein level in the offspring of anemic mothers strongly suggests that the liver despite decreased iron content still remains an efficient source of iron for satisfying erythropoietic demand. On the other hand, decreased hepatic transferrin receptor 1 protein level in pups after gestational ID point to impaired iron uptake for rebuilding hepatic iron stores. We propose that relatively quick normalization of RBC status in pups born to anemic mothers is due to the efficient mobilization of iron from endogenous sources.

PL ISSN 1898-0295

Effect of H_2O_2 on sulfurtransferases activity, thioredoxin system and proliferation of human neuroblastoma cells

Leszek Rydz[1,*], Halina Jurkowska[1], Maria Wróbel[1], Ewa Jasek-Gajda[2]

[1]*Jagiellonian University, Medical College, Faculty of Medicine, Chair of Medical Biochemistry, Kopernika 7 St., 31-034 Kraków, Poland*

[2]*Jagiellonian University, Medical College, Faculty of Medicine, Department of Histology, Kopernika 7 St., 31-034 Kraków, Poland*

**e-mail: leszek.rydz@uj.edu.pl*

Sulfurtransferases - 3-mercaptopyruvate sulfurtransferase (MPST) and thiosulfate sulfurtransferase (TST) are involved in L-cysteine desulfuration – in the formation and/or transfer of sulfane sulfur atoms in persulfides, polysulfides, thiosulfate, and H_2S production. These enzymes participate also in cyanide detoxification, and iron-sulfur clusters formation (a components of mitochondrial respiratory chain). The catalytic site cysteinyl residue (Cys^{247}) of MPST and TST is redox sensitive. The reduced, active form of MPST is maintained by the thioredoxin system (which include thioredoxin, NADPH and thioredoxin reductase).

We studied the effect of hydrogen peroxide (H_2O_2) on MPST activity (modified method of Valentine and Frankenfeld), TST activity (modified Sörbo method), reactive oxygen species (ROS) production (flow cytometry and fluorescence microscopy analysis), thioredoxin expression (RT-PCR), and proliferation (crystal violet method) of human neuroblastoma SH-SY5Y cells.

The cells were culture with different concentrations of H_2O_2 during 12, 24, and 48 hours. We found that proliferation of H_2O_2-treated cells was decreased during these culture conditions. The inhibition of cell proliferation was associated with increased ROS production (including the increased level of superoxide anion determined by flow cytometry) at higher concentrations of H_2O_2. Simultaneously, the activity of MPST and TST was decreased; additionally, the expression of thioredoxin was increased.

In the presence of H_2O_2, a catalytic site Cys^{247} of mitochondrial MPST and TST is oxidized- the cysteine-sulfenate is formed (Cys^{247}-S^- Cys^{247}-SO^-), and consequently the sulfurtransferases activity is inhibited. It seems that H_2O_2 damaged mitochondria leading also to the generation of superoxide anion, which can result in impaired functioning of the respiratory chain and inhibition of cell proliferation.

PL ISSN 1898-0295

ANALYSIS OF CHANGES IN THE EXPRESSION OF YAPSIN (YPS) GENES IN *CANDIDA GLABRATA* UNDER VARIOUS CULTURE CONDITIONS

DOROTA SATAŁA*, AGNIESZKA KABUT, MARIA RĄPAŁA-KOZIK

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

**e-mail: dorota.satala@uj.edu.pl*

Candida glabrata is the second, after *C. albicans*, the major causative agent of systemic fungal infections in humans. Its pathogenic nature results from the ability to produce many virulence factors, one of the most important of which are the *YPS* genes (*YPS1-YPS11*) encoding extracellular glycosylphosphatidylinositol-linked aspartyl proteases. So far, the role of *YPS* has been poorly characterized, and a few reports suggest their participation in maintaining cell wall integrity and glucose homeostasis.

The aim of the study was to analyze the level of *YPS* genes expression after culturing fungal cells in various culture media – rich YPD, YPDA with reduced nitrogen, RPMI1640 typically used for inducing pseudohyphal growth of *Candida* species, and an amino acid-based synthetic medium – as well as after contact with neutrophils which are the major effector cells recruited to the inflammation sites during fungal infections.

In response to changing external conditions, significant overexpression was noticed for *YPS* genes located in a cluster unique to *C. glabrata*, especially after overnight culture in RPMI1640 medium (*YPS4*, *YPS6*, *YPS9* and *YPS11*) and after 4 hours of culture in YPDA medium (*YPS6*, *YPS7*, *YPS10*, *YPS11*). Contact with neutrophils was manifested by increased *YPS1*, *YPS3*, *YPS5*, and *YPS11* expression and decreased *YPS2* expression.

Our finding that *C. glabrata YPS* genes undergo significant changes in the response to external environment conditions provides new targets for further research at the protein and cellular levels.

ACKNOWLEDGEMENTS

This work was financially supported by the National Science Centre, Poland (grant number 2020/39/D/NZ6/00854 awarded to D.S.).

PL ISSN 1898-0295

SYNERGISTIC INTERACTION BETWEEN INTERFERON-Γ AND PROINFLAMMATORY CYTOKINES IN GINGIVAL FIBROBLASTS AS A POSSIBLE MECHANISM UNDERLYING RUNAWAY INFLAMMATION IN PERIODONTITIS

AURELIUSZ SCHUSTER*, ALEKSANDER M. GRABIEC

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

**e-mail: aureliusz.schuster@doctoral.uj.edu.pl*

Periodontitis is a chronic inflammatory disease of the gum, driven by dysbiotic oralome. Gingival fibroblasts (GFs) participate in developing inflammation by producing proinflammatory mediators in response to bacteria and inflammatory environment. Interferon-gamma (IFNγ) causes synergetic amplification of this response by GFs infected with the oral pathogen *Fusobacterium nucleatum* (*Fn*), which is mediated through the production of secondary inflammatory mediators.

Here, we analyzed the interplay between different inflammatory mediators and IFNγ, elevated levels of which are detected in gingival cervical fluid in patients with periodontitis, on GF inflammatory responses.

