Student scientific international conference

"GenICa"

19-21 June 2024

High Tatras, Slovakia

BOOK OF ABSTRACTS

Martina Šemeláková (ed.)

Iceland Liechtenstein Norway grants

Norway grants

Working together for a green, competitive and inclusive Europe

"Bilateral relations and common knowledge between Slovakia and Iceland research at Universities on topic "Genomic instability and cancer "GenICa"

Funded under the EEA and Norwegian 2014-2021 Financial Mechanism Programme (FBR02, bilateral initiatives under the Fund for Bilateral Relations at national level co-financed by EEA and Norwegian Financial Mechanism 2014-2021), FBR-PDI-012

Editor:

RNDr. Martina Šemeláková PhD.
Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovakia

Reviewers:

prof. MVDr. Ján Mojžiš, DrSc.
Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice

RNDr. Štefan Čikoš, DrSc.
Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences

Organizing committee:

RNDr. Martina Šemeláková, PhD.
doc. RNDr. Peter Solár, PhD.
RNDr. Jozef Židzik, PhD.

Available at: www.unibook.upjs.sk
Publication date: 17.06.2024

DOI: https://doi.org/10.33542/SSI-0333-3
ISBN 978-80-574-0333-3 (e-publication)

Abstracts

Proceedings contain abstracts from participants of the scientific conference with international participation under the name Student scientific international conference *GenICa"*, supported by the project "Bilateral relations and common knowledge between Slovakia and Iceland research at Universities on the topic "Genomic instability and cancer "GenICa", funded under the *EEA and Norwegian 2014-2021* Financial Mechanism Programme (*FBR02, bilateral initiatives under the Fund for Bilateral Relations at national level co-financed by EEA and Norwegian Financial Mechanism 2014-2021)* which took place on 19 - 21 June 2024 in the premises of Hotel Sorea Trigan, Štrbské Pleso, Slovakia

(picture by Šemeláková Martina, Štrbské pleso, High Tatras, Slovakia)

Pavol Jozef Šafárik University in Košice, Slovakia

The Pavol Jozef Šafárik University in Košice was established in 1959 as the second institution of higher education in Slovakia. The current structure of the University consists of five faculties – the Faculty of Medicine, the Faculty of Science, the Faculty of Law, the Faculty of Public Administration, the Faculty of Arts, and the Institute of Physical Education and Sports.

The University offers education in three main levels – bachelor's, master's and PhD study programs in various fields and also medical education. Tuition is provided in both Slovak and English. The Pavol Jozef Šafárik University in Košice provides higher education study programs based on up to date scientific findings in a broad international context. The University is actively involved in cooperations with academic institutions from all over the world.

The Faculty of Medicine at the Pavol Jozef Šafárik University in Košice began teaching medical students in September 1948. Since 1992 it has also accepted international medical students, with classes and tuition provided in English language. Students from approximately fifty different countries are currently studying at the University. The history of the Faculty of Medicine is closely linked to the development of human medicine in eastern Slovakia in the years after World War II. The establishment of the Faculty of Medicine in Košice as a branch of the Slovak University in Bratislava in 1948 was a response to the shortage of medical doctors in Eastern Slovakia in this period. The Faculty of Medicine consists of several institutes, departments, research and experimental workplaces, and special-purpose facilities. Its modern high-rise building is located close to the L. Pasteur University Hospital, the institution in which students receive the majority of their practical training.

University of Iceland, **Reykjavik, Iceland**

The University of Iceland was founded on 17 June 1911, on the centenary of 19th-century Icelandic statesman Jón Sigurðsson, usually referred to as "president". The University was located in the Parliament House at Austurvöllur for the first 29 years. The University of Iceland was formed in a merger of the Seminary, the School of Medicine, and the School of Law, which each formed a faculty, in addition to the newly-established Faculty of Philosophy. Only 45 students, were enrolled during 1911-1912; in the academic year 2023-2024 almost fourteen thousand students, studying at the University of Iceland. The University offers a variety of academic programs on the undergraduate, graduate, and doctoral levels. A new structure and governance system for the University of Iceland entered into force on 1 July 2008. At the same time, the University merged with Iceland University of Education on its centenary. The new University of Iceland has five academic schools, each comprised of a number of faculties. The academic schools are the School of Education, the School of Engineering and Natural Sciences, the School of Health Sciences, the School of Humanities, and the School of Social Sciences. In addition, the University operates a number of research and service institutions. The Faculty of Medicine is composed of four departments: Medicine, Physiotherapy, Radiography, and Biomedical Science. The BioMedical Center (BMC) of the University of Iceland is an official collaboration between research groups working in biomedical molecular life sciences within the University, the National Hospital, Reykjavik University, and other research institutions. BMC provides access to various core research facilities, hosts regular seminars and offers excellent opportunities for masters students, PhD fellows and postdoctoral fellows to participate in cutting-edge research.

List of participants:

Stefan Thorarinn Sigurdsson
Jozef Madžo
Peter Solár
Martina Šemeláková
Lucia Klimčáková
Ján Šalagovič
Helena Mičková
Sigurdur Runar Gudmundsson
Evangeline Breeta Raja David Isac
Lilit Ghukasyan
Seyedeh Parinaz Mahdavi
Kardo Parison Foroutan Pajoohian
Jana Matejovská
Martina Bago – Pilátová
Martin Kello
Dominika Šebová
Kriváková Eva
Pavol Harvanik
Ivana Večurkovská
Morávek Marko

Petur Henry Petersen
Júlia Starková
Jozef Židzik
Terézia Hudáková
Maonian Xu
Salanci Šimon
Martin Bačkor
Francois Olivier Mohan Singh
Jenny Lorena Molina Estupinan
Hildur Run Helgudottir
Poorya Foroutan Pajoohian
Denisa Harvanová
Zuzana Solárová
Eva Slabá
Ester Tomajková
Sankar Rathinam
Ondrej Franko
Monika Majirská
Monika Švecová
Gulová Slavomíra

Program

***19 June 2024*:** initial meeting of working groups

***20 June 2024*:**

9:30 – invitation (Lecture hall)

Plenary lectures:

9:40 -10:40

1. **Jozef Madžo:** Epigenetically driven transposable elements dysregulation in cancer
2. **Stefán Þórarinn Sigurðsson:** The regulation of DNA repair by epigenetics and epitranscriptomics

Presentations/student lectures:

10:40 – 11:20

3. **Maonian Xu, Elín S. Ólafsdóttir:** Biochirality of usnic acid enantiomers in lichens
4. **Franko Ondrej, Čižmarikova Martina, Michalková Radka, Garberová M, Vilková M, Szemerédi N, Spengler G:** Natural compounds and their synthetic derivatives as potential agents overcoming drug resistance mediated by membrane transporters
5. **Parinaz Mahdavi, Eirikur Steingrimsson:** The regulatory link between MITF and VEGFA/FLT1 in melanoma cell lines

11:45 – 13:00

6. **Hildur Run Helgudottir, Runolfur Palsson, Vidar Orn Edvardsson, Thorarinn Gudjonsson:** 2,8-Dihydroxyadenine Impacts Kidney Cell Health and Barrier Integrity in a Cell Culture Model of APRT Deficiency
7. **Harvanik Pavol, Šemeláková Martina, Solár Peter:** The influence of the erythropoietin receptor (EPOr) on chemotherapy resistance of ovarian adenocarcinomas
8. **Jenny Lorena Molina Estupiñan, Audur Anna Aradottir Pind, Poorya Foroutan Pajoohian, Ingileif Jonsdottir and Stefania P. Bjarnarson:** The adjuvants dmLT and mmCT enhance humoral immune responses to a pneumococcal conjugate vaccine after both parenteral or mucosal immunization of neonatal mice
9. **Artimovič Peter**, **Krivakova Eva, Badovska Zuzana, Marekova Maria, Rabajdova Miroslava:** Effect of 17β-estradiol on two endometrial epithelial cell lines with different expression of estrogen receptors
10. **Lilit Ghukasyan, Remina Dilixiati, Eiríkur Steingrímsson:** Is SETDB2 an MITF-dependent binary switch responsible for melanoma plasticity?
11. **Švecová Monika, Dubayová Katarína, Urdzik Peter, Mareková Mária:** Non-invasive cancer diagnostics utilizing 3D fluorescent analysis

Plenary lecture:

14:00 – 14:30

12. **Julia Starkova:** Metabolic Adaptations in Leukemia: Implications for Treatment Response and Drug Resistance

Presentations/student lectures:

14:30 – 15:30

13. **Evangeline Breeta Raja David Isac, Sara Sigurbjörnsdóttir, Ramile Dilshat, Eirikur Steingrimsson:** MITF regulates CDH1 expression in melanoma through intronic elements
14. **Majirská Monika, Vojtek Martin, Pereira Diniz Carmen, Zuzana Kudličková, Bago Pilátová Martina**: Targeting Acute Leukemias with Isoxazole Derivative DHI1
15. **Večurkovská Ivana, Roskovicova Veronika, Stupak Marek, Maslankova Jana:** TGF-β RII as a potential biomarker for colorectal carcinoma progression
16. **Poorya Foroutan Pajoohian, Audur Anna Aradottir Pind, Jenny Lorena Molina Estupiñan, Dennis Christensen, Thorunn A. Olafsdottir, Ingileif Jonsdottir, Stefania P. Bjarnarson:** The benefits of employing a heterologous prime-boost approach in vaccination during early life
17. **Morávek Marko**, **Slovinska Lucia, Kolesár-Fecskeová Lívia, Matejová Jana, Harvanová Denisa:** A comparative analysis of conditioned medium and extracellular vesicles from osteoarthritic tissues and cells
18. **Sankar Rathinam, Aathira Pradeep, Jayakumar Rangasamy, Már Másson:** Ciprofloxacin Conjugated on Chitosan through Click Chemistry; Evaluation of Antibacterial Activity and Drug Release

15:50 – 16:50

19. **Kriváková Eva**, **Artimovic Peter, Badovska Zuzana, Kubala Lukas, Rabajdova Miroslava:** Role of hyaluronic acid in endometrial receptivity
20. **Gulova Slavomira, Slovinska Lucia, Matejova Jana, Harvanova Denisa:** The pivotal role of synovitis in the pathogenesis of osteoarthritis
21. **Šebová Dominika**, **Zilakova Simona, Goga, Michal, Kello Martin:** Evaluation of antiproliferative and pro-apoptotic activity of *Pseudevernia furfuracea* extract and physodic acid in *in vitro* breast carcinoma models
22. **Tomajková Ester, Mareková Mária, Hudák Vladimír, Capková Judita, Kyseľ Roman:** Calcitonine Gene-Related Peptide as new potential biomarker of vasospasm after subarachnoid hemorrhage
23. **Salanci Šimon**, **Schwarzbacherová Viera, Wolaschka Tomáš, Michalková Radka**: Miconazole and acetamiprid as regulators of cell cycle, apoptosis and signaling pathways associated with their cytotoxic effects in vitro

21 June 2024: 10:00-13:00 final meeting: conference summary, meeting of working groups

Abstracts

P1:

Biochirality of usnic acid enantiomers in lichens

MAONIAN XU[1], ELÍN S. ÓLAFSDÓTTIR [1]

[1] Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, maonian@hi.is

Background: Chiral compounds of natural origins usually occur in one major enantiomeric form with exceptions where both enantiomers are produced. Enantiomers may have drastically different bioactivity and toxicological profiles and to figure out their enantioselective biosynthesis could have profound pharmaceutical and biotechnological implications. Studies of biosynthetic pathways of enantiomers are rare and have not been conducted in lichens before. Usnic acid (UA) enantiomers in lichens are potentially excellent candidates to study enantiomer biosynthesis for the following reasons: 1) both enantiomers are produced in multiple lichen-forming fungal families as predominant secondary metabolites [1], 2) enantiomers have different antibiotic activities, and only the (+)-UA has been associated with therapeutical applications, 3) certain lichen-forming fungi tend to produce one major enantiomer, either (+)- or (-)-UA, depending on their phylogenetic placement.

Objective: This study aims to characterize the biosynthesis of usnic acid enantiomers in the lichen-forming fungal family Parmeliaceae.

Material & Methods: Determination of usnic acid enantiomers are performed by a chiral high-performance liquid chromatography (HPLC) method [2]. A fungal phylogenetic tree is constructed by concatenating three fungal loci, i.e. nrITS, MCM7 and RPB1. Purified enantiomers are used for antimicrobial assay tests against bacterial and fungal pathogens. Polyketide synthase genes are annotated and their phylogenetic relationship is analyzed.

Results: A chiral HPLC method has been developed and validated for quantitative analysis of usnic acid enantiomers. There is a strong phylogenetic pattern for enantiomeric biosynthesis: the cetrarioid clade is producing the (-)-usnic acid, while the parmelioid clade the (+)-usnic acid. Phylogenetic analysis of biosynthetic genes also revealed the same pattern. (+)-usnic acid has stronger antibacterial activity than the (-)-usnic acid, which may explain the herbal selection of (+)-usnic acid-containing lichens.

Summary & Conclusion: The study revealed the biosynthetic divergence of usnic acid enantiomers in the family Parmeliaceae, and key variations in the biosynthetic genes have been identified. Stronger antibacterial activity of (+)-usnic acid may explain the herbal selection of (+)-usnic acid-containing lichens.

References:

1. Xu et al., Phytomedicine, 23:441-459, 2016.
2. Xu et al., Phytochemistry, 200:113210, 2022.

Support & Funding: This study was supported by Icelandic Research Fund (grant number 2310001-051), University of Iceland Research Fund (grant number 92257).

P2:

Natural compounds and their synthetic derivatives as potential agents overcoming drug resistance mediated by membrane transporters

FRANKO ONDREJ[1], ČIŽMÁRIKOVÁ MARTINA[1], MICHALKOVÁ RADKA[1], GARBEROVÁ M[2], VILKOVÁ M[2], SZEMERÉDI N[3], SPENGLER G[3]

[1]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia; ondrej.franko@student.upjs.sk
[2]Department of Biochemistry, Institute of Chemistry, Faculty of Science, Pavol Jozef Šafárik University in Košice, Slovakia
[3]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Hungary

Background: Multidrug resistance (MDR) is a phenomenon occurring in cancer cells and pathogenic microorganisms. One avenue of research to overcome MDR involves targeting ATP-binding cassette (ABC) transporters, which are membrane proteins that act as efflux pumps to expel xenobiotics, including chemotherapeutics, from cells. Chalcone molecule offers a diverse and easily modifiable structure for optimizing pharmacological and pharmacodynamic properties, enhancing effectiveness and safety. Recently, we determined that these derivates can potentially possess anti-cancer activity [1] and inhibitory activity on ABCB1 (P-gp) efflux transporter, demonstrated by the use of a rhodamin 123 efflux assay with 1C chalcone derivate [2]. Chalcone derivates also exhibit antimicrobial activity [3] and can inhibit bacterial efflux [4]. Therefore, these molecules could be promising for developing clinically valuable MDR inhibitors, to overcome drug resistance.

Objective: The recent experiments conducted by our scientific group aimed to reaffirm the anti-efflux properties of compound 1C on ABCB1 transporter using an alternative laboratory method. Concurrently, we aimed to assess its effect on ABCB1 protein expression in a time-dependent manner in colorectal cancer cells overexpressing this efflux pump. As a secondary objective, we aimed to evaluate the antimicrobial and anti-efflux capabilities of the tested chalcone derivative on strains of Staphylococcus aureus ATCC 25923 and Escherichia coli K-12 AG100. Another goal was to identify new derivatives within a series of acridine chalcones capable of inhibiting the growth of tumor cells expressing the ABCB1 transporter. This exploration is crucial for future investigations into the correlation between chemical structure and inhibitory activity on the transporter.

Material & Methods: This study employed two human colorectal carcinoma cell lines: a parental cell line Colo 205 and a Colo 320 cell line characterized by overexpression of the ABCB1 transporter. The ability of the tested chalcone derivates to modulate the efflux activity of the ABCB1 transporter was evaluated using the Multidrug Efflux Transporter ABCB1 Ligand Screening Kit. Western blot technique was used to determine the protein expression of the ABCB1 in cells exposed to 1C chalcone derivate at a concentration of 8 mM. The protein expression was evaluated at 24-, 48-, and 72-hour exposure times. Non-cytotoxic value of 1C was determined based on findings from previous studies and utilized in the current research. The minimum inhibitory concentration (MIC) of the compounds of concentrations from 100 μM to 0.195 μM was evaluated according to the standard guidelines. The impact of 1C chalcone (50 and 100 μM) on efflux activity was determined using the Real-time ethidium bromide (EB)

accumulation assay. To determine IC50 values of other chalcone derivates, we employed a screening antiproliferative colorimetric test using methylthiazoltetrazolium bromide dye (MTT).

Results: Initially, an enhanced accumulation of the fluorogenic ABCB1 substrate was noted upon exposure to 1C chalcone derivate – (E)-3-(acridin-9-yl)-1-(2,6-dimethoxyphenyl) prop-2-en-1-one, commencing at a concentration of 1 μM, as compared to the control. Next, we verified the presence of the protein expression of the ABCB1 transporter in the Colo 320 cell line but did not observe its presence in the parental Colo 205 cells. We have also observed a time-dependent reduction in protein expression of ABCB1 exposed to 1C chalcone derivate (c= 8 μM) in the Colo 320 cell line. Interestingly, the compound did not demonstrate any inhibitory effects on the growth of the bacterial strains under investigation. At high concentrations of 1C (≥50 μM), a mild to moderate inhibition of substrate efflux was noticeable only in *S. aureus* ATCC 25923. Additionally, our results confirmed significant antiproliferative effects of two additional acridine chalcone derivates in both parental and ABCB1-overexpressing cancer cell lines. Upon final comparison of IC50 values, it was evident that 1C exhibited the highest potency among the chalcone derivates tested.

Summary & Conclusion: Our results suggest that acridine chalcone derivates could be promising molecules against colon cancer cells, including those with elevated expression of the ABCB1 transporter. Additionally, 1C chalcone repeatedly demonstrated inhibitory activity on the tested efflux transporter. We hypothesize, that this effect may be associated with the reduction in protein expression of ABCB1. Overall, the impact of 1C on cell growth and efflux was notably more pronounced in cancer cells compared to prokaryotic organisms. Nevertheless, further studies are needed.

References:

1. Vilková M et al., Medicinal Chemistry Research, 2022.
2. Čižmáriková M. et al., Anticancer Res, 2019.
3. Henry EJ et al., Journal of Antibiotics, 2020.
4. Le MT et al., Biomed Res Int, 2022.

Support & Funding: This study was supported by VEGA, the Scientific Grant Agency of the Ministry of Education of the Slovak Republic by grant No. VEGA 1/0446/22.

P3:

The regulatory link between MITF and VEGFA/FLT1 in melanoma cell lines

PARINAZ MAHDAVI[1], EIRIKUR STEINGRIMSSON [1]

[1]Department of Biochemistry and Molecular Biology, Biomedical Center, Faculty of Medicine, School of Health Sciences, University of Iceland, Sturlugata 8, 102 Reykjavik, Iceland, Parinaz@hi.is

Background: Melanoma, the deadliest form of skin cancer, originates from melanocytes [1] Studies in cell culture suggest that melanoma cells have the ability to switch their phenotype from proliferative, non-invasive cells to quiescent, invasive cells and back, thus escaping therapeutic intervention; this has been termed phenotype switching [2] The Microphthalmia-associated transcription factor (MITF) has been shown to play an important role in melanoma and to be instrumental in phenotype switching [3-5]. How MITF mediates the switching is not known, although the tumour microenvironment has been

proposed to play a role. Recent work from the laboratory has shown that MITF represses the expression of genes involved in establishing the extracellular matrix (ECM) and focal adhesions [6], including soluble FLT1 (sFLT1) and its ligand VEGFA. sFLT1 (VEGFR1) is proposed to create non-signaling complexes with VEGFR2, thereby regulating VEGFA signaling pathways involved in angiogenesis, proliferation, and migration via focal adhesion proteins [7]. Additionally, sFLT1 acts as a decoy receptor, reducing available VEGFA for binding, and may contribute to melanoma progression by interacting with a5b1 integrin, promoting tumor cell adhesion and migration [8].

Objective: In this study, we use data from our lab and publicly available databases and perform experiments on melanoma cell lines with and without MITF to uncover the regulatory link between MITF and the sFLT1 and VEGFA genes. The effects of sFLT1 and VEGFA on proliferation, migration, and angiogenesis will be investigated.

Material & Methods: Various RNA-seq datasets were analyzed to evaluate the expression of sFLT1 and VEGFA mRNAs. Next, quantitative PCR (qPCR) was utilized to measure sFLT1 and VEGFA expression at the mRNA level, while ELISA was employed to assess protein expression in melanoma cells with and without MITF. In the next step, MITF binding sites were identified using ChIP-Seq data, and these sites were mutated to evaluate the impact of these mutations on sFLT1 and VEGFA expression using Luciferase reporter assays. To investigate the role of FLT1-VEGA in cellular processes, the stable FLT1 knocked-down cells were created in MITF knockout cells using short hairpin RNAs (shRNAs). Additionally, to further follow the cellular processes, the full-length and soluble FLT1 clones were transfected into cells and treated with recombinant VEGFA protein. In the following, co-culture experiments with endothelial HUVEC cells were conducted to evaluate potential differences in angiogenesis between cells expressing high and low levels of full-length or soluble FLT1, followed by tube formation assays.

Results: Analysis of both our RNAseq and the CCLE data revealed a negative correlation between the expression of FLT1 and VEGFA on the one hand and MITF on the other hand. qPCR and Luciferase assays showed elevated FLT1 and VEGFA expression in MITF knock-out cells compared to controls, with FLT1 showing a more pronounced increase. Silencing FLT1 increased cell proliferation. We are currently conducting wound healing, invasion, and angiogenesis assays to assess FLT1's impact on these parameters post MITF knock-out as well as measuring the protein expression of sFLT1 in the cells.

Summary & Conclusion: Our analysis suggests that MITF expression possesses the ability to inhibit the expression of both FLT1 and VEGFA, with a notable preference towards sFLT1. Additionally, our observations indicate that in the absence of MITF, cell proliferation increases notably in the absence of FLT1. This suggests a potential regulatory relationship where the expression of FLT1 may influence the expression of MITF, subsequently impacting cell growth.

References:

1. Bertrand JU et al., Acta Derm Venereol, 100, 2020.
2. Rambow F et al, Genes Dev, 33(19-20):1295-1318, 2019.
3. Steingrímsson et al, Annu Rev Genet, 38:365-411, 2004.
4. Bertolotto C et al., Nature, 480(7375):94-8, 2011.
5. Garraway LA et al., Nature, 436(7047):117-22, 2005.
6. Dilshat R et al., Elife, 10., 2021.
7. Oltean MS, Cell, 8, 2019.
8. Ruffini F et al, Brit J Dermatol, 1061-1070, 2010.

Support & Funding: This study was supported by the Research Fund of Iceland and a fellowship from the University of Iceland PhD Student Fund.

P4:

8-Dihydroxyadenine impacts kidney cell health and barrier integrity in a cell culture model of APRT deficiency

HILDUR RUN HELGUDOTTIR[1], RUNOLFUR PALSSON[1,2], VIDAR ORN EDVARDSSON[1,3], THORARINN GUDJONSSON[1,4]

[1]Biomedical Center, Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavik, Iceland; [2]Division of Nephrology, [3]Children's Medical Center, and [4]Division of Laboratory Hematology, Landspitali University Hospital, Reykjavik, Iceland.

Background: Adenine phosphoribosyltransferase (APRT) deficiency is a rare genetic disorder that results in excessive generation and excretion of 2,8-dihydroxyadenine (DHA), leading to kidney stone formation and crystal nephropathy [1]. This condition causes inflammation and fibrosis of the kidneys. Although the effects of other types of crystal nephropathies and kidney stone diseases have been studied in cell culture models, the mechanism of DHA-mediated kidney injury is not well understood.

Objective: This study aimed to establish a comprehensive cell culture model to investigate DHA crystal-induced kidney injury and to identify therapeutic targets for clinical intervention.

Material & Methods: Three kidney cell lines, MDCK, HK-2, and HEK293, were tested in both monolayer and 3D cultures, including liquid-liquid interface (LLI) and "on-top" of Matrigel methods. Cells were exposed to DHA at concentrations similar to those found in the urine of APRT-deficient patients. Cell viability and migration assays, RT-PCR, western blotting, phase-contrast microscopy, and immunostaining were used as read-out assays. Immunohistochemistry (IHC) was used to analyze kidney tissue samples from APRT deficiency patients and healthy controls obtained from Landspitali University Hospital Biobank.

Results: DHA exposure decreased kidney cell viability and migration across all cell lines. Rising DHA concentrations increased CD44 expression, suggesting enhanced crystal-binding potential of the kidney cells[2]. In a 3D environment "on-top" of Matrigel, MDCK cells maintained polarized structures despite DHA accumulation and did not show an increase in the EMT phenotype compared with TGFβ-treated cells. MDCK cells formed a polarized cell layer grown on Transwell polyester membranes and demonstrated trans-epithelial electrical resistance (TEER) in LLI, which decreased when the cells were treated with DHA at 120 and 480 µg/mL. Analysis of patient kidney tissue samples revealed increased collagen I and III expression, well-known markers of fibrosis, in APRT deficiency patients.

Summary & Conclusion: Our findings demonstrate deleterious effects of DHA on kidney cell health, barrier function, and its potential role in promoting fibrosis in APRT deficiency. Future studies in 3D cultures will focus on further exploring the phenotypic changes in kidney cells after exposure to DHA both at the cellular and molecular level.

References:

1. Runolfsdottir HL, et al., Am J Kidney Dis 67(3):431-8, 2016.
2. Asselman M et al., J Am Soc Nephrol 14(12):3155-3166, 2003.

Support & Funding: This project, grant no. 217707-051, was supported by the Icelandic Research Fund and Landspitali University Hospital Research Fund.

P5:

The effect of erythropoietin receptor on chemotherapeutic resistance of ovarian adenocarcinoma A2780 cells

HARVANIK PAVOL[1], ŠEMELÁKOVÁ MARTINA[1], SOLÁROVÁ ZUZANA[2], BHIDE MANGESH[3], SOLÁR PETER[1]

[1]Department of Medical Biology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia pavol.harvanik@student.upjs.sk
[2]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovakia
[3]Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

Background: The glycoprotein erythropoietin (EPO) has long been exclusively associated with the process of erythropoiesis [1]. However, recent evidence of its action in various non-hematopoietic cells and tissues has pointed to the existence of a much wider spectrum of effects [2]. EPO stimulates cells to grow through a functional EPO receptor (EPOR) expressed on the cell surface [3,4]. The wide action of EPO and last but not least the detection of EPOR in many types of tumor cells drew attention to the possibility of influencing tumor growth. Moreover, the presence of EPOR and the possible activation of signaling pathways in tumor cells can also result in the support of tumor survival, growth and progression [5]. In addition to growth stimulation itself, EPOR-expressing tumor cells can potentially be favored in relation to antitumor therapy.

Objective: The aim of our project was to verify the role of EPOR in ovarian tumor cells. For that reason, we prepared a stably EPOR overexpressed human ovarian adenocarcinoma cell line by transfecting parental A2780 cells with the expression vector containing human EPOR cDNA.