Using RNA-seq. we found amplified expression of components of the Jak-STAT pathway and important proinflammatory cytokines TNFα and IL-1α among others in GFs stimulated with IFNγ in the presence of *Fn*. These transcriptional effects translated into a significant amplification of IFNγ-induced CXCL9 and CXCL10 production by GFs infected with *Fn*. TNFα secretion was confirmed through TNFα-R silencing, resulting in reduced GF response to IFNγ/*Fn* stimulation. Next, qPCR analysis revealed synergetic amplification of CXCL9, CXCL10 and CXCL11 expression and/or production in GFs exposed to IFNγ and TNFα or IL-1α, while western blot analysis identified synergetic increase in STAT1/3/5 phosphorylation and increased p38 activation. The obtained results show preserved synergetic effect when replacing *Fn* with TNFα or IL-1α when stimulating GFs with IFNγ.

These results show that TNFα and IL-1α synergize with IFNγ to amplify GF activation in a similar manner as the presence of *Fn*, identifying specific components of the host innate immune response responsible for uncontrolled inflammation in the development of periodontitis.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Science Centre, Poland to AMG (2019/35/B/NZ5/01823).

PL ISSN 1898-0295

BALBIANI BODY OF BASALLY BRANCHED INSECTS: A GERM-LINE-SPECIFIC COMPLEX OF ORGANELLES LINKED TO THE SELECTION OF MITOCHONDRIA

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 in Krakow, Gronostajowa 9, 30-387 Krakow, Poland

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

Balbiani body (Bb) is a transient, non-membrane bound complex of organelles, positioned at one side of the oocyte nucleus. It consists mostly of mitochondria and aggregates of nuage material but also Golgi complexes, cisternae and/or vesicles of endoplasmic reticulum and even cytoskeletal fibers. Previous studies have shown that the Bb performs disparate functions in various animal taxa; it may be implicated in the delivery of localized mRNAs and organelles (mitochondria, germinal granules) to the vegetal cortex of the oocyte, lipidogenesis or preferential transmission of wild-type mitochondria to the offspring. To gain further insight into the last possibility we performed a series of ultrastructural and histochemical studies using developing oocytes of a bush cricket, *Meconema meridionale*. We show that mitochondria within the Bb are interconnected and form an intricate mitochondrial network. As oogenesis progresses, this network undergoes fragmentation into individual, bean-shaped mitochondria. At least some of the mitochondrial divisions involve lysosomes as well as ATG proteins, and lead to the elimination of defective mitochondria. Other, involve microfilaments and a highly conserved dynamin-related protein 1 (Drp1) and result in multiplication of mitochondria. Our results provide evidences for the involvement of the Bb in multiplication and selection of mitochondria in female germline cells. Interpretation of the obtained data in a phylogenetic context indicates that the transmission of "healthy" mitochondria to the next generation represents an ancestral function of the Bb. Other, previously proposed functions of this organelle assemblage seem to be evolutionary derived.

ACKNOWLEDGEMENTS

The study was supported by NCN grant No. 2021/41/N/NZ3/00360.

PL ISSN 1898-0295

"Next generation" bisphenols modulate proliferation and decrease glucose uptake in human ovarian granulosa cells

Joanna Smoleniec[1,*], Paulina Głód[1,2], Anna Ptak[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Faculty of Biology, Institute of Zoology and Biomedical Research, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

**e-mail: joanna.smoleniec@student.uj.edu.pl*

Bisphenol A (BPA) is an endocrine disrupting chemical that has been used as a component in polycarbonate plastics and was detected in human serum. BPA has been proven to impair fertility and cause numerous negative health effects. Due to its toxicity, it is gradually being replaced by presumably safer analogues known as "next generation" bisphenols that have been detected in human serum. It is known that the ovary is the target tissue for these chemicals, however the effect of "next generation" bisphenols on human ovary is still uncertain. In the present study, our aim was to determine how these bisphenols affect viability, glucose uptake, and mitochondrial activity in human ovarian granulosa cells (HGrC1).

Cells were cultured on 96-well plates and were exposed to each bisphenol (BPAF, BPAP, BPB, BPP, BPZ) in doses 0.1, 1, 10 nM for 24 and 48 hours. Viability was measured with PrestoBlue HS Cell Viability Reagent and glucose uptake was measured with Glucose Uptake-Glo Assay. Mitochondrial activity was obtained with JC-1 dye and mitochondrial structure with MitoTracker Red dye.

In this study we have found that nearly all tested bisphenols increased viability of HGrC1 cells (0.1 nM, 1 nM, 10 nM), with BPB and BPZ having the strongest influence. Furthermore, BPB and BPZ (1 nM, 10 nM) decreased glucose uptake, however mitochondrial activity and structure remained unchanged (10 nM).

In conclusion, "next generation" bisphenols can alter the functioning of granulosa cells by increasing proliferation and decreasing glucose uptake. However, further studies are needed to determine the mechanism through which bisphenols affect granulosa cells.

ACKNOWLEDGEMENTS

The research has been supported by grant N18/DBS/000008 from the Faculty of Biology Jagiellonian University in Krakow, Poland.

PL ISSN 1898-0295

MORPHOGENESIS OF THE BALBIANI BODY IN DEVELOPING OOCYTES OF *PHANEROPTERA FALCATA* (PODA, 1761)

MAGDA SOCHACZEWSKA[1,2,*], WACŁAW TWORZYDŁO[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

**e-mail: magda.sochaczewska@doctoral.uj.edu.pl*

Early oocytes of almost all animal species are asymmetric. This asymmetry is strongly related to the structure found in the close vicinity to the nucleus of the oocyte, termed the Balbiani body. The latter structure is a complex composed of diverse organelles that are not membrane-limited. It always contains numerous mitochondria and electron-dense material, called the nuage. Comparative studies have shown that even in closely related species – the Balbiani body has various composition, shape and location within the oocyte.

Here, we analyzed the morphology and ultrastructure of the Balbiani body in the long horn bush cricket *Phaneroptera falcata*, a member of the orthopteran family, the Tettigoniidae.

The results indicate that the Balbiani body in the studied species is composed of typical organelles characteristic for this structure that are unevenly distributed around almost the entire surface of the oocyte nucleus. It is important to note that in *Ph. falcata*, the Balbiani body does not disperse very quickly, but remains in the oocytes until the late stages of oogenesis. We use the term "late Balbiani body" to highlight the contrast to other species where the Balbiani body is a short-lasting feature.