Material & Methods: Expression vector pUNO1 (Invivogen, Toulouse, France) containing a fully sequenced open reading frame of EPOR was transfected into human ovarian adenocarcinoma cell line A2780 using Lipofectamine LTX DNA transfection reagents (ThermoFisher, Waltham, MA, USA). 25 µg of blasticidin was used for a selection of EPOR overexpressed A2780 clones. The cell lysates with equal protein amounts (30 µg) supplemented with 0.01% bromphenol blue, 1% 2-mercaptoethanol, 0.4% SDS and 5% glycerol were then separated via 10% SDS-PAGE gel and transferred onto a NC membrane (Bio-Rad Laboratories, Hercules, CA, USA) by dry transfer (iBlot 2 *Gel* Transfer Device, Thermo Fisher Scientific, Waltham, MA, USA). NC membrane blots were incubated overnight at 4°C with primary antibody anti-EPOR (Amgen Inc., Thousand Oaks, CA, USA). After washing in wash buffer, the membranes were incubated with secondary antibody for 1 h at RT (goat anti-rabbit IgG 1:2000, 31461 Thermo Fisher Scientific, Waltham, MA, USA). Equal loading was verified by the detection of β-actin. The data were analyzed by using the RTCA software Pro 1.2.1 (ACEA Bioscience). Statistical analysis were carried out by a non-parametric method, one-way ANOVA using SigmaPlot (Ver. 12.0).

Results: After the transfection of A2780 cells with EPOR expression vector we prepared twenty different clones using the protocol for limiting dilution and the blasticidin resistance selection marker. By testing each individual clone via western blot analysis and the specific anti EPOR antibody A82, we observed the occurrence of three different patterns of EPOR expression. The first pattern (clone C) was characterized by the presence of two 68 and 28 kDa EPOR isotypes, the second contained only the 68 kDa EPOR fragment (clone T) and the third one (clone V) a combination of 68 and 50 kDa EPOR proteins. Sensitivity tests of EPOR clones to chemotherapy treatment showed that C clone was more

resistant to carboplatin, paclitaxel and other types of chemotherapy compared to the parental A2780 cell line. In addition, the C clone with elongated spindle shape also differed morphologically compared to the control and the other clones with spherical shapes. We hope that transcriptomic analysis of EPOR clones will soon reveal significant differences in overall gene expression in EPOR overexpressed A2780 cells compared to control and suggest the most likely mechanisms explaining altered properties of EPOR clones.

Summary & Conclusion: We are the first ever to transfect human ovarian adenocarcinoma cell line A2780 with the human EPOR gene and show the altered properties of resulted clones as a result of EPOR overexpression. The increased resistance of the A2780-EPOR clones to chemotherapy as well as the significantly altered morphology of such cells closely correlated with the specific pattern of EPOR protein isotypes.

References:

1. Hidalgo et al, Nat Commun, 12:7334, 2021.
2. Debeljak et al, Curr Pharm Design, 14:1302-1310, 2008.
3. Tóthová et al, Int J Mol Sci, 22:7109, 2021.
4. Tóthová et al, Int J Mol Sci, 22:7682, 2021.
5. Chan et al, Oncotarget, 8:38251-38263, 2017.

Support and Funding: This study was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic by grant No. VEGA1/0500/23 and Slovak Grant Agency KEGA under contracts No. 024UPJŠ-4/2024.

P6:

The adjuvants dmLT and mmCT enhance humoral immune responses to a pneumococcal conjugate vaccine after both parenteral or mucosal immunization of neonatal mice

JENNY LORENA MOLINA ESTUPIÑAN[1,2], AUDUR ANNA ARADOTTIR PIND[1,2], POORYA FOROUTAN PAJOOHIAN[1,2], INGILEIF JONSDOTTIR[1,2] AND STEFANIA P. BJARNARSON[1,2]

[1]Department of Immunology, Landspitali, The National University Hospital of Iceland, Reykjavik, Iceland, [2] Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavik, Iceland. jlm4@hi.is

Background: Immaturity of the neonatal immune system contributes to increased susceptibility to infectious diseases and poor vaccine responses. Therefore, better strategies for early-life vaccination are needed. Adjuvants can enhance the magnitude and duration of immune responses [1].

Objective: In this study, we assessed the effects of the adjuvants dmLT and mmCT and different immunization routes, subcutaneous (s.c.) and intranasal (i.n.), on neonatal immune response to a pneumococcal conjugate vaccine Pn1-CRM_{197}.

Material & Methods: Neonatal mice were immunized s.c or i.n. with the pneumococcal conjugate vaccine Pn1-CRM_{197}, with or without 5µg of dmLT or mmCT. Serum and saliva were collected biweekly after immunization for measurements of Pn1-specific IgG and IgA antibodies by ELISA, and Pn1-specific

IgG^+ and IgA^+ antibody-secreting cells (ASCs) in spleen and bone marrow (BM) were enumerated by ELISpot both 2 and 8 weeks after immunization. Germinal Center formation was studied by staining spleen sections (collected 2 weeks after immunization) with peanut agglutinin (PNA) and anti-IgM.

Results: Pn1-specific antibody (Ab) levels of neonatal mice immunized with Pn1-CRM_{197} alone were low. The adjuvants enhanced IgG Ab responses up to 8 weeks after immunization, more after s.c. than i.n. immunization. On the contrary, i.n. immunization with either adjuvant-enhanced serum and salivary IgA levels more than s.c. immunization. In addition, both dmLT and mmCT enhanced germinal center formation and accordingly, dmLT and mmCT enhanced the induction and persistence of Pn1-specific IgG+ ASCs in spleen and BM, irrespective of the immunization route at that early timepoint, not explaining the difference in IgG serum Abs at later time points. Furthermore, i.n. immunization enhanced Pn1-specific IgA+ ASCs in BM more than s.c. immunization. Mucosal delivery of vaccines faces several obstacles, including stability and dosage for optimal vaccine uptake at the mucosal inductive sites and since i.n. immunization with 0.75 µg of Pn1-CRM_{197} and 5 µg of dmLT or mmCT induced lower IgG Abs in serum than s.c. immunization was suboptimal for reaching protective levels against bacteremia and induced more heterogeneous responses, we decided to assess i.n. immunization with higher doses of Pn1-CRM_{197} (1 µg, 2 µg and 4 µg) with 5 µg of dmLT or mmCT, and evaluated the immune response, by measuring Pn1-specific IgG and IgA Abs in serum bi-weekly, IgG+ and IgA+ ASCs in spleen and BM 8 weeks after immunization, as well as salivary IgA. We demonstrated that by increasing the dose of the vaccine when administered i.n. both dmLT and mmCT were able to enhance vaccine-specific IgG responses to comparable levels of those reached by s.c. immunization with a lower dose of Pn1-CRM_{197} and the adjuvants, in addition to the benefits achieved in mucosal and systemic IgA immune responses.

Summary & Conclusion: We conclude that dmLT and mmCT enhance both induction and persistence of the neonatal immune response to the vaccine Pn1-CRM_{197}, following mucosal or parenteral immunization. This indicates that dmLT and mmCT are promising adjuvants for developing safe and effective early-life vaccination strategies.

References:

1. Molina Estupiñan JL et al, Front Immunol, 13:1078904, 2023.

Support & Funding: This study was financially supported by grants from the Icelandic Research Fund (RSJ207287) and the University of Iceland Research Fund (2019-21).

P7:

Effect of 17β-estradiol on two endometrial epithelial cell lines with different expression of estrogen receptors

ARTIMOVIC PETER[1], KRIVAKOVA EVA[1], BADOVSKA ZUZANA[1], MAREKOVA MARIA[1], RABAJDOVA MIROSLAVA [1]

[1]Department of Medical and Clinical Biochemistry, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Košice, Slovakia peter.artimovic@student.upjs.sk

Background: The endometrial tissue which lines the uterus, undergoes various morphological and biochemical changes throughout the menstrual cycle, preparing it for the 'window of implantation'—a

brief phase during which the embryo can adhere to epithelial cells. It is during this window that the endometrium is considered receptive [1]. A lack of receptivity is a common issue in fertility treatments globally, particularly in the process of in vitro fertilization [2]. Cyclical changes of the endometrium during the menstrual cycle are primarily regulated by two steroid hormones – estrogen and progesterone. Their optimal levels provide the necessary stimuli to achieve receptive endometrium [3]. Estrogen is the main hormone regulating changes also in the proliferative phase of the menstrual cycle, during which endometrial cells rapidly proliferate and change the expression of molecules important for endometrial receptivity [4].

Objective: The main objective of the presented pilot study in vitro was to verify the hypothesis whether two cell lines of the endometrial epithelium may differ primarily in their sensitivity to 17β-estradiol (E2) due to the difference in the expression of estrogen receptors ER1, ER2 and GPER1 since alterations in their levels can have a causal effect on changes in the receptivity of the endometrium and contribute to affecting female infertility and infertility treatment failure.

Material & Methods: The receptive cell line RL95-2 and the non-receptive cell line AN3CA were grown in a monolayer culture, and their gene expression was examined through RT-qPCR. The effect of 17β-Estradiol (E2) on these cells was assessed using the MTT assay. The signaling pathways activated by E2 were investigated via Western blot analysis. Alterations in the expression levels of endometrial receptivity markers were evaluated using both RT-qPCR and Western blot techniques. To test functional receptivity, a Calcein-stained Jar cell assay was utilized, with choriocarcinoma Jar cells representing trophoblast cells in this context.

Results: The expression of estrogen receptors varies between RL95-2 and AN3CA cells. Corresponding to the specific receptors present in each cell line, characteristic signaling pathways were activated. The E2 treatment was able to induce the proliferation of RL95-2 cells, in contrast to the AN3CA cell line. Regarding receptivity, E2 provided a slight enhancement of the receptivity only of RL95-2 cells as evidenced by our experimental results.

Summary & Conclusion: In summary, the effect of 17β-Estradiol (E2) on two distinct endometrial cell lines correlates with their varying expression of estrogen receptors. E2 by itself does not significantly influence receptivity, but we propose that it might play a crucial role in priming the cells for subsequent treatment with progesterone, which we intend to explore more in our upcoming research.

References:

1. Tamm K et al., Reprod. Biol. Endocrinol, 7:150; 2009.
2. Griesinger G. et al., Hum. Reprod. Open; 2020.
3. Lessey BA and Young SL, Fertil. Steril., 111:611-617; 2019.
4. Blanco-Breindel MF et al, In StatPearls; StatPearls Publishing: Treasure Island (FL), 2023.

Support & Funding: This study was supported by APVV-22-0357.

P8:

Is SETDB2 an MITF-dependent binary switch responsible for melanoma plasticity?

LILIT GHUKASYAN[1], REMINA DILIXIATI[1, 2], EIRÍKUR STEINGRÍMSSON[1]

[1]Department of Biochemistry and Molecular Biology, BioMedical Center, Faculty of Medicine, School of Health Sciences, University of Iceland, Sturlugata 8, 102 Reykjavík.
[2]Present address: EBML, Heidelberg, Germany, lig45@hi.is

Background: Melanoma is the most aggressive type of skin cancer. Melanoma cells have the ability to modulate their phenotype in response to stress, transitioning from a proliferative non-invasive state to a quiescent invasive state and back [1]. Microphthalmia-associated transcription factor (MITF) is known as the master regulator of melanocytes [2] and is instrumental in phenotype switching [3]. Recently our group demonstrated that SETDB2 might be a potential direct target of MITF and that SETDB2 activity may play pivotal role in plasticity observed in melanoma. SETDB2 is a lysine methyltransferase that methylates the amino group of lysine 9 on histone H3 (H3K9me3), thereby suppressing the expression of target genes [4].

Objectives: We hypothesize that SETDB2 is a critical and reversible regulator of melanoma phenotype switching by changing the chromatin landscape. To test this model, the effect of SETDB2 on proliferation and migration of melanoma cells was determined as well as its effects on gene expression and histone modifications with a particular focus on cell cycle and extracellular matrix (ECM) genes.

Material & Methods: SETDB2-knockout cell lines were generated using CRISPR-Cas9 technology. Cell lines with SETDB2- and MITF-overexpression constructs were generated and the effects investigated by RNAseq, qPCR, Western blotting as well as cell proliferation and wound healing assays. We also performed Cut'n'Run assays to investigate the chromatin landscape.

Results: SETDB2-depleted 624Mel cell lines exhibited decreased proliferation rates. However, no significant changes were observed in wound healing time. Previously performed RNAseq on MITF knockout cells showed upregulation of CDH2. Western blotting and qPCR revealed overexpression of CDH2 in SETDB2-depleted cells. However, introduction of MITF-overexpressing vectors into SETDB2 knockout cells did not rescue the depletion of CDH2. This observation suggests that MITF's regulation of CDH2 might be mediated by SETDB2. CUT'n'RUN experiments on SETDB2 knockout cells revealed differential enrichment of H3K9me3 and H3K4me3 histone marks. The overlapping analysis identified 91 MITF and SETDB2 commonly regulated genes that have H3K4me3 differently bound sites. The overlapping gene set includes CDH2, NRP1 and other genes associated with neuron differentiation and development, cell motility, cell migration, cell-cell adhesion and focal adhesion.

Summary & Conclusion: We have shown that SETDB2 affects proliferation but does not affect wound healing, suggesting that it is not involved in migration. The CUT'n'RUN results suggest that SETDB2 is instrumental in both gene repression (H3K9me3) and activation (H3K4me3). Furthermore, analysis of genes regulated by MITF and SETDB2 and shown to have differential histone modification sites identifies key genes like CDH2 and NRP1 associated with neurogenesis and neuron differentiation. These findings suggest that changes in SETDB2 activity may lead to upregulation of neuron-specific genes in melanoma cells, which in turn may alter the cell phenotype, potentially leading to treatment evasion.

References:
1. Sun Ch et al, Nature, 508(7494):118–122, 2014.
2. Steingrimsson E et al, Annu Rev Genet, 38: p. 365-411, 2004.
3. Rambow et al, Genes & Dev, 33(19-20):1295–1318, 2019.
4. Dillon SC et al, Genome Biol. 6(8): 227, 2005.

Support & Funding: This study was supported by Rannís

P9:

Non-invasive cancer diagnostics utilizing 3D fluorescent analysis

ŠVECOVÁ MONIKA[1], DUBAYOVÁ KATARÍNA[1], URDZIK PETER[2], MAREKOVÁ MÁRIA[1]

[1]Department of Medical and Clinical Biochemistry, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Košice, Slovakia, monika.svecova@student.upjs.sk
[2]Department of Gynaecology and Obstetrics, UPJŠ in Kosice, Faculty of Medicine, UNLP, Košice, Slovakia

Background: Endometrial cancer (EC) poses a significant health challenge globally, particularly prevalent in developed nations where it ranks as the most common cancer of the female reproductive tract [1]. Despite advancements in cancer biology and molecular technology over the past decade, the absence of a validated screening test underscores the urgency to explore alternative diagnostic options. Present diagnostic methods involve often invasive, and discomforting procedures, which hinders their application in clinical prevention [2]. Recent insights highlight native fluorescence as a potential non-invasive diagnostic avenue, reflecting physiological and pathological conditions via fluorescent metabolites found in body fluids [3]. This approach holds promise for revolutionizing EC diagnostics, facilitating early detection, and enhancing patient outcomes.

Objective: The objective of this study was to explore and validate the effectiveness of specific metabolic biomarkers identified through fluorescence analysis of blood serum samples. This research aimed to expand the range of diagnostic tools available for detection of EC in clinical practice.

Material & Methods: Spectral analysis was performed on the blood serum samples of gynecological patients with EC (n=70) which were compared with a control group of healthy volunteers (n=47). Patients were recruited during their examination at the Department of Gynaecology and Obstetrics of the Louis Pasteur University Hospital in Košice. The control group consisted of healthy female volunteers who had negative findings during the USG examination of the pelvis, negative blood oncomarkers and were not subjectively experiencing any health issues. Synchronous excitation spectra (SES) were measured with a Perkin Elmer LS 55 luminescence spectrophotometer in the excitation range and processed in the WinLab software. Statistical analysis involved GraphPad Prism 8.0.1, presenting data as mean fluorescent profiles and ratios ± standard deviation. Data analysis of Receiver operating characteristic (ROC) curves assessed the diagnostic efficiency of fluorescent ratios. Clustering data analysis (PCA, PLS-DA) was created in Python (version 3.9) installed and operated via Anaconda Navigator 2.4.2 utilizing Juptyer Notebook 6.5.4 program.

Results: The SES of a diluted serum measured at two different Δλ provided a discernible difference

between the observed groups. Based on the mean profiles two fluorescent ratios were defined at specific wavelengths. One of these ratios characterizes NADH and FAD fluorescence, often referred to as redox status. At 290 nm excitation, malignant samples had the lowest fluorescence intensity and controls the highest, which indicates altered Trp metabolism in this carcinogenesis. The created ratios were both able to distinguish between controls and EC samples with high statistical significance ($p<0.0001$). The area under the curve (AUC) for the ratios was 0.893 and 0.909, which represents a significant level of separation between controls and EC samples for both ratios. The clustering models successfully distinguished between the samples based on similar characteristics, demonstrating a visible separation among distinct clusters. Additionally, the model's performance metrics, such as the explained variation ($R2 = 0.72$) and predictive capability ($Q2 = 0.68$), were found to be notably high, indicating strong reliability in the clustering analysis.

Summary & Conclusion: The current findings of this research specify that fluorescence of NADH, FAD and metabolism of tryptophan are misregulated in EC patients as compared to healthy individuals. These experimental results provide significant discriminatory spectral features in the serum samples for the detection of endometrial cancer. The importance of this non-traditional technique lies in its extreme simplicity, cost-effectiveness, and non-invasive approach. Substantial variation between the observed groups supports the premise for integrating this fluorescent non-invasive diagnostic monitoring into clinical practice.

References:

1. Bray et al, CA Cancer J Clin, 68(6):394–424; 2018.
2. Jones et al, Obstet Gynaecol, 23(2):103–12; 2021.
3. Birková et al, Int J Mol Sci, 22(4):1884; 2021.

Support & Funding: This study was supported by project VEGA 1/0435/23.

P10:

MITF regulates CDH1 expression in melanoma through intronic elements.

EVANGELINE BREETA RAJA DAVID ISAC[1], SARA SIGURBJÖRNSDÓTTIR[1], RAMILE DILSHAT[1,2], EIRIKUR STEINGRIMSSON[1]

[1]Department of Biochemistry and Molecular Biology, BioMedical Center, Faculty of Medicine, School of Health Sciences, University of Iceland. [2]Present address: EMBL, Heidelberg

Background: Melanoma cells have the ability to change their phenotype from proliferative to migrative cells and vice versa, a process known as phenotype switching. Through this, the cells can metastasize to a new site and evade drug treatment[1]. The Microphthalmia-associated transcription factor (MITF), master regulator of melanocytes, plays a vital role in the phenotype-switching process[2,3]. However, little is known about how MITF mediates the switch.

Objective: Previous studies from our laboratory have revealed that loss of MITF affects the expression of the epithelial-to-mesenchymal transition (EMT) markers, E-Cadherin (CDH1) and N-Cadherin (CDH2)[4]. However, the role of CDH1 and CDH2 in phenotype switching or their relationship with MITF

is not fully established. Our main objective was to determine the role of MITF in regulating CDH1.

Methods: the prospective MITF-binding sites in CDH1 and CDH2 were identified using Cut-and-Run and ChiP-seq data. To determine their function as direct targets of MITF, the regions under the peaks were cloned into a pGL3 promoter vector and transactivation assays were performed in melanoma cells. Potential MITF-binding sites were mutated to find the most important binding sites.

Result: Transactivation assays showed that a 1kb intronic CDH1 fragment (CDH1-B) leads to MITF-dependent activation of CDH1 expression. To validate this result, all the E-boxes present in the CDH1-B fragment were mutated and tested using the luciferase assay. This showed that MITF activates CDH1 by binding to specific E-boxes (CAGCTG and CATGTG) in this regulatory region. To further support these observations, CRISPRi was designed to target these prospective MITF binding sites.

Conclusion: Transactivation assays and CRISPRi show that MITF activates CDH1 directly by binding to multiple E-boxes present in the specific intronic region. Based on our results we are currently creating a reporter vector by combining the MITF-responsive CDH1 regulatory region with eGFP to better understand the role of MITF in phenotype switching.

Reference:

1. Rambow F et al, Genes & Develop. 33, 1295-1318, 2019.
2. Yokoyama S et al., Nature 480, 99-103, 2011.
3. Garraway LA et al., Nature 436, 117-122, 2005.
4. Dilshat R et al., Elife 10: e63093, 2021.

Support & Funding: This study was supported by a project grant from the Icelandic Research Fund number 207067.

P11:

Targeting acute leukemias with isoxazole derivative DHI1

MAJIRSKÁ MONIKA[1], VOJTEK MARTIN[2], PEREIRA DINIZ CARMEN[2], ZUZANA KUDLIČKOVÁ[3], BAGO PILÁTOVÁ MARTINA[1*]

[1] Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia; monika.majirska@student.upjs.sk, martina.pilatova@upjs.sk *(corresponding author)*
[2] LAQV/REQUIMTE**,** Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; mvojtek@ff.up.pt and cdiniz.@ff.up.pt
[3]Institute of Chemistry, Faculty of Science, Pavol Jozef Šafárik University in Košice, Slovakia, zuzana.kudlickova@upjs.sk

Background: Acute leukemias encompass a diverse range of malignant conditions affecting hematopoietic progenitor cells, each characterized by distinct molecular genetic abnormalities, clinical features, and variable outcomes. Despite advancements in standard therapies, relapse or resistance to treatment is common, leading to poor prognosis and significant adverse effects [1]. The isoxazole derivatives present a compelling class of compounds with diverse biological activities, particularly

demonstrating potential for anticancer effects [2]. In our study, we focused on the potential of using an isoxazole derivative DHI1 in the treatment of hematological malignancies.

Objective: The study aimed to target acute leukaemias using the selective isoxazole derivative DHI1 and to investigate its mechanism of action. The objective was to demonstrate DHI1's potential to impede the progression of these diseases by impacting crucial anticancer targets.

Material & Methods: For the chemotaxis assay, Jurkat and HL60 cells were seeded onto the upper part of the insert in a serum-free medium and treated with DHI1. The bottom part of the chamber was filled with cell culture medium supplemented with 20% of FBS. Migrated cells were collected and counted. For the cell invasion assay, Matrigel was used to coat the upper part of the insert's membrane followed by the same steps as for the chemotaxis assay. For Hoechst and F-actin staining analysis, cells were treated with DHI1, then centrifugated, fixed and permeabilized. Cells were incubated with F-Actin solution, Phalloidin-iFluor 488 conjugate, and Hoechst 33258 solutions. Analysis was conducted using the LionheartFX automated microscope (BioTek, Winooski, VT, USA). The phosphorylation of ERK 1/2 (Thr 202/Tyr/204) was observed using the AlphaScreen SureFire p-ERK1/2 Kit, following the manufacturer's instructions. Briefly, cells were stimulated with PMA and/or inhibited with lidocaine. DHI1 was investigated as a potential inhibitor or stimulator. GraphPad Prism 9 software (San Diego, CA, USA) was utilized to generate graphs and perform statistical analysis.

Results: Treatment of Jurkat and HL-60 cells with DHI1 reduced cell migration and invasion in a concentration-dependent manner. Compared to untreated cells, DHI1, at the highest concentration, significantly reduced chemotaxis and invasion in both leukaemic cell lines. DHI1 treatment also caused disruption, disorganisation and damage to F-actin (a protein of the cell cortex consisting of F-actin filaments beneath the plasma membrane [3], suggesting that the anticancer activity of DHI1 may be due, at least in part, to this mode of action. DHI1 was also able to inhibit pERK, thus affecting the ERK1/2 signaling pathway, suggesting a link to the induction of apoptosis and inhibition of cancer progression.

Summary & Conclusion: this study suggests that the antiproliferative and anti-invasive effect of DHI1 on leukemia cells is mediated by i) inhibiting chemotaxis and invasion, ii) damaging the F-actin protein (which is associated with cell motility) and iii) inhibiting the phosphorylation of the ERK1/2 signalling pathway. Thus, DHI1 is an isoxazole derivative with promising potential (seems to interact with multiple targets) in the fight against haematological malignancies due to its ability to inhibit cancer progression and spread.

References:

1. Gilliland DG, Tallman MS. Cancer Cell. 1(5):417-420; 2002.
2. Arya GC et al., Eur J Med Chem. 221:113511; 2021.
3. Bisaria A et al., Science 368(6496):1205-1210; 2020.

Support & Funding: This study was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic by grant No. VEGA No 1/0539/21 and by UIDB/50006/2020 from the Portuguese Foundation for Science and Technology (FCT). Moreover, this publication is the result of the project implementation: "Open scientific community for modern interdisciplinary research in medicine (OPENMED)", ITMS2014+: 313011V455, supported by the Operational Programme Integrated Infrastructure, funded by the ERDF.

P12:

TGF-βRII as a potential biomarker for colorectal carcinoma progression

VECURKOVSKA IVANA[1], ROSKOVICOVA VERONIKA[2], STUPAK MAREK[1], MASLANKOVA JANA[1]

[1]Department of Medical and Clinical Biochemistry, Pavol Jozef Safarik University in Kosice, Faculty of Medicine, Kosice, Slovakia, ivana.vecurkovska@student.upjs.sk
[2]Department of Surgery, Pavol Jozef Safarik University in Kosice, Faculty of Medicine and UNLP, Slovakia

Background: According to WHO, Slovakia has long been one of the five countries with the highest colorectal carcinoma (CRC) incidence and mortality rates in the world. Therefore, it is very important to specify markers for early diagnosis, but also markers capable of predicting disease progression and patient response to treatment. Several studies describe the important roles of the TGF-β signaling pathway in CRC. Based on the literature, up to 20-30% of CRCs contain mutations in the TGF-βRII gene. Some studies even suggest that one of the important factors contributing to CRC transformation is precisely the inactivation of TGF-βRII, which increases cell proliferation [1,2]. Preclinical studies show that TGF-βRII mutations are not sufficient to initiate the malignant transformation of intestinal epithelial cells. However, in combination with mutations of other tumor suppressor genes, TGF-βRII mutations lead to tumor progression [3,4].

Objective: In this work, we focused on determining TGF-β receptor type II expression in biological samples of CRC patients depending on their clinical stage.

Material & Methods: Tissue homogenates and blood serum samples from 103 patients operated for colorectal lesions were analyzed by ELISA method. Patients were divided into benign (BTG; 29 patients, 28.2%) and malignant (MTG; 74 patients, 71.8%) tumor groups based on the standard TNM classification. Within the MTG, 21 patients were categorized into the first clinical stage, 29 patients into the second stage, and 24 patients into the third stage of CRC. IBM SPSS Statistics 23 was used to generate Kaplan-Meier univariate survival analyses and a log-rank test to determine differences between survival curves. GraphPad Prism 8.0.1.244 was used for statistical analysis of the other results: Shapiro-Wilk normality test to evaluate the distribution of values in individual groups; Unpaired t-test, ANOVA, Dunn's multiple comparison test in case of normal distribution of values; Mann-Whitney test, Kruskal-Wallis test, Dunnett's T3 multiple comparison test in case of non-normal distribution of values; ROC curves to evaluate the sensitivity and specificity of the tests

Results: BTG had significantly higher tissue expression compared to the MTG (p=0.0140), but this test is moderately sensitive and specific (AUC 72.44%, 95% CI 0.5718-0.8771, p=0.0150). Within the MTG, TGF-βRII expression correlated with stages. For patients in the first stage, the tissue average expression was 931.23 (SD 216) pg/mL, in the second stage 476.11 (SD 168) pg/mL, and in the third stage 405.59 (SD 193) pg/mL. A statistically significant difference was confirmed when comparing the group of patients in the first stage with the group of patients in the second stage (p=0.0028) and the third stage (p=0.0005). Based on ROC curves, the most reliable results are provided by the comparison of patient groups in the first and second CRC stage (AUC 97.96%; 95% CI 0.9282-1.000; p=0.0005). Patients with TGF-βRII expression lower than 700 pg/ml had a slightly lower survival time (28.103 months; 95% CI 0.2313 – 0.3303) compared with patients with higher TGF-βRII expression (31.620 months; 95% CI 0.2532 – 0.3791; p>0.05). In addition, the same trend in TGF-βRII concentrations was observed using serum samples.