We interpreted our results in the comparative context. We suggest that the Balbiani body and ensuing oocyte asymmetry are very stable and evolutionarily conservative features.

PL ISSN 1898-0295

THE ROLE OF BRASSINOSTEROIDS IN THE COLD ACCLIMATION AND DEACCLIMATION OF OILSEED RAPE

JULIA STACHURSKA[1,*], IWONA SADURA[1], MAGDALENA RYS[1], BARBARA JURCZYK[2], JANA OKLESTKOVA[3], ANNA JANECZKO[1]

[1]*Department of Developmental Biology, The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland*

[2]*Department of Plant Physiology, Faculty of Agriculture and Economics, University of Agriculture in Kraków Al. Mickiewicza 21, 31-120 Kraków, Poland*

[3]*Laboratory of Growth Regulators, Faculty of Science, Institute of Experimental Botany of the Czech Academy of Sciences, Olomouc, Czech Republic*

**e-mail: j.stachurska@ifr-pan.edu.pl*

Nowadays, more frequent episodes of higher temperatures in autumn and winter occur due to climate change that may interrupt the cold acclimation of winter plants. This leads to a deacclimation process and may be connected to a decrease in plants' frost tolerance. Relatively little is known about the physiological/biochemical basics of deacclimation, especially in the aspect of hormonal management. The aim of the research was to characterize the cold acclimation-induced and deacclimation-induced changes in brassinosteroid biosynthesis and expression of brassinosteroid receptor *BRI1* in order to verify the potential role of these hormones in the changes of freezing tolerance of oilseed rape. Samples of leaves were collected from oilseed rape (*Brassica napus* L.) plants non-acclimated (three weeks of growth at 17°C) cold-acclimated (4°C d/n, three weeks), and deacclimated (16/9°C d/n, one week). The expression of *BRI1* gene that encodes brassinosteroid receptor protein (BRI1) was lowered after cold acclimation and after deacclimation, especially in more frost tolerant cultivar. Simultaneously the accumulation of BRI1 protein decreased in cold acclimated plants, but it increased after deacclimation. Deacclimation resulted in a slight increase in the content of active brassinosteroid (growth-promoting brassinolide, ligand of receptor BRI1) which together with an increase of accumulation of BRI1 may be one of many factors involved in deacclimation-induced decrease of frost tolerance in oilseed rape.

ACKNOWLEDGEMENTS

Studies were funded by the National Science Center (2019/35/B/NZ9/02868).

PL ISSN 1898-0295

CINDERELLA AMONG CELLULAR BLOOD COMPONENTS: SCRUTINIZING INVOLVEMENT OF RED BLOOD CELLS IN IMMUNE RESPONSES

ANNA SUCH[1,2,*], ELZBIETA KOLACZKOWSKA[1]

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

[2]*Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

**e-mail: anna.such@doctoral.uj.edu.pl*

Red blood cell (RBC) biology is undergoing a quiet revolution as RBCs are emerging as essential regulators of the innate immune response. *Ex vivo* RBCs were shown to impact neutrophil effector functions during sepsis but these data is yet to be confirmed *in vivo*. Therefore, the aim of the study was to investigate neutrophil-RBC interplay *in vivo* at different stages of endotoxemia, and furthermore, to study effects of their co-incubation *ex vivo*. *In vivo* RBCs were visualized with intravital microscopy (IVM) in the vasculature of live mice and then detailed mechanisms of these interactions were studied on isolated cells. Moreover, we applied phenylhydrazine-induced anemia model in order to test whether partial RBC depletion affects the course of sepsis. We report that *ex vivo* murine RBCs increased neutrophil viability and release of IL-1beta but diminished lipopolysaccharide-induced Siglec E-dependent release of neutrophil extracellular traps (NETs). *In vivo* RBC-leukocyte interactions were clearly observed in the liver vasculature during sepsis (leading to either phagocytosis of RBCs or were transient in nature) and they outnumbered those observed in healthy animals. As a results of RBC depletion, longer neutrophil-RBC interactions, down-regulation of NET formation and enhancement of phagocytosis were detected. The studies confirm the modulatory effects of RBCs on functioning of murine neutrophils and strongly indicate an interplay between the two cell types during sepsis, but further studies are required to verify if this crosstalk is beneficial or pathological for the sepsis outcome.

ACKNOWLEDGEMENTS

The study was supported by National Science Centre of Poland, OPUS 22, 2021/43/B/NZ6/00782.

PL ISSN 1898-0295

New aspects of medulla dopaminergic cells in the *Drosophila melanogaster* brain

Kornel Szypulski*, Milena Damulewicz

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

**e-mail: kornel.szypulski@student.uj.edu.pl*

Drosophila melanogaster brain is composed of almost 200 000 neurons, half of which are found in the optic lobes. These neurons are known to be involved in many different processes such as vision and circadian rhythmicity. In our study we focused on the medulla dopaminergic cells (MDC), which precise function is still unknown. We discovered that some of them express vesicular glutamate transporter – marker protein of glutamatergic neurons. Using cell-specific GFP expression and immunostaining we showed that there are no other cell populations in the brain which are both dopaminergic and glutamatergic in their nature. To understand the role of MDC in the visual system we took advantage of *trans*Tango genetic transsynaptic tracing method, which enabled us to visualize synaptic partners of MDC. Together with further research this may shed light on their function in *Drosophila* nervous system. In humans neurons producing both dopamine and glutamate seem to be less prone to neurodegeneration during Parkinson's disease progression. Existence of similar cells in fruit fly's nervous system provides a promising perspective for the new approach in research on the mechanisms of neurodegeneration and neuroprotection in neurological disorders.