Summary & Conclusion: In the presented work we confirmed that TGF-βRII expression decreases with increased CRC clinical stage, and that lowered TGF-βRII expression is associated with poor survival of CRC patients. Due to this, our results of tissue TGF-βRII expression indicate that by monitoring patients at an early stage for a longer period, we would be able to detect the progression of CRC and thus assess patients' response to treatment. Promising for future research is the finding that serum samples provide a similar trend to tissue homogenates. Therefore, by increasing the size of the research sample and optimizing the processing of blood serum, we could achieve the prediction of CRC by less invasive sampling in the future.

References:

1. Fricke et al., Int J of Oncology, 55(4): 925-937; 2019.
2. Waldner et al., Cells 12(8): 1139; 2023.
3. Yu et al., Oncogene, 33(12): 1538-47; 2014.
4. Schmitt and Greten, Nat Rev Immu, 21(10): 653-667; 2021.

Support & Funding: This study was supported by project VEGA 1/0435/23.

P13:

The benefits of employing a heterologous prime-boost approach in vaccination during early life

POORYA FOROUTAN PAJOOHIAN[1,2], AUDUR ANNA ARADOTTIR PIND[1,2], JENNY LORENA MOLINA ESTUPIÑAN[1,2], DENNIS CHRISTENSEN[3], THORUNN A. OLAFSDOTTIR[1,4], INGILEIF JONSDOTTIR[1,2,4], STEFANIA P. BJARNARSON[1,2]

[1]Faculty of Medicine, School of Health Sciences, University of Iceland, *pooryaf@landspitali.is*
[2]Department of Immunology, Landspitali, the National University Hospital of Iceland
[3]Statens Serum Institute, Copenhagen, Denmark
[4]deCODE genetics, Reykjavík, Iceland

Background: Most pathogens enter the body through the mucosal surfaces. Mucosal IgA antibody is one of the key elements in the first line of defense [1,2] and IgG antibody is critical for systemic immunity [3,4]. Heterologous prime/boost strategies have been proposed to optimally shape the desired immune response induced by vaccination [5].

Objective: The study aims to assess if a heterologous route prime-boost strategy can induce strong and persistent vaccine-specific immune responses.

Material and Methods: We immunized neonatal mice with a pneumococcal conjugate vaccine, Pn1-CRM_{197}, and two adjuvants, by heterologous subcutaneous (s.c) priming with CAF01 followed by intranasal (i.n.) booster with mmCT, or two homologous immunizations, either s.c. or i.n. Blood and saliva were collected at different time points and spleen, bone marrow (BM) and lungs five weeks after booster to assess vaccine-specific immune responses by ELISA and ELISPOT.

Results: The heterologous s.c.-i.n. immunization elicited higher vaccine-specific IgA in serum two weeks after booster and in saliva four weeks after booster than homologous s.c. immunization, and two weeks after booster then homologous i.n. immunization. Vaccine-specific IgG in serum was in general lower after heterologous than homologous immunization, but higher in lung homogenate than after homologous i.n. immunization with mmCT. Heterologous s.c.-i.n. immunization elicited a higher frequency of vaccine-specific IgG antibody-secreting cells (ASCs) than homologous s.c. immunization with CAF01 and a higher number of vaccine-specific IgA ASCs in bone marrow than homologous s.c. immunizations five weeks after booster.

Summary and Conclusion: Although, with the vaccine and adjuvants tested, the heterologous s.c.-i.n. strategy was inferior to homologous s.c. immunization, it induced a higher IgA response than homologous s.c. immunization, but comparable to homologous i.n. immunization.

References:

1. Moor K et al., Nature. 544(7651):498–502, 2017.
2. Lavelle EC and Ward RW, Nat Rev Immunol, 22(4):236–50, 2022.
3. Jódar L et al., Vaccine, 21(23):3265–72.17, 2003.
4. Musher DM et al., J Infect Dis, 154(2):245–56,1986.
5. Lu S, Curr Opin Immunol, 21(3):346–5116, 2009.

Support & Funding: This study was supported by the doctoral grant of the University of Iceland research fund, the Icelandic research fund, and the Landspitali science fund.

P14:

A comparative analysis of conditioned medium and extracellular vesicles from osteoarthritic tissues and cells

MORÁVEK MARKO[1], SLOVINSKA LUCIA [1], KOLESÁR-FECSKEOVÁ LÍVIA[1], MATEJOVÁ JANA[1], HARVANOVÁ DENISA[1]

[1]Associated tissue bank, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia marko.moravek@student.upjs.sk

Background: Osteoarthritis (OA) is a degenerative disease of the musculoskeletal system that affects a large number of people worldwide. Research focused on the accurate diagnosis and efficient treatment of this disease thus constitutes a significant societal challenge. Extracellular vesicles (EVs), serving as cell products that encompass a diverse array of nucleic acids, proteins, and lipids, play a crucial role in facilitating intercellular communication and, consequently, exert an impact on the biological activity of cells. Investigating the composition of these vesicles and elucidating their functions in OA aims to contribute to a better understanding of the disease's pathogenesis and aid advancements in its diagnosis.

Objective: This study aims to determine the optimal source of biomarkers and EVs by evaluating conditioned media (CM) from OA tissues (synovial membrane, infrapatellar fat pad, and cartilage) and cells isolated from these tissues.

Material & Methods: OA tissues from total knee replacements were used to isolate OA cells characterized by flow cytometry and PCR. EVs were then obtained from CM of OA tissues and cells using the precipitation method. Following this, both CM and EVs were characterized using NTA, multiplex immunoassay, and flow cytometry.

Results: The cells primarily exhibited the surface markers CD73, CD90, CD105, and CD44, while lacking expression of the hematopoietic marker CD45. Gene expression comparison results showed elevated expression of CCL2, IL6, MMP13, and Ki67 in cells compared to tissues. Simultaneously, the expression of MMP3, TIMP1, and TNFα was higher in tissues than in cells. Concentrations of chosen biomarkers (IL6, IL8, IL10, IL-1β, IL-12p70, IL-1Ra, TNF, G-CSF, CCL5, and VEGF-A) were markedly higher in CM from OA tissues compared to that from OA cells. The quantity of obtained EVs was also notably higher from OA tissues. The synovial membrane exhibited the highest production of EVs, while the cartilage showed the lowest production. The concentrations of G-CSF, IL-1Ra, and VEGF-A were elevated in lysed EVs as compared to CM. Phenotypic characterization revealed that EVs predominantly expressed CD9, CD63, and CD81, along with MCSP, CD29, and CD44. EVs derived from tissues additionally displayed the surface marker HLA-DRDPDQ.

Summary & Conclusion: The study suggests that gene expression patterns differ between tissues and cells depending on the specific gene of interest. Analyte concentrations and isolated EVs quantities support the notion that tissues offer more advantageous information for OA diagnosis research than cells. Elevated biomarker levels in EVs compared to CM highlight their potential as diagnostic tools. The diverse characterization of EVs reveals variability in both content and phenotype. Preliminary findings suggest that tissues may be a more suitable source of biomarkers and EVs compared to cells.

Support & Funding: This study was supported by the Slovak Research and Development Agency under Contract No. APVV-17-0118. This publication is the result of the project implementation: "Open scientific community for modern interdisciplinary research in medicine (OPENMED)", ITMS2014+: 313011V455 supported by the Operational Programme Integrated Infrastructure

P15:

Ciprofloxacin conjugated on chitosan through click chemistry; evaluation of antibacterial activity and drug release

SANKAR RATHINAM[1], AATHIRA PRADEEP[1], JAYAKUMAR RANGASAMY[2], MÁR MÁSSON [1]

[1] Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, sankar@hi.is

[2] Centre for Nanoscience and Molecular Medicine, Amrita Vishwa Vidyapeetham, Kochi, Kerala, India

Background: Ciprofloxacin is an antibiotic belonging to the fluoroquinolone class. It's commonly used to treat a variety of bacterial infections [1]. Drug delivery methods for ciprofloxacin can vary depending on the specific condition being treated and the desired therapeutic outcomes [2]. Chitosan is a natural polysaccharide derived from chitin, which is found in the exoskeletons of crustaceans such as shrimp, crab, and lobster, as well as in the cell walls of certain fungi. It is a biocompatible, biodegradable, and non-toxic polymer that has gained attention for its wide range of potential applications in various fields, including pharmaceuticals, food and beverage, cosmetics, agriculture, and wastewater treatments [3]. Chitosan is used in the formulation of drug delivery systems due to its ability to form nanoparticles,

microspheres, and hydrogels [4]. These drug delivery systems can improve drug solubility, stability, and bioavailability and provide controlled release profiles [5].

Objective: The project aims to synthesize water-soluble chitotriazolan-drug conjugates that can be formulated into dual-action to treat infectious diseases. To design a drug delivery system that ensures controlled and sustained release of ciprofloxacin over an extended period. This controlled release helps maintain therapeutic drug levels within the target tissues for a prolonged duration, improving efficacy and potentially reducing dosing frequency.

Material & Methods: Chitosan was provided by Primex ehf Siglufjördur, Iceland. Ciprofloxacin and other reagent solvents were purchased from Sigma Aldrich (Germany) and TCI (Belgium). The synthesis aims to synthesize a low degree of azidation with HTC for the first C-2 primary amino groups have been modified to N-(2-hydroxy-3-(*N,N,N*-trimethylammoniumyl)propyl chitosan (HTC) [6] and then remaining primary amino groups that could be converted to azide (low degree of azidation) and finally triazole via copper (I) catalyzed azide-alkyne cycloaddition reaction. The Chitosan-ciprofloxacin conjugate was characterized by IR and NMR spectroscopy. The antibacterial activity of derivatives was tested against four bacterial strains: Gram-positive bacteria *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC29212), and Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC27853) and Zone inhibition assay.

Results: The study focused on creating and developing a new group of chitosan drug conjugates. First, the HTC derivative was synthesized with a low degree of azidation via a diazo-transfer reaction. HTC azide biopolymer has been modified by conjugating it with Ciprofloxacin through a click reaction copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction [7,8] was performed using HTC azide and Propargyl ester-ciprofloxacin. The chitosan-ciprofloxacin conjugates were characterized by IR spectroscopy and proton NMR. The degree of substitution of conjugates was approximately 20%, as determined by proton NMR spectroscopy. The antibacterial activity of the conjugate was evaluated against *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa* at pH 7.2. The conjugate showed better activity against *E. coli* bacteria compared to other bacteria. The drug conjugate can enter cellular compartments through phagocytosis and endocytosis and will be degraded through pH change or enzymatic action to release the antimicrobial drug and the antimicrobial cationic chitotriazolan derivative. The drug release was performed using lysozyme and pH conditions through a zone diffusion assay.

Summary & Conclusion: We were able to successfully bind ciprofloxacin to chitosan biopolymer through a copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. The degree of substitution of the grafted ciprofloxacin was determined to be around 20% using proton NMR. We tested the antibacterial activity against four different strains and found that the conjugate showed good activity against E. coli. The drug release was significantly increased when lysozyme was present.

References:

1. Rancan F et al., Pharmaceutics, 11(10):527, 2019.
2. Egorov A et al., Molecules, 27, 8865, 2022,
3. Inmaculada Aranaz et al., Polymers, 13:3256, 2021.
4. Munawar A Mohammed et al., Pharmaceutics, 9,53, 2017.
5. Vivek S Gaware et al., Biomacromolecules, 18, 4, 1108–1126, 2017.
6. Sankar Rathinam et al., Int J Bioll Macromol, 160, 548–557, 2020.
7. Sankar Rathinam et al., Carbohydrate Polymers, 267, 118162. 2021.
8. Sankar Rathinam et al., European Polymer *J* 196, 112311, 2023.

Support & Funding: The research work was funded by the Icelandic Research Fund (Rannis Grant No. 1709-0210)

P16:

Role of hyaluronic acid in endometrial receptivity

KRIVAKOVA EVA[1], ARTIMOVIC PETER[1], BADOVSKA ZUZANA[1], KUBALA LUKAS[2], RABAJDOVA MIROSLAVA[1]

[1]Department of Medical and Clinical Biochemistry, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Kosice, Slovakia, eva.krivakova@student.upjs.sk
[2]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic

Background: Infertility is a global problem, affecting more than 48 million couples worldwide. Despite the use of assisted reproduction and artificial insemination techniques, the number of infertile couples remains high, with one in seven couples experiencing infertility. Despite extensive research, the success rate of embryo implantation during the in vitro fertilization (IVF) process remains suboptimal. One of the factors that affect the success of IVF is the receptivity of the endometrium, the layer of tissue lining the uterus. However, a significant number of patients suffer from a condition known as a non-receptive endometrium. Glycosaminoglycans, present on the surface of both the embryo and endometrial epithelial cells, play a key role in their interaction. Among these glycosaminoglycans, hyaluronic acid (HA) is a major component. Analyses of the endometrium from women undergoing IVF reveal differences in HA metabolism between fertile and infertile women.

Objective: The main objective of the presented pilot study in vitro was to verify the hypothesis that the modulation of HA metabolism and the expression of HA receptors are crucial for successful embryo implantation. We believe that pathological alterations in these processes lead to endometrial non-receptivity. Infertility due to a non-receptive endometrium can be caused by defects in both the production and degradation of HA. Both insufficient and excessive amounts of HA on the endometrium surface can obstruct successful embryo implantation.

Material & Methods: To assess the significance of HA-related genes and the hormonal regulation of their expression, we established a functional receptivity assay using Jar cells, a choriocarcinoma cell line commonly used as a surrogate for trophoblasts. We utilized two endometrial epithelial cell lines as models of receptive and non-receptive endometrium: RL95-2 (CD44+, representing receptive endometrium) and AN3CA (CD44-, representing non-receptive endometrium). We employed molecular and cytometric approaches to study the differences between receptive and non-receptive endometrium. Functional testing of different receptivity levels in these two cell lines was performed using Calcein-stained Jar cells, and the rate of adhesiveness was determined. The expression of CD44, RHAMM, HAS3, Hyal2, and TMEM2 in epithelial cells was analyzed using RT-PCR for gene expression, and Western blot and flow cytometry for protein expression. The presence of HA on the cell surface was analyzed by ICC and flow cytometry.