PL ISSN 1898-0295

IN VIVO EFFECT OF VITAMIN D_3 ON HORMONAL AND REPRODUCTIVE PARAMETERS IN PCOS RAT MODEL

WIKTORIA SZYRZISKO[1,*], KINGA KAMIŃSKA[1], AGATA SZLAGA[2], ANNA BŁASIAK[2], AGNIESZKA RAK[3], MAŁGORZATA GRZESIAK[1]

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

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

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

**e-mail: wiktoria.szyrzisko@student.uj.edu.pl*

Polycystic ovary syndrome (PCOS) is a common endocrinopathy in women of reproductive age characterized by reproductive, hormonal and metabolic disturbances. Recent reports indicate that vitamin D_3 (VD) could be an alternative therapy in the PCOS treatment. Furthermore, its deficiency has been observed in many women suffering from PCOS. This study aimed to investigate the effect of VD supplementation on hormonal and reproductive parameters in PCOS rats. Animals were divided into 4 groups (n = 8/each): control (C), supplemented with VD (VD), with letrozole-induced PCOS (L), and PCOS-induced treated with VD (VD+L). Within the experiment, the estrous cycle was monitored by microscopic analysis of vaginal smears. After 21 consecutive days, blood was collected for estradiol and testosterone levels assessment, whereas ovaries and periovarian adipose tissue (POAT) were fixed for histological analysis. PCOS rats had high testosterone and low estradiol levels and were acyclic. In the VD+L group, we observed a decrease in testosterone level compared to the L group, however estradiol concentration did not increase. In the VD and VD+L groups, estrous cycles were shorter and more frequent. Histologically, the L and VD+L groups were characterized by the presence of numerous ovarian cysts. However, in the VD+L group single antral follicles were also found. The size of adipocytes in POAT was the greatest in the L groups in comparison to others. Obtained results suggest a positive effect of vitamin D_3 on the tested parameters in PCOS and its effectiveness in the PCOS treatment.

ACKNOWLEDGEMENTS

Funded by the Priority Research Area BioS (U1U/P03/NO/03.09 to MG).

PL ISSN 1898-0295

MiR6996-3p regulates the activity of *Pxt1* gene

Igor Tomczyk[1], Mikołaj Rokicki[1], Wioleta Sieńko[1], Katarzyna Rożek[2], Anna Nalepa[3], Jasmin Wiench[1], 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 Plant Ecology, Institute of Botany, Jagiellonian University, Gronostajowa 3, 30-387 Krakow, Poland*

[3]*Department of Chemical Technology and Environmental Analytics, Cracow University of Technology, Warszawska 24, 31-155, Krakow, Poland*

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

Mouse *Pxt1* (*Peroxisomal, testis specific 1*) encodes for a protein containing functional BH-3 like domain and induces cell death. This gene is expressed exclusively in male germ cells and the it's targeted deletion resulted in increased proportion of sperm with enhanced DNA strand breaks. We concluded that the function of *Pxt1* is the elimination of male germ cells with damaged chromatin. Previous study demonstrated that the pro-apoptotic activity of PXT1 protein is prevented by BAG6, an anti-apoptotic protein that binds PXT1. However we ask the question whether it is the only mechanism regulating *Pxt1* activity. Here we identified that 3'UTR of *Pxt1* transcript is a target for miR6996-3p. Using the luciferase reporter assay we demonstrated that miR6996-3p binds to *Pxt1* mRNA. For the first time we demonstrated that in adult mouse testis the PXT1 protein is present at very low abundance. Then, using a site directed mutagenesis and the miR6996-3p inhibitor we confirmed that the interaction of this miRNA with *Pxt1* mRNA suppresses *Pxt1* translation to a significant, although not complete extent.

ACKNOWLEDGEMENTS

This work was supported by polish National Science Centre grant nr 2015/19/B/NZ4/00576 (PG).

PL ISSN 1898-0295

S-palmitoylation of DGKε in the endoplasmic reticulum and in the Golgi apparatus inhibits DGKε activity

Gabriela Traczyk*, Aneta Hromada-Judycka,
Anna Ciesielska, Anna Świątkowska, Katarzyna Kwiatkowska

Laboratory of Molecular Membrane Biology, Nencki Institute of Experimental Biology PAS, Ludwika Pasteura 3, 02-093 Warsaw, Poland

**e-mail: g.traczyk@nencki.edu.pl*

Diacylglycerol kinase-ε (DGKε) catalyzes phosphorylation of diacylglycerol to phosphatidic acid with a unique specificity toward 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG) which is a backbone of phosphatidylinositol (PI). DGKε plays a crucial role in maintaining the cellular level of phosphorylated PI derivatives of signaling activity, and in lipid metabolism. DGKε dysfunction is linked with the development of several human diseases, including kidney diseases and obesity. Despite the DGKε significance, data on the regulation of its functioning by possible co/post-translational modifications are scarce. We cloned the *Dgke* gene from mouse J774 macrophage-like cells, introduced the Myc tag at the C-terminus of the kinase and ectopically expressed DGKε-Myc in HEK293 cells. With this approach, we found that DGKε is *S*-palmitoylated at Cys38 located at the cytoplasmic end of its N-terminal transmembrane fragment. The *S*-palmitoylation of DGKε was revealed by metabolic labeling of cells with a palmitic acid analogue followed by click chemistry allowing detection of acylated DGKε with a fluorescent dye. Furthermore, palmitoyl *S*-acyltransferases zDHHC7, 17 and 6/16 were found to catalyze DGKε *S*-palmitoylation. Additionally, zDHHC17 co-immunoprecipitated with DGK-Myc regardless of the kinase *S*-palmitoylation. Our confocal microscopy observations showed that DGKε-Myc is localized in the endoplasmic reticulum where zDHHC6 resides, and in the Golgi apparatus where zDHHC7 and zDHHC17 are found. Co-expression of DGKε with zDHHC17 inhibited while the Cys38Ala substitution upregulated the phosphorylation of SAG by DGKε, revealing an inhibitory effect of the acylation on the DGKε activity. Taken together, our data indicate that *S*-palmitoylation fine-tunes DGKε activity and can affect its cellular localization.