Results: RL95-2 and AN3CA cells express HA receptors and genes related to HA metabolism differently. This results in varied expression of HA on the cell surface and the ability to bind exogenous HA. The receptivity of the two cell lines varies and is influenced by treatment with exogenous HA.

Summary & Conclusion: In conclusion, HA on the endometrial surface can play a key role in successful embryo implantation. However, the amount of HA present must be optimal. These observations could be utilized in future treatment research.

References:
1. Johnson LA and Jackson DG, Cells 10:2061; 2021.
2. Afify A et al., Annals Diagnos Pathol 9:312–318; 2005.
3. Berneau SC et al., MHR: Basic science of reproductive medicine 25:265–273; 2019.
4. Wang H et al., Molecular Human Reproduction 18:33–43; 2012.

Support & Funding: This study was supported by projects APVV-22-0357 and VEGA 1/0747/24

P17:

The pivotal role of synovitis in the pathogenesis of osteoarthritis

GULOVA SLAVOMIRA[1], SLOVINSKA LUCIA[1], MATEJOVA JANA[1], HARVANOVA DENISA[1]

[1]Associated Tissue Bank, Faculty of Medicine, Pavol Jozef Safarik University and Louis Pasteur University Hospital, Tr. SNP 1, 040 11 Kosice, Slovakia, slavomira.gulova@student.upjs.sk

Background: Osteoarthritis (OA) is pain causing, mobility reducing, degenerative disease affecting articular joints in adults. Patients suffer from the symptoms which significantly influence their life quality. OA affects the entire joint, including articular cartilage, subchondral bone, ligaments, capsule, and synovial membrane (SM) [1]. The SM comprises metabolic highly active cells and is responsible for content and volume of synovial fluid. During OA, SM undergoes hypertrophy, hyperplasia and infiltration by immune cells. Inflammation of the SM (synovitis) significantly influence the progress of OA by releasing factors promoting cartilage degradation [2]. Currently, the treatment of OA is mostly symptomatic and at the end stage of disease, total joint replacement is the only option. Therefore, there is need to find a new therapeutic tool with regenerative and anti-inflammatory properties to treat this disease as a suitable alternative to conventional, symptomatic treatment.

Objective: OA is a complex disease and different ways of inflammation stimulation could lead to different results in therapeutic effect of analysed therapy. In the pathogenesis of OA, IL-1β plays crucial role and the broad research community commonly uses IL-1β for stimulation of OA inflammation *in vitro*. However, SM from OA patients release a variety of bioactive molecules, that can influence cells in different ways. The conditioned media from SM (CM/SM) contains besides soluble factors also extracellular vesicles (EVs). EVs serve as intercellular communicators with bioactive content (e.g., proteins, lipids, nucleic acids) reflecting the parental cell´s state. Their properties have captured the attention of researchers as potential candidates for cell-free therapy or diagnostic tools in various diseases [3]. The aim of this study was to establish a suitable *in vitro* model of OA utilizing CM/SM, which is enriched with pro-inflammatory molecules naturally occurring in knee joints affected by OA.

Material & Methods: Synovial fibroblasts and chondrocytes obtained from OA patients during total knee joint replacement were used as a target cells for *in vitro* OA model. Cells were treated either with synthetic cytokine IL-1β (5ng/ml or 0,5ng/ml), CM/SM or EVs separated from CM/SM within 24h. Untreated cells were used as a control group. EVs were separated from CM/SM using a combination of precipitation (with PEG) and ultracentrifugation methods. The CM/SM (pool, n=9), was analysed for protein determination. Concentrations of 12 biomarkers (IL-6, IL-8, IP-10, IL-10, IL-1ra, IL-4, MCP-1, RANTES, Eotaxin, FGF-2, EGF, VEGF-A) were measured using MILLIPLEX® Assays and the MAGPIX Luminex platform. To evaluate the impact of each inflammatory environment (IL-1β, CM/SM, EVs), we

analysed gene expression of cytokines (IL-8, YKL-40, TSG-6), chemokines (MCP-1, RANTES) and matrix degrading enzymes (MMP-1, MMP-3, MMP-13) in target cells by RT-qPCR.

Results: High levels of proinflammatory proteins (IL-6, IL-8, MCP-1, RANTES, IL-1Ra) and growth factors (FGF-2, VEGF-A) were detected in CM/SM, which was used as the inflammatory agent in the OA *in vitro* model. Synovial fibroblasts treated with IL-1β or CM/SM had upregulated genes expression of inflammatory cytokine (IL-8), chemokines (MCP-1, RANTES) and matrix-degrading enzyme (MMP-3) compared to the control group. No significant changes in gene expression were observed in synovial fibroblasts treated with EVs separated from CM/SM. In chondrocytes treated with IL-1β or CM/SM, a significant upregulation of gene expression of inflammatory cytokines (YKL-40,TSG-6,RANTES) and matrix-degrading enzymes (MMP-1, MMP-3, MMP-13) was observed compared to the control group.

Summary & Conclusion: The analysis of new drugs or the investigation of the pathophysiology of a disease requires the preparation of an *in vitro* model in laboratory conditions that can faithfully mimic the actual state of the disease occurring in the human body. In our presented study we have created an *in vitro* model of OA, by stimulating synovial fibroblasts and chondrocytes with synthetic IL-1β, CM/SM or EVs separated from CM/SM. Although, the impact of CM/SM and synthetic cytokine IL-1β on the inflammatory stimulation of target cells was comparable; CM/SM represents a more natural alternative for mimicking the inflammatory microenvironment of the OA knee joint. Our *in vitro* model exhibits potential for further use in OA research.

References:

1. Martel-Pelletier *et al.*, Nat Rev Dis Primers 2 (1):16072, 2016.
2. Sellam J and F. Berenbaum, Nat Rev Rheumatol 6 (11): 625–635, 2010.
3. Dixson AC et al., Nat Rev Mol Cell Biol, 2023.

Support & Funding: This study was supported by the Internal Scientific Grant System VUaVP35 UPJS No. vvgs-2023-2730. This study was supported by the Slovak Research and Development Agency under Contract No. APVV-17-0118. This publication is the result of the project implementation: "Open scientific community for modern interdisciplinary research in medicine (OPENMED)", ITMS2014+: 313011V455 supported by the Operational Programme Integrated Infrastructure, funded by the ERDF.

P18:

Evaluation of antiproliferative and pro-apoptotic activity of *Pseudevernia furfuracea* extract and physodic acid in *in vitro* breast carcinoma models

SEBOVA DOMINIKA [1], ZILAKOVA SIMONA[1], GOGA MICHAL[2], KELLO MARTIN [1]

[1]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia dominika.sebova@student.upjs.sk
[2]Department of Botany, Faculty of Science, Pavol Jozef Šafárik University in Košice, Slovakia

Background: Breast malignancies are the most diagnosed oncological diseases in women [1]. Statistical data show an increase in the number of newly diagnosed patients, while mortality remains high, despite advances in diagnostic and therapeutic approaches [1-3]. Breast carcinoma is divided at the molecular level into 3 subtypes – HR+, HER2+ and triple-negative. Appropriate therapeutics are

indicated depending on diagnosed subtype and the stage. For HR+ patients, antiestrogens (fulvestrant, tamoxifen) or aromatase inhibitors (anastrozole, letrozole) are used. Targeted pharmacotherapy, such as HER2 antibodies (trastuzumab, pertuzumab) and inhibitors of tyrosine kinase domains (lapatinib), is available in HER2+ tumors. Conventional chemotherapy (e.g. docetaxel, cisplatin) is the only therapeutic option in triple-negative tumors [4]. The currently used treatment faces several problems, including tumor resistance to cytostatics and related relatively high recurrence rates, as well as a high risk of serious side effects of conventional chemotherapy. These limitations lead to an increasing interest in natural substances as potential anticancer agents [5].

Objective: Several lichen extracts (e.g. *Pseudevernia furfuracea*, *Evernia prunastri*, *Lobaria pulmonaria*) as well as isolated secondary metabolites (e.g. physodic acid, evernovic acid, gyrovocic acid) are demonstrating their antiproliferative and pro-apoptotic abilities in *in vitro* breast cancer models [6]. Combinations of phytopharmaceuticals and conventional chemotherapy are indicating synergism and significant reduction in the risk of tumor resistance, as well as adverse effects of conventional cytostatics [7]. The main objective of this study is to evaluate the mechanism of pro-apoptotic and antiproliferative effect *of Psedevernia furfuracea* extract as well as a secondary metabolite physodic acid in *in vitro* breast cancer models, focusing on all molecular subtypes (ER+, HER2+, triple-negative).

Material & Methods: As an *in vitro* breast cancer models, MCF-7, MDA-MB-231 and SK-BR-3 cell lines were used. Antiproliferative effect of tested compounds as well as IC50 values were assessed by BrdU colorimetric assay. To determine colony forming ability of treated cells, we performed clonogenic assay. Cells were analysed using the automated Cytation™ 3 Cell Imaging in both analyses. To evaluate changes in protein levels, western blot analysis was performed. Protein concentrations were determined using a colorimetric Pierce® BCA protein assay. The expression of proteins was detected using iBright™ FL1500 Imaging System. iBright Analysis software was used for normalization and quantification of protein levels. TPN was performed using No-Stain™ Protein Labeling Reagent. Changes in mitochondrial membrane potential were evaluated using JC-1 fluorescent probe. For flow cytometric analyses (cell proliferation analysis, cell cycle analysis, detection of mitochondrial membrane potential changes, Annexin V/PI staining, analysis of cytochrome c release, detection of caspase 3/7 activity, detection of DNA damage markers, detection of ROS and RNS) FACSCalibur flow cytometer was used.

Results: In this study, we evaluated the mechanism of pro-apoptotic and antiproliferative effects of *Psedevernia furfuracea* extract (PSE) and a secondary metabolite physodic acid (PHY) in *in vitro* breast cancer models. Tumour cell lines representing breast cancer (BC) were selected based on basic screening of metabolic and antiproliferative activity after treatment. We confirmed concentration and time-dependent cytotoxic and antiproliferative potential of PHY and PSE in BC cell lines, while MCF-7 cell line was the most sensitive. IC50 values of PHY and PSE were calculated for MCF-7, MDA-MB-231, SK-BR-3 cell lines as well as MCF-10A and BJ-5ta non-cancer cell lines and used for further experiments. The results show, that IC50 concentrations of PHY and PSE inhibited proliferation and inducted apoptotic cell death, which is demonstrated by phosphatidylserine externalization, disruption of the mitochondrial membrane potential, increased activity of executioner caspases 3 and 7, cytochrome c release from mitochondria to cytoplasm, cleavage of PARP, as well as pro-apoptotic BAX/Bcl-2 ratio. Furthermore, involvement of ROS production in PHY and PSE mediated apoptosis was demonstrated.

Summary & Conclusion: In the present study, we demonstrated antiproliferative and pro-apoptotic activity of *Pseudevernia furfuracea* acetone extract as well as isolated secondary metabolite, physodic acid, in an *in vitro* breast carcinoma models representing all 3 subtypes of BC. Moreover, our results show that ROS production is involved in PHY and PSE mediated apoptosis. In conclusion, PHY and PSE are showing potential as anticancer agents, however further studies of molecular mechanism need to be conducted. Furthermore, PHY and PSE could have synergistic effect in combination with conventional cytostatics, which would increase effectiveness of BC therapy and minimize serious

adverse effect of conventional chemotherapy. In addition to that, tested compounds could possibly act in sensitization of the resistant cell line to cytostatics, which could contribute to the improvement of the recurrent BC therapy management, as well as limiting the development of tumour resistance. The study of potential synergism of natural substance/ cytostatic drug combination as well as potential sensitization of resistant BC cell line will be the subject of the further study.

References:

1. Shen Y et al., Genomics, 112(3):2640-2646; 2020.
2. Kubatka et al., Bratislava: Univerzita Komenského; 2017
3. Sung H et al., CA Cancer J Clin, 71(3):209-249; 2021.
4. www.esmo.org
5. Nan Y et al., Front Oncol, 12:1049888; 2022.
6. Kello M et al., Pharmaceutics, 13(12); 2021.
7. Islam MR, et al., Molecules, 27(7); 2022.

Support & Funding: This study was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic by grant No. VEGA 1/0498/23.

P19:

Calcitonine gene-related peptide as new potential biomarker of vasospasm after subarachnoid hemorrhage

TOMAJKOVÁ ESTER[1], MAREKOVÁ MÁRIA[2], HUDÁK VLADIMÍR[1], CAPKOVÁ JUDITA[1], KYSEĽ ROMAN[1]

[1] 1st Department of Anaesthesiology and Intensive Medicine, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia tomajkova.ester@student.upjs.sk

[2] Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia

Background: Subarachnoid hemorrhage (SAH) is an acute cerebrovascular disorder, in most cases caused by the intracranial artery aneurysm rupture. Although the rupture can be effectively treated, the mortality remains unchanged. Moreover, patients are at risk from subsequent ischemia caused by arterial vasospasm. The spasm of vessels subsequently leads to hypoxia in the area supplied with blood, which significantly increases the morbidity and mortality of patients. These spasms occur most often in the interval of 3-7 days after the onset of SAH, but they can occur at any time up to 21 days after the initial damage. The incidence of symptomatic vasospasm is up to 28.5% and in the second week more than 67%. The American Stroke Association recommendations 2023 identify gaps in the knowledge of pathophysiology and the need to study new vasospasm biomarkers to improve patient survival and quality of life. There is currently no proven parameter fulfilling a diagnostic or prognostic function [1-4].