PL ISSN 1898-0295

THE IMPACT OF KETOGENIC DIET ON THE BONE REGENERATIVE POTENTIAL OF BONE MARROW CELLS

KAROLINA TRUCHAN*, ANNA MARIA OSYCZKA

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

**e-mail: karolina.truchan@doctoral.uj.edu.pl*

The ketogenic diet (KD) is a high-fat, moderate-protein, and low-carbohydrate diet. Initially recommended for drug-resistant epilepsy, KD is now widely used for diabetes and obesity, which are associated with skeletal disorders. However, the impact of KD on bone marrow osteoprogenitors remains largely unexplored. We have examined the effects of KD, implemented during pregnancy and lactation of Wistar rat females, on the osteogenic potential of bone marrow cells (BM) of 30-day-old Wistar rat offspring. In female offspring, KD supported osteogenesis in BM cells, but reduced their multipotency. In contrast, in male offspring, KD downregulated osteogenesis, enhanced cell multipotency, and increased mRNA expression of osteoclast-specific enzymes. When normal adult rat BM cell cultures were treated with sodium beta-hydroxybutyrate (BHB), the primary ketone body produced during KD, we observed that treatment with BHB stimulated osteogenesis and inhibited the expression of mRNA of proinflammatory and osteoclast markers. Remarkably, BHB treatment led to increased extracellular matrix mineralization compared to untreated cultures. Overall, our findings demonstrate the effect of KD on BM cells of both adult and newborn rats and the osteogenic role of BHB in cultures of adult BM cells. Our results suggest sex differences in the response of BM cells of the offspring to KD. Given the existing literature controversies regarding the effects of KD on skeletal tissues, the current study should prompt further research on the potential role of KD and specific ketone bodies on bone marrow osteoprogenitor cells and their bone regenerative potential.

ACKNOWLEDGEMENTS

The study was supported by ID.UJ Research Support Module.

PL ISSN 1898-0295

Potential anticancer activity of fexofenadine against A549 cells

Ewa Trybus*, Wojciech Trybus, Teodora Król

Department of Medical Biology, Jan Kochanowski University of Kielce, Uniwersytecka 7, 25-406 Kielce, Poland

**e-mail: ewa.trybus@ujk.edu.pl*

Fexofenadine is a third-generation selective histamine H1 receptor antagonist. Due to its anti-inflammatory properties, it is used in anti-allergic treatment. Because cancer, like allergy, may result from chronic inflammation, and histamine itself is involved in the carcinogenesis process, these types of drugs are an appropriate starting point in the search for new anticancer therapies. The aim of the present study was to demonstrate the effect of fexofenadine on lung cancer cells. The study was conducted on the A549 line, which was treated with fexofenadine at concentrations of 90-400 μM for 48 h. Cells were cultured on DMEM medium. In order to demonstrate the cytotoxic effect of the compound on the above-mentioned cells, MTT assay, annexin V assay, ROS level assay, and H&E staining were used. As a result of fexofenadine, a progressive with concentration reduction in viability of cells was observed. Morphological study showed increased vacuolization and induction of apoptosis, which were confirmed also by flow cytometry. The analyze clearly show that the greatest number of cells with a late apoptotic phenotype occur at 400 μM. At the same time, it was shown that the tested compound generates concentration dependent reactive oxygen species, the highest level of which correlated with the level of apoptotic cells.

The conducted research shows that fexofenadine has a cytotoxic effect on A549 cells by reducing viability, inducing oxidative stress and a pro-apoptotic effect. The demonstrated new properties of this compound may find practical application in anticancer therapy in the future, which requires further research.

PL ISSN 1898-0295

POTENTIAL ANTICANCER EFFECT OF QUINALIZARIN AGAINST PROSTATE CANCER CELLS

WOJCIECH TRYBUS*, EWA TRYBUS, TEODORA KRÓL

Department of Medical Biology, Jan Kochanowski University of Kielce, Uniwersytecka 7, 25-406 Kielce, Poland

**e-mail: wojciech.trybus@ujk.edu.pl*

Cancer is a serious health problem for society, and prostate cancer ranks high in terms of morbidity and mortality. One of the reasons for the failure of anticancer therapy is drug resistance, which requires a constant search for new compounds with anticancer potential. An example is quinalizarin, which belongs to the group of anthraquinones. Since this compound has numerous pharmacological properties, including anticancer potential, it seemed advisable to analyze its effect on prostate cancer cells. For this purpose, DU145 line cells were cultured in DMEM medium supplemented with 10% fetal serum and a mixture of antibiotics.

After 48 hours of exposure to quinalizarin at concentrations of 25-100 μM, the cells were subjected to both biochemical and morphological tests. Based on the MTT test, it was found that quinalizarin had cytotoxic effects on the tested cancer cells, significantly inhibiting their viability, which progressed with increasing concentration. Morphological analysis (DAPI staining) showed nuclear changes typical of the apoptosis process, such as chromatin condensation and fragmentation of the cell nucleus. They were confirmed by the annexin V test, which showed a highly statistically significant increase in cells with a late apoptotic phenotype (the highest at a concentration of 100 μM). The cytometric method also showed increased permeability of mitochondrial membranes and the generation of reactive oxygen species, which indicates that quinalizarin intensifies oxidative stress in the tested cells.

Summarizing, quinalizarin is an anthraquinone that has a potential anticancer effect based on the induction of apoptosis and pro-oxidant activity, which depended on the concentration used.

PL ISSN 1898-0295

THE TESTED [60]C FULLERENES DO NOT SHOW PHYTOTOXICITY TO SELECTED PLANT SPECIES IN TISSUE CULTURE CONDITIONS

MONIKA TULEJA[1,*], MONIKA BOJKO[2], GRZEGORZ GÓRALSKI[1], EMILIA GULA[3], MAGDALENA KĘDRA[2], BEATA MYŚLIWA-KURDZIEL[2], MARTA LIBIK-KONIECZNY[3], MATEUSZ MALISZ[1], DIANA SAJA-GARBARZ[3], MACIEJ SERDA[4], IGA SŁOMCZYŃSKA[1]

[1]*Department of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland*

[2]*Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland*

[3]*The F. Górski Institute of Plant Physiology PAN, Niezapominajek 21, 30-239 Kraków, Poland*

[4]*Faculty of Science and Technology, University of Silesia, Szkolna 9, 40-006 Katowice, Poland*

**e-mail: monika.tuleja@uj.edu.pl*

Fullerenes gained significant attention due to their unique properties and beneficial applications in biotechnology, medicine, and agriculture. However, despite the growing commercial application of carbon nanomaterials, their influence on organisms is still difficult to predict. Therefore, it is important to precisely determine the nature of their impact on plant tissues. The aim of the study was to investigate the effect of fullerenes on monocotyledons (*Lilium martagon*), dicotyledonous (*Arabidopsis thaliana*), and medicinal *Euphorbia milii* in *in vitro* conditions. It was found that in three species studied, the presence of fullerenes increased mitotic activity and accumulation of starch and protein grains, stimulated the growth of callus mass in the initial stages of their cultures, and extended the lifetime of the advanced *A. thaliana* culture. The tested fullerenes concentrations did not change the nature of induced morphogenesis, additionally resulted in the preservation of the original mesophyll structure pattern in advanced *in vitro* culture, although slightly decreased regenerative efficiency (10 roots/explant while with nanoparticles 6.2) in spurge. Fullerenes also slightly decreased photochemical activity in *L. martagon* and *E. milii* but did not cause changes in the total antioxidant activity of cells in morphogenic conditions although, the activity of the SOD isoforms: MnSOD and CuZnSOD was significantly reduced in the presence of fullerenes on non-morphogenic media. The content of secondary metabolites evolved under the influence of fullerenes and was related to phytohormones used. Generally, the nature of the effect of fullerenes ([60]C+D-glucosamine) on the examined plants was not phytotoxic, and in some cases highly beneficial.

PL ISSN 1898-0295

ORAL SUPPLEMENTATION WITH SUCROSOMIAL® IRON ALLEVIATES IRON DEFICIENCY ANEMIA IN A PREMATURE PIGLET MODEL

XIUYING WANG[1], JAROSŁAW WOLIŃSKI[2], PAWEŁ LIPIŃSKI[1], RAFAŁ MAZGAJ[1], MAGDALENA OGŁUSZKA[3], ZUZANNA KOPEĆ[1], BEATA ŻELAZOWSKA[1], GERMANO TARANTINO[4], ELISA BRILLI[4], RAFAŁ R. STARZYŃSKI[1*]

[1]*Institute of Genetics and Animal Biotechnology PAS, Department of Molecular Biology, Laboratory of Iron Molecular Biology, Jastrzębiec, Poland*

[2]*Kielanowski Institute of Animal Physiology and Nutrition PAS, Department of Animal Physiology, Laboratory of Large Animal Model, Jabłonna, Poland*

[3]*Institute of Genetics and Animal Biotechnology PAS, Department of Genomics and Biodiversity, Jastrzębiec, Poland*

[4]*Scientific Department, Pharmanutra S.p.A. Via delle Lenze, 216/B, Pisa, Italy*

**e-mail: r.starzynski@ighz.pl*

Unlike full-term newborns, premature babies are at greater risk of iron imbalance due to insufficient iron storage. Sucrosomial® Iron (SI) is an innovative oral iron-containing carrier. We hypothesized that SI may alleviate iron deficiency anemia in the early postnatal period. Premature piglets were born by cesarean section on day 109 of gestation, while full-term piglets were born naturally. Newborn piglets were assigned to 5 treatment groups: (1) full-term piglets without iron supplementation, (2) full-term piglets with SI supplementation (2 mg Fe/piglet/day), (3) premature piglets without iron supplementation, (4) premature piglets supplemented with $FeSO_4$, (5) premature piglets supplemented with SI. The piglets were fed human milk every 2 hours. Blood and tissue samples were collected on day 11. Premature infants deprived of iron supplementation developed early iron deficiency anemia, as indicated by lower red blood cells indices, plasma iron levels, and transferrin saturation. Interestingly, premature piglets showed higher non-heme iron content in the spleen and muscles compared to full-term piglets. Oral administration of SI improved plasma iron parameters and iron content in the liver, spleen, kidneys and muscles in full-term and premature piglets. No significant changes in iron parameters were observed in piglets supplemented with $FeSO_4$. We provided evidence that SI is a promising iron supplement for alleviating anemia in preterm piglets and improving body iron status. These results indicate that preterm infants are an appropriate animal model for the study of iron deficiency anemia in preterm infants.

ACKNOWLEDGEMENTS

The study was carried out under the National Science Center grant no. 2020/39/B/NZ5/02469.

PL ISSN 1898-0295

BIOCOMPATIBILITY OF INJECTABLE AND LYOPHILIZED CHITOSAN BIOMATERIALS MODIFIED WITH BIOGLASS AND ZN OR SR IONS

ALEKSANDRA WIDŁAK[1], SZYMON SALAGIERSKI[2], BARBARA ZAGRAJCZUK[2], MICHAŁ DZIADEK[2], KATARZYNA CHOLEWA-KOWALSKA[2], ELŻBIETA MENASZEK[1,*]

[1]*Department of Pharmacobiology, UJ CM, Medyczna 9, 30-688 Krakow, Poland*

[2]*Department of Glass Technology and Amorphous Coatings, AGH, Krakow, Poland*

**e-mail: elzbieta.menaszek@uj.edu.pl*

Chitosan has numerous promising properties such as biocompatibility, biodegradation, antimicrobial and antioxidative behaviors which made it a potential biomaterial for applying in medicine. The advantage of the polymer is that, depending on the manufacturing process, it can be used to obtain scaffolds with different physical and mechanical properties. Chemical properties of the biomaterial can be modified with additives such as bioglass and metal ions.

In the study effects of lyophilized or injectable hydrogel chitosan biomaterials modified with calcium-rich bioactive glass (A2) and Zn or Sr ions on cell viability and oxidative stress were examined.

Capacities of modified chitosan biomaterials were tested using three cell types: human fibroblasts (Hs 680), aortic endothelial cells (HAEC) and murine macrophages (RAW 264.7).

Obtained results indicate that lyophilized materials, probably due to their mechanical properties, provide better conditions for fibroblast culture.

The hydrogel form of biomaterials to some extent imitates the conditions provided by the extracellular matrix in the natural environment. These conditions enable endothelial cells to form three-dimensional structures leading to the formation of capillary blood vessels. Microscopic observations confirmed the formation of such structures in HAEC culture on the chitin biomaterial modified with A2 and Zn. It indicates that bioglass and zinc additives promote differentiation of HAEC. In addition, the biomaterials with bioglass and Zn or Sr ions enhanced cell viability.