Objective: Current knowledge about the exact pathophysiological processes that occur in patients with SAH is ambiguous, and therefore it is not possible to predict the development of vasospasm. Some vasospasms that occur after SAH are clinically silent, but despite this, arteriospasms are the dominant factor adversely contributing to the development of the resulting neurological deficit. Elucidation of the pathophysiology and proof of the molecule connected to vasospasms can be a milestone for further

pharmacological research and the development of targeted vasospasm therapy. The specific objectives are as follows: identification of a molecule that correlates with the development of clinical and subclinical vasospasms in the human population; description of the dynamics of concentrations of the selected molecule in body fluid; determination of the nature of the biomarker (prognostic, predictive, diagnostic) and limitations of the given biomarker.

Material & Methods: As part of the preclinical phase of our observational prospective study, we identified articles using the Cochrane database, Web of Science, and Google Scholar that address potential biomarkers of cerebral vasospasm in association with SAH. Based on the study of selected articles and meta-analyses published between the years 2000-2023 (74 studies in total), we selected the Calcitonine Gene-Related Peptide (CGRP) molecule. Subsequently, we used another 11 articles to study the structure, properties, and biological effect of CGRP to ensure a comprehensive view and the specificity of the investigation of this molecule in connection with SAH, and any disorders of the central nervous system.

Results: Calcitonin gene-related peptide belongs to vasoactive neuropeptides. CGRP was discovered in the thyroid gland of aging rats, and subsequently, the occurrence of this peptide in nervous tissue was demonstrated and its significant cardiovascular activity was proven. It is a highly potent microvascular vasodilator and neuromodulator that is abundantly expressed and stored in the central and peripheral nervous systems. CGRP is involved in wound healing processes, the pathophysiology of atherosclerosis and vascular wall remodeling, pulmonary hypertension, migraine, diabetes and obesity, sepsis, and others. Among the most important effects associated with SAH is the influence of CGRP in the cerebral circulation, where it is released from presynaptic vesicles located in sensory perivascular fibers. The response is a noticeable relaxation of the smooth muscle of the vessels, which leads to dilation of the arteries, gradually increasing cerebral blood flow. In aneurysmal SAH, it has been proven that CGRP is excessively released into the CSF during the first 10 days after the initial stroke with a potentially neuroprotective effect in terms of preventing cerebral vasospasms and ischemia.

Summary & Conclusion: Physiologically, together with other neuropeptides such as neuropeptide Y, CGRP restores cerebrovascular tone in response to excessive vasoconstriction. The triggering factor of releasing CGRP is not entirely clear, it may be blood in the subarachnoid space or even direct disruption of the perivascular nerve fibers. CGRP participates in neurogenic inflammation too. Although the neuroprotective and vasodilatory effects of CGRP are assumed, the dynamics and target values in cerebrospinal fluid have not yet been determined. Despite many studies devoted to the influence of CGRP on the human organism and its role in many pathophysiological processes of various organ systems, many studies devoted to CGRP about the central nervous system and more specifically to SAH have been conducted in animal models and are not available clear data on specificity, sensitivity and dynamics in the human organism. For this reason, it is crucial that the research also takes place in the human population.

References:

1. Ashley JR et al., Medscape, 9:12, 2022.
2. Bründl et al., Neurosurg. Rev, 44 (3):1479–1492, 2021.
3. Hirsch Y et al., Neurocritical Care, 34(3): 89–898, 2021.
4. Hoh B et al., Stroke, 54 (7), 2023.

P20:

Miconazole and acetamiprid as regulators of cell cycle, apoptosis and signaling pathways associated with their cytotoxic effects in vitro

SALANCI ŠIMON[1], SCHWARZBACHEROVÁ VIERA[2], WOLASCHKA TOMÁŠ[3], MICHALKOVÁ RADKA[1]*

[1]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia; simon.salanci@student.upjs.sk, radka.michalkova@upjs.sk
[2] Department of Biology and Physiology, Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia; viera.schwarzbacherova@uvlf.sk
[3]Department of Pharmaceutical Technology, Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia; tomas.wolaschka@uvlf.sk
* Correspondence: radka.michalkova@upjs.sk

Background: Human activity can significantly affect the ecosystem and the quality of the environment. In addition to the environment itself, the health of plants, animals and humans is also affected [1,2]. For many compounds, the positive effect associated with their use is accompanied by several properties that negatively affect the ecosystem. Among the xenobiotics contaminating the environment, we undoubtedly include products from the pharmaceutical industry [3]. Due to their extensive use and practically continuous supply to the environment, they represent a special class of environmental contaminants. Azole antifungals are used in medicine and, together with neonicotinoids, are among the substances widely applied in agro-industry. For this reason, we rank both groups among significant environmental pollutants associated with many negative effects on the ecosystem. By using an in vitro model, we aimed to experimentally test the potential cytotoxic effects of the antifungal miconazole, the insecticide preparation Mospilan® and their combination on healthy human cells.

Objective: The main goal of our experimental work is to study the impact of the potential cytotoxic effects of the azole antifungal miconazole and the neonicotinoid insecticide acetamiprid on healthy human cells in vitro. Partial objectives include evaluating the metabolic and antiproliferative activity of the tested compounds on healthy cells, and analyzing the potential mechanism of their action alone and in combination. Analysis of their effect involves monitoring the ability to induce apoptosis using specific markers. Another objective was to study the effect of the selected compound on the cell cycle progression of healthy MCF-10A epithelial cells by analyzing changes in cell cycle phases and levels of related proteins. Another goal was to evaluate changes in the levels of proteins associated with autophagy and signaling pathways involved in cell death, proliferation, and survival.

Material & Methods: Healthy human mammary gland epithelial cells MCF-10A were used to assess the potential cytotoxic effect of miconazole, acetamiprid in the Mospilan 20SP® suppository and their combination. The substances were dissolved in DMSO and at a final concentration of 10 µg/mL were added to the cells individually or in combination. Changes in viability were assessed using the metabolic MTT assay. For a detailed understanding of their effect, further experiments were carried out using flow cytometry, thanks to which it is possible to evaluate changes in the distribution of the cell cycle and the rate of apoptosis. Changes in protein levels and their phosphorylation were assessed by Western blot analysis using specific primary and HRP-conjugated secondary antibodies. The results of the experiments were evaluated as mean ± standard deviation (SD). We performed a statistical analysis of the data using the Student´s t-test, where we consider the difference to be statistically significant if $p < 0.05$.

Results: The study demonstrated the ability of the tested substances to induce apoptosis in cells. An increase in the number of apoptotic cells was observed after 24 hours with a maximum after 72 hours. Analysis of BC demonstrated that both miconazole and the combination can block the cell cycle in the G1 phase already after 24 hours. Blockade was observed after 24 and 72 hours of incubation even with Mospilan. Similar to the analysis of apoptosis, the effect observed in Mospilan and its combination with miconazole disappeared after 48 hours, and after 72 hours it was observed only in miconazole and Mospilan samples. Western blot analysis showed a decrease in phospho-Bad expression and an increase in the cleaved form of PARP. Furthermore, a significant increase in p21 protein expression after 72 hours of exposure, with a simultaneous decrease in PCNA, phospho-Rb and phospho-Wee1 protein expression was observed. The potential effects of the tested substances on the induction of autophagy was demonstrated by a significant decrease in the expression of phospho-ULK1 Ser757 and an increase in the expression of LAMP1 in cells treated with miconazole and in combination. The presence of changes in cells exposed to these two conditions was confirmed by an increase in the expression of phospho-Akt and phospho-Erk proteins, which are involved in processes related to cell proliferation and survival.

Summary & Conclusion: In this in vitro study, we analyzed the potential cytotoxic effects of the azole antifungal miconazole, the insecticide Mospilan® (acetamiprid) and their combinations on non-neoplastic human mammary epithelial cells. These substances are widely used in medicine and industry in the world and, due to accumulation in the environment, represent a potential environmental risk. In conclusion, miconazole had a cytotoxic effect on the MCF-10A cell line. Cells after its exposure showed signs of oxidative stress and damage to mitochondrial functions [4]. An effect on the induction of apoptosis and autophagy was also demonstrated [5]. Miconazole also had a significant effect on the cell cycle with its blockade in the G1 phase [6]. The combination of miconazole and Mospilan® did not show an increase in cytotoxicity. According to the presented results, it can be concluded that Mospilan® paradoxically reduced the toxicity of miconazole in some cases. Expanding the set of data obtained so far could lead to a better understanding of the complex effects of miconazole, Mospilan® and the combination of these compounds.

References:

1. Trösken ER, et al. Endocr Res. 30(3):387-394, 2004.
2. Zuščíková L, et al. Toxics. 11(7):598, 2023.
3. Borpatragohain BR. et al., Int J Chem Stud. 7 (3):4935–4941, 2019.
4. Phogat, A., et al. Environ Chem Lett 20:1453–1478, 2022.
5. Jung HJ, et al. Oncol Lett.; 21(4):335, 2021.
6. Petricca S, et al., Int J Mol Sci. 2022;23(10):5429.

Support & Funding: This research was funded in part by the Grant Agency of the Ministry of the Education, Science, Research and Sport of the Slovak Republic VEGA 1/0166/21, VEGA 1/0653/19, VEGA 1/0498/23 and VEGA 1/0446/22 and the Slovak Research and Development Agency under the contract No. APVV-16-0446. Moreover, this publication is the result of the project implementation: "Open scientific community for modern interdisciplinary research in medicine (OPENMED)", ITMS2014+: 313011V455, supported by the Operational Programme Integrated Infrastructure, funded by the ERDF. Furthermore, this publication is the result of the project implementation: "Medicínsky univerzitný vedecký park v Košiciach (MediPark, Košice—Fáza II.)", kód ITMS2014 + 313011D103 supported by the Operational Programme Research & Innovation, funded by the ERDF.
We would like to thank Mgr. Martina Hájiková for her excellent technical assistance.

P21:

The effect of different roasting and processing method of coffee on the growth of colon tumor cells in vitro

VÁRADY MATÚŠ[1], ŠEMELÁKOVÁ MARTINA[2], SOLAR PETER[2], HUDÁKOVÁ TERÉZIA[2], POPELKA PETER[1]

[1]Department of Food Hygiene, Technology and Safety, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovak Republic

[2]Department of Medical Biology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia

Background: Coffee is one of the most popular drinks in the world due to its sensory properties and the stimulating effect of caffeine [1]. Coffee contains many beneficial substances but can also contain potentially harmful substances due to improper processing, subsequent handling, or low-quality green beans [2,3]. Coffee beverages are one of the most popular commodities consumed in recent years. *Coffea arabica* specialty coffees have a standardized process of production characterized by the quality and uniqueness of origin, from criteria for selecting plantations to preparation. To maintain the high quality of specialty coffees, older processing methods (natural, washed, and honey) are gradually being replaced by new methods, e.g. anaerobic fermentation, Various methods of post-harvest processing can determine the sensory profile, producing coffees with highly differentiated characteristics, classified as specialty coffees [4].

Objective: The aims of the present study were as follows: (1) to determine the influence of various processing methods natural and anaerobic fermentation, on the content of bioactive compounds in specialty coffees and (2) to determine their effect on healthy cells (fibroblasts) and colon cancer cells (HCT116) *in vitro*.

Material & Methods: Samples of beans of 100% C. arabica Burundi (BUR) beans were obtained from the Kayanza coffee region and the Nemba processing station. The beans were the Red Bourbon variety harvested in 2020 and were processed by two methods, natural and anaerobic fermentation. The cherries processed by the natural dry method were harvested by hand and sorted by size and quality. In the natural or dry process, whole coffee cherries are left to dry in the sun, leaving the fruit on the beans, allowing them to "raisin" around the bean. They were spread on raised African beds, where they were dried for 3–6 wks. The beans processed by anaerobic fermentation were fermented in closed barrels containing Cima yeast (*S. cerevisiae*), where they were dried. The chlorogenic acid (5-O-Caffeoylquinic acid, 5-O-CQA), caffeine (CAF), gallic acid (GLA) and cinnamic acid (CNA) in coffee samples were analyzed by HPLC. Each coffee sample (100 mg of freshly roasted beans) was treated with 10 mL of boiling distilled water. A sample of 100 µL was transferred to the vials for subsequent HPLC analysis. High-performance liquid chromatography (HPLC-UV/Vis) was performed using an Ultimate 3000 system, controlled using Chromeleon 7.2 software, detection of analytes was performed at 194 and 500 nm. Quantification was performed at 260 and 300 nm [5]. The data were evaluated using Chromeleon 7.2 software. The amount of chlorogenic acid (5-O-CQA) and caffeine (CAF), were expressed as µg/1000 µg of coffee. The effect of coffee on the culture of fibroblasts BJ-5ta and cancer cell line HCT116 was tested. Proliferative activity was tested by the MTT assay on 96-well plates to set the concentration required for a suboptimal dose. The cells were seeded in 6-well plates and 24 hours after incubation cells were treated with a determined concentration of coffee, boiled and sterilized solution (1 g/10 ml H_20). Cells were harvested 48 hrs after treatment, and proteins were isolated and

sequenced.

Results: We compared coffee processed by natural and anaerobic fermentation from Burundi. The results of bioactive substances (specifically chlorogenic acid and caffeine) did not show significant differences between the samples. The amount of chlorogenic acids ranged between 6.47 – 6.66 µg/1mg and caffeine ranged from 9.83 – 10.1 µg/1mg of coffee. Proteomic analysis showed in the colon cancer cell line HCT116 (ATCC): treatment with roasted/unroasted natural/Burgundy anaerobic coffee/control (untreated HCT116 cancer cells) leads to changes in the expression of up- and down-regulated genes. Proteomic analysis showed on tested healthy BJ-5ta (ATCC) fibroblast cell lines: treatment with roasted/unroasted natural/Burgundy anaerobic coffee/control (untreated cells) leads to leads to changes in the expression of up- and down-regulated genes.

Summary & Conclusion: The new fermentation methods can probably help to improve most of the quality parameters, but further study is needed to understand the impact of fermentation on specialty coffees from different areas and on other qualitative and sensory parameters. The effect on gene expression of roasted or non-roasted coffee, BURN or BURA was different and further in-depth study of the effect of coffee on gene expression is needed.