Our investigations show that chitosan biomaterials modified with bioglass and metal ions have the potential for use in tissue engineering.

ACKNOWLEDGEMENTS

The study was supported by NCN grant No. 2019/32/C/ST5/00386 (MD).

PL ISSN 1898-0295

FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS REVEALS GLYCOSYLATION-RELATED STRUCTURAL CHANGES IN MELANOMA CELL AND EXTRACELLULAR VESICLE PROTEINS

MAGDALENA WILCZAK[1,*], ANDRZEJ WRÓBEL[2], MAGDALENA SURMAN[1], EWA STĘPIEŃ[2], MAŁGORZATA PRZYBYŁO[1]

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

[2] *Department of Medical Physics, Jagiellonian University, S. Łojasiewicza 11, 30-348 Krakow, Poland*

**e-mail: magdalena.wilczak@doctoral.uj.edu.pl*

Introduction: Glycosylation is one of the most common protein modifications, which, among other functions, affects protein folding. During neoplastic transformation, there are rapid changes in the biosynthesis of glycans. These changes are observed not only at the cellular level but also in isolated populations of extracellular vesicles (EVs).

Methods: EVs were isolated from conditioned media of WM266-4 melanoma cells treated with tunicamycin for 24, 48, and 72 hours, by differential centrifugation and low-vacuum filtration. Changes in surface glycosylation of cells and EVs were analyzed by flow cytometry. Fourier Transform Infrared Spectroscopy (FTIR) was applied to gain insight into how tunicamycin affects the secondary structure of proteins and lipid saturation in melanoma cells and EVs they release.

Results: Dynamic changes in cell and EV glycosylation were observed throughout the experimental period. EV lipid saturation analysis showed changes indicative of endoplasmic reticulum stress. Tunicamycin treatment resulted in a decrease in the amount of α-helix and an increase in side chains in cellular proteins. For EVs, a reduction in the amount of random coils, side chains, and inter β-sheets was observed. Moreover, in the EV samples, the protein-to-lipid ratio showed changes in the protein content of the cargo.

Conclusions: FTIR analysis showed that the secondary structure of proteins is highly susceptible to changes and depends on the proper functioning of mechanisms related to glycosylation. Additionally, changes in the secondary structure of proteins are definitely more visible and significant in EV cargo.

ACKNOWLEDGEMENTS

The research was funded by the BioS Priority Research Area under the program "Excellence Initiative – Research University" at the Jagiellonian University in Krakow (U1U/P03/NO/03.22).

PL ISSN 1898-0295

THE EFFECTS OF ADIPOKINES ON THE CYTOKINES SECRETION FROM LYMPHOCYTES DURING PIG INFLAMMATION

JOANNA ZUBEL-ŁOJEK*, KRYSTYNA KOZIEC

Department of Animal Physiology and Endocrinology, University of Agriculture, Al. Mickiewicza 24/28, 30-059 Krakow, Poland

**e-mail: joanna.zubel@urk.edu.pl*

Adipose tissue is a highly active metabolic and endocrine organ. It is now recognized that adipose tissue produces multiple bioactive peptides, termed adipokines, several of them are linked to inflammation and immune response. The aim of this study was to examine the influence of adipokines (resistin, visfatin, adiponectin) supplementation on the lymphocytic CRP, TNF-α, NFκB, IL-6 and IL-10 *in vitro* secretion during acute and chronic inflammation. Experiment was carried out on 24 piglets at the age of 10 weeks. Animals were divided into 4 groups: I- control, II- with overweight (chronic inflammation), III- acute inflammation and IV- overweight with acute inflammation. Piglets from I and III groups were fed with a commercial feed whereas from II and IV with high-calories diet to develop overweight. In order to induce acute inflammation animals received a single injection of streptozotocin (100 mg/kg b.w., i.p.). Peripheral blood lymphocytes were isolated from fresh heparinized blood using standard gradient sedimentation technique, placed in medium and cultured for 72 hours with or without 1mM of adipokines. Cytokines levels were estimated in culture supernatant (ELISA). The obtained results showed that adipokines (resistin, visfatin, adiponectin) act directly on immunological cells *in vitro* and alter the degree of secretion of proinflammatory cytokines from leukocytes.

ACKNOWLEDGEMENTS

Supported by grant NN 311 227 138 and DS 3243/KFiEZ.

PL ISSN 1898-0295

Index of Authors

A
Abram Michał 32
Adamek Mikołaj 44
Ajersch Paula 22
Antos Piotr 48

B
B. Łagosz-Ćwik Katarzyna 50
Barg Ewa 58
Błasiak Anna 73
Błasiak Tomasz 55
Bojko Monika 79
Bonarek Piotr 14
Brankiewicz Aleksandra 23
Brela Mateusz 14
Brilli Elisa 80
Burczyk Gabriela 24
Burda-Uryga Anna 25

C
Capcarová Marcela 41
Chadzinska Magdalena 49
Cholewa-Kowalska Katarzyna 81
Ciepiela Katarzyna 32
Ciesielska Anna 75
Czernicka Małgorzata 26-27
Czerwińska Joanna 28

D
Damulewicz Milena 72
Domagalska Aleksandra 49
Drab Dominika 29
Dubniewicz Klaudia 31
Dupák Rudolf 41
Dziadek Michał 81
Dziekońska Anna 46
Dziurka Michał 26

F
Flisikowska Tatiana 34
Flisikowski Krzysztof 34

G
G. Scanes Colin 61
Gapińska Nikola 32
Gdula-Argasińska Joanna 33, 37, 58, 62
Gilun Przemysław 34, 46
Głód Paulina 68
Gmiter Dawid 53-54, 59
Gogola-Mruk Justyna 35
Góralski Grzegorz 25, 79
Górka-Dynysiewicz Joanna 33, 37, 58
Górska-Andrzejak Jolanta 51
Gozdur Kacper 36
Grzesiak Małgorzata 22, 73
Grzmil Paweł 74
Gucwa Daniel 38
Guevara-Lora Ibeth 23
Gula Emilia 79
Gurgul Artur 31

H
Herman Sylwia 39, 63
Hoyer-Fender Sigrid 12
Hromada-Judycka Aneta 75
Hura Tomasz 56

J
J. Arent Zbigniew 31
Jakubiec Marcin 32
Jakubowska Monika 39
Janeczko Anna 70
Jasek-Gajda Ewa 42, 64
Jasielczuk Igor 31
Jaśkowska Jolanta 20
Jasmin Wiench 74
Jaszcza Klaudia 41
Jurczyk Barbara 70
Jurkowska Halina 42, 64

K
K. Jaglarz Mariusz 40
Kabut Agnieszka 65
Kaczor-Kamińska Marta 14
Kalafová Anna 41
Kamińska Kinga 22, 73
Kamiński Kamil 14
Kamiński Krzysztof 32
Kanty Trościński Jan 27
Karkowska-Kuleta Justyna 45
Kędra Magdalena 79
Kęska-Izworska Kinga 26–27
Kmiecik Michał 22
Kołaczkowska Elżbieta 14, 24, 29, 38, 71
Kołton Anna 26
Kopeć Zuzanna 19, 43, 63, 80
Kot Karolina 44
Kowalik Katarzyna 45

PL ISSN 1898-0295

Koziec Krystyna 83
Kozieradzka-Kiszkurno Małgorzata 27
Koziorowska-Gilun Magdalena 34, 46
Król Teodora 77-78
Krzanowska Katarzyna 62
Krzysztofik Daria 39
Kulig Kamila 45
Kumor Izabela 35
Kustolainen Elena 47
Kwiatkowska Katarzyna 13, 75
Kwolek Dagmara 25

L
Lenartowicz Małgorzata 39, 63
Libik-Konieczny Marta 79
Librowski Tadeusz 33, 37, 58
Link-Lenczowski Paweł 18, 47
Lipiński Paweł 19, 43, 63, 80

M
M. Biliński Szczepan 17, 67
M. Drapała Dominika 30
M. Grabiec Aleksander 16, 30, 50, 66
Machura Marta 48
Maciak Patrycja 17
Malec Joanna 27
Malisz Mateusz 79
Manrique-Moreno Marcela 23
Marcinkowska Magdalena 49
Maria Osyczka Anna 76
Marzec Martyna 55
Massányi Peter 41
Mazgaj Rafał 19, 43, 80
Mazur-Biały Agnieszka 47
Melnykova Mariia 50
Menaszek Elżbieta 81
Miarka Przemysław 62
Mieszkowska Anna 49
Mitka Monika 51
Mogielnicki Andrzej 14
Mojżesz Miriam 44
Mosurek Aleksandra 52
Musiał Klaudia 53
Myśliwa-Kurdziel Beata 79

N
Nalepa Anna 74
Nawrot Sylwia 54
Nieboga Elwira 16
Nowakowska Maria 14
Nowalińska Karolina 55

O
Ogłuszka Magdalena 19, 63, 80
Ogórek Mateusz 39
Oklestkova Jana 70
Opydo Małgorzata 14, 18
Opydo Małgorzata 52
Ostrowska Agnieszka 56
Owczarczyk-Saczonek Agnieszka 28

P
Pacak Ilona 54
Pałka Sylwia 22
Papież Monika A. 57
Pardyak Laura 31
Pastuła Agnieszka 60
Pawlik-Sobecka Lilla 33, 37, 58
Pazgan-Simon Monika 37
Petruńko Leon 59
Piekarska Julia 60
Pierzchała-Koziec Krystyna 61
Pietrzycka Agata 58
Pietrzycka Agata 33, 62
Pirga Natalia 63
Piwowarczyk Renata 25
Płonka Wiktoria 63
Pocheć Ewa 18, 44, 47
Popielarska-Konieczna Marzena 26–27
Potempa Jan 49
Prajsnar Tomasz 49
Przybyło Małgorzata 82
Ptak Anna 35, 68
Pycior Aleksandra 14
Pytka Karolina 20

R
R. Starzyński Rafał 19, 43, 63, 80
Rak Agnieszka 73
Rakus Krzysztof 44, 49
Rapala-Kozik Maria 45
Rąpała-Kozik Maria 65
Rokicki Mikołaj 74
Rożek Katarzyna 74
Rydz Leszek 42, 64
Rys Magdalena 70

S
Sadura Iwona 70
Saja-Garbarz Diana 79
Sałaciak Kinga 20
Salagierski Szymon 81
Sapa Jacek 20
Satała Dorota 65
Schuster Aureliusz 16, 66
Sekuła Małgorzata 17, 67
Serda Maciej 79
Sieńko Wioleta 74
Siwek Agata 20
Ślesak Halina 36
Słomczyńska Iga 79
Smoleniec Joanna 68
Socała Katarzyna 32
Sochaczewska Magda 17, 69

PL ISSN 1898-0295

Stachurska Julia 70
Stępień Ewa 82
Stolarek Marta 14
Such Anna 38, 71
Surman Magdalena 82
Surowiec Magdalena 45
Świątkowska Anna 75
Szczubiałka Krzysztof 14
Szklarczyk Marek 25
Szlaga Agata 73
Szopa Agnieszka 36
Szypulski Kornel 72
Szyrzisko Wiktoria 73

T
Tarantino Germano 80
Tomczyk Igor 74
Traczyk Gabriela 75
Truchan Karolina 76
Trybus Ewa 77–78
Trybus Wojciech 77–78
Trzos Sara 18, 47
Tuleja Monika 79
Tworzydło Wacław 17, 67, 69

W
Walczak Magdalena 55
Wang Xiu 63
Wang Xiuying 19, 80
Wąsowska Barbara 34
Widłak Aleksandra 81
Widziolek Magdalena 49
Wikar Anna 33
Wilczak Magdalena 82
Wilmowicz Emilia 27
Wlaź Piotr 32
Wojtaszek Gabriela 35
Wojtowicz Natalia 18
Woliński Jarosław 19, 80
Wróbel Andrzej 82
Wróbel Maria 42, 64
Wyska Elżbieta 32

Y
Ylla Guillem 50

Z
Zagrajczuk Barbara 81
Zając Paulina 46
Zawisza Maria 44, 49
Żelazowska Beata 19, 80
Żmudzka Elżbieta 20
Zubel-Łojek Joanna 83

PL ISSN 1898-0295