References:

1. Rodak K et al., Nutrients, 13 (9):3088; 2021.
2. García M et al., Appl. Sci, 9 4195; 2019.
3. Merhi A, Front. Public Health, 10: Article 1004570; 2022.
4. Cortés-Macías ET et al., Postharvest Biol Technol, 187: article 111852; 2022.
5. Tauchen J et al., J Food Comp An, 41:113-121; 2015.

Support & Funding: This study was supported by funds from The Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0073/22) and Slovak Grant Agency KEGA under contracts No. 024UPJŠ-4/2024.

P22:

The effect of candidate biomarkers BLVRA and SCARB2 on platinum-based chemotherapy resistance in ovarian adenocarcinoma cells.

HARVANIK PAVOL[1], ŠEMELÁKOVÁ MARTINA[1], SOLÁROVÁ ZUZANA[2], BHIDE MANGESH[3], SOLÁR PETER[1]

[1]Department of Medical Biology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia pavol.harvanik@student.upjs.sk
[2]Department of Pharmacology, Faculty of Medicine, P.J. Šafárik University in Košice, 04001 Košice, Slovakia
[3]Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, 04001 Košice, Slovakia

Background: Ovarian tumors represent one of the most common forms of gynecological tumors and have a high mortality rate [1]. Cisplatin (CDDP) is one of the oldest and most widely applied chemotherapeutic drugs used for treatment of solid tumors [2]. Despite a positive primary response, some patients treated with CDDP develop chemotherapy resistance, leading to treatment failure and increased mortality [3]. The molecular principle of the emergence of such resistance is a change in gene

expression of tumor cells and subsequent inhibition of the effect of CDDP. Most often, these mechanisms are divided into four groups: reduced intracellular accumulation of CDDP; increased accumulation of sulfur-containing macromolecules; increased activity of repair mechanisms removing CDDP-adducts of DNA; regulation of apoptosis and cell survival signals [4]. Probably the biggest challenge in the field is the discovery and understanding of the molecular mechanisms that cause the ineffectiveness of CDDP treatment. Known biomarkers of CDDP resistance include, for example, CD70, whose increased expression correlates with the development of such resistance [5,6]. The MEST gene, whose expression reduction inactivated the Wnt/β-catenin signaling pathway, resulted in the repression of CDDP resistance [7,8]. The others such as PHGDH [9] and UCHL1 [10] are also well known.

Objective: The aim of our work was to find new biomarkers of CDDP resistance and verify their role in ovarian tumor cell morphology, chemotherapeutic resistance and signaling. After the selection of two candidate biomarkers (BLVRA and SCARB2), we prepared a stably overexpressed human ovarian adenocarcinoma cell line using the transposon-based mechanism in parental A2780 cells containing human BLVRA and SCARB2 cDNA.

Material & Methods: We performed a whole proteome comparative analysis of A2780 and A2780 cis cell lines, selected the most upregulated and downregulated genes and chose two BLVRA and SCARB2 that are not prominently mentioned in scientific literature. We have also compared their metabolic activity using the MTS test. Moreover, we used a transposon-based mechanism to overexpress our BLVRA and SCARB2 genes in human ovarian adenocarcinoma cell line A2780 using Lipofectamine LTX DNA transfection reagents (ThermoFisher, Waltham, MA, USA). 25 µg of blasticidin was used for a selection of BLVRA and SCARB2 overexpressed A2780 clones. The cell lysates with equal protein amounts (30 µg) supplemented with 0.01% bromphenol blue, 1% 2-mercaptoethanol, 0.4% SDS and 5% glycerol were then separated via 10% SDS-PAGE gel and transferred onto a NC membrane (Bio-Rad Laboratories, Hercules, CA, USA) by dry transfer (iBlot 2 Gel Transfer Device, Thermo Fisher Scientific, Waltham, MA, USA). NC membrane blots were incubated overnight at 4 °C with primary antibodies anti-BLVRA and anti-SCARB2. After washing in wash buffer, the membranes were incubated with a secondary antibody for 1h at RT. Equal loading was verified by the detection of β-actin. The data were analyzed by using the RTCA software Pro 1.2.1 (ACEA Bioscience). Statistical analysis was carried out by a non-parametric method, one-way ANOVA using SigmaPlot (Ver. 12.0).

Results: We compared both metabolic activity and proteomic data of the A2780 cell line vs. its cisplatin-resistant derivate - A2780cis. According to metabolic activity (MTS assay) of the parental and resistant cells after the exposure to CDDP for 72 hours we found that IC50 of A2780cis cells was 3.8 times higher compared to A2780 cells. Comparative proteomic analysis revealed more than 20 significantly upregulated and downregulated genes. Among these genes, we found some that have already been mentioned in the scientific literature, but also some that have not been published yet. Indeed, western blot analysis and quantitative real-time PCR confirmed changes in protein composition and gene expression between A2780 and A2780cis cell lines (e.g. EMT markers like ZO1 and vimentin). However, only BLVRA and SCARB2 as a new marker were selected for further analyses.

Summary & Conclusion: To the best of our knowledge we are the first ever to transfect human ovarian adenocarcinoma cell line A2780 with human BLVRA and SCARB2 genes to study their impact on chemotherapy resistance, morphology and signaling pathways.

References:

1. Gaona-Luviano P et al., Chin Clin Oncol. 9(4), 2020.
2. Ghosh S, Bioorg Chem. 88; 2019.
3. Song M et al., Eur J Med Chem. 232; 2022.
4. Solár P et al., Cancer Lett. 309(1):11-18; 2011.
5. Aggarwal S et al., Gynecol Oncol. 115(3):430-437; 2009.
6. Liu N et al., Onco Targets Ther. 6:615-619; 2013.

7. Zhang J et al., Gynecol Oncol. 164(3):566-576; 2022.
8. Ruan Z et al., Cancer Gene Ther. 28:785-798; 2021.
9. Bi F et al., Front Oncol. 11; 2021.
10. Jin C et al., J Cancer. 4(8):662-670; 2013.

Support and Funding: This study was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic by grant No. VEGA1/0500/23 and Slovak Grant Agency KEGA under contracts No. 024UPJŠ-4/2024.

P23:

Analysis of molecular mechanisms leading to endometrial pathologies

ABRAHAMOVSKÁ MICHAELA[1], BARANOVIČOVÁ BARBORA[2], GREŠOVÁ ANDREA[3], RABAJDOVÁ MIROSLAVA[1]

[1]Department of Medical Biology, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Slovakia michaela.abrahamovska@student.upjs.sk
[2]Department of Gynaecology and Obstetrics, UPJŠ in Kosice, Faculty of Medicine, UNLP, Košice, Slovakia
[3]East Slovakia Institute of Oncology a.s., Košice, Slovakia

Background: Gynaecological diseases include diseases mediated by the inflammatory process, which have the highest prevalence in women [1]. These inflammatory processes can lead to chronic inflammatory responses that cause local tissue hypoxia and stimulate cells to compensate for the lack of active new blood vessel formation [2]. This process occurs through increased activity of signalling pathways involved in neovascularization and angiogenesis [3]. Angiogenesis is one of the key processes in endometrial cancer and endometriosis. It is necessary to ensure an adequate supply of oxygen and nutrients to endometriosis deposits and the growth of tumour tissue [4,5]. Therefore, systematic investigation of these angiogenic processes is critical not only for understanding the pathogenesis of these diseases, but also for discovering new possibilities in targeted therapy and treatment [6].

Objective: The differences between tumour and physiological angiogenesis have been the subject of extensive research. Nevertheless, differences in the molecular mechanisms of angiogenesis between endometriosis and endometrial carcinoma remain relatively unspecified. For this reason, the goal of our work was the identification and characterization of factors that are key for angiogenic processes in these pathological conditions.

Material & Methods: As part of our research, we analysed mRNA sequencing data in Gene Expression Omnibus (GEO) databases in the initial phase, focusing on angiogenic factors (VEGF, PLGF, ANG1, TGFβ, ENG, NRF2, NOTCH3) in patients with endometrial cancer of *corpus uteri* and endometriosis of *corpus uteri*. Subsequently, gene expression experiments were performed. The experimental group consisted of patients with histologically confirmed endometrial carcinoma of the uterine body and patients with histologically confirmed endometriosis. After the isolation of total mRNA from endometrial tissue, mRNA was transcribed into cDNA using a commercial ProtoScript® II First Strand cDNA Synthesis Kit. Gene expression quantification was detected by real-time PCR using the SensiMix™ SYBR® No-ROX kit. Data obtained were analysed using GraphPad Prism version 8 software (GraphPad

Software). The Mann Whitney U test was used for the statistical analysis of the obtained data. A p-value of 0.05 was defined as a statistically significant difference between the analysed groups.

Results: The Gene Expression Omnibus (GEO) database was searched using the keywords 'endometrial tissue,' 'endometriosis,' and 'endometrial carcinoma of *corpus uteri* and endometriosis of *corpus uteri*.' The selection was made only for human sequencing analyses to ensure the relevance of the results to our study. Out of the seven analysed genes, results from data processing in the GEO databases indicate significant changes in the expression of four genes. Within the NOTCH3, PLGF, and TGFβ genes, we noted significant upregulation in endometrial carcinoma tissue compared to endometriosis. The NRF2 gene was downregulated in endometrial carcinoma tissue compared to endometriosis. Statistical analysis of mRNA relative gene expression in endometrial tissue showed a significant change between the monitored groups in only two analysed genes, where we recorded a statistically significant upregulation of the PLGF gene and downregulation of the NRF2 gene in endometrial carcinoma tissue versus endometriosis.

Summary & Conclusion: Our study revealed significant differences in the expression of genes associated with angiogenesis in patients with endometrial cancer and endometriosis. These findings may contribute to the understanding of the pathophysiological mechanisms of these gynaecological diseases from the perspective of angiogenesis, and also indicate that the analysed parameters have the potential to be key subjects of future research in the context of these pathologies.

References:

1. Begum et al., Front. Cell Develop Biolog 9:780510, 2021.
2. Li et. al., Cell Death Discovery 8(1):29, 2022.
3. Okada et. al., Uterine Endometrial Function 83-103, 2016.
4. Olsen et. al., Clin. Biochem Rev 38(3):131-142, 2017.
5. Garcia-Gómez et al., Front. Endocrinol. 10:935, 2020.
6. Fallah et. al., Biomed & Pharmacother. 110:775-785, 2019.

Support & Funding: This study was supported by projects VEGA1/0435/23 and APVV-22-0357.

P24:

Biological activity of lichens extracts *Cladonia stellaris* and *Pseudevernia furfuracea*

BAČKOROVÁ MIRIAM[1], BENKOVÁ-RYBÁROVÁ ZUZANA[1], PETROVOVÁ EVA[2]

[1] Department of Pharmaceutical Technology, Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy in Košice, Slovakia, miriam.backorova@uvlf.sk
[2] Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Košice, Slovakia

Background: Lichens are a unique type of plants in nature. It is a symbiotic organism with a stable and peculiar structure, consisting of a fungal partner (mycobiont) and an algal or cyanobiont partner (photobiont). Most mycobionts are ascomycetes, and a few are basidiomycetes, deuteromycetes, and

phycomycetes, while photobionts are prokaryotic cyanobacteria and eukaryotic green algae. They interact positively with each other through a symbiotic relationship. A unique group of natural organic substances consists of the products of the secondary metabolism of lichens, which are characterised by significant biological effects. They are characterised by antimicrobial, antibacterial, antiproliferative, anti-inflamatory and antioxidant properties. Due to their metabolites, they may be beneficial in the treatment of cancer.

Objective: This study deals with the biological effects of lichens and their possible benefit in the therapy of oncological diseases. It evaluates the antiangiogenic, antioxidant and vasoactive effects of extracts from the lichens *Cladonia stellaris* and *Pseudevernia furfuracea*. The antioxidant effects were monitored by the DPPH radical method and the FRAP method. Content substances, especially flavonoids and polyphenols, are related to antioxidant effects. The total amounts of these substances *Cladonia stellaris* and *Pseudevernia furfuracea* lichens were determined by spectrophotometric methods. The antiangiogenic activity of the extracts was evaluated using the Japanese quail CAM model *ex ovo* culture. Vasoactivity and irritant potential of lichen extracts were monitored using the domestic chicken CAM model by the *in ovo* method.

Material & Methods: The lichen species *Cladonia stellaris* and *Pseudevernia furfuracea* were used in the determination. *Cladonia stellaris* was obtained from the herbarium items of the Department of Pharmaceutical Technology, Pharmacognosy and Botany UVLF in Košice. *Pseudevernia furfuracea* was collected on Minčol of the Čergov Mountains at an altitude of 1157.2 m.a.s.l. The lichen drug was pulverized in an electric grinder. It was extracted with 500 ml of methanol for 60 minutes in an ultrasonic bath (KRAITEK 18, Germany) at a temperature of 40°C. The extract was filtered and the filtrate was transferred to a round bottom flask and concentrated to dryness in a rotary vacuum evaporator (Heidolph, Hei-VAP Pression, Germany). The dry extract was weighed and redissolved in 10 mL of methanol and allowed to evaporate. A dry extract containing 1 g of the drug in 1 ml of methanol was obtained.

Results: In the determination of total flavonoids and polyphenols, *Pseudevernia furfuracea* was found to contain more flavonoids and *Cladonia stellaris* to contain more polyphenols. Higher antioxidant activity of P*seudevernia furfuracea* extract in both antioxidant methods, where the % inhibition of DPPH radical was 86.69% and value of the FRAP was 1.61. From the perspective of the assessment of angiogenesis on the CAM model, there was a significant inhibition of vessel density after the application of *Cladonia stellaris* extract at a concentration of 200 µg/ml. Total vessel length was not significantly affected and vessel branching was significantly affected by *Pseudevernia furfuracea* extract at a concentration of 200 µg/ml. During monitoring of the irritant potential according to Luepke's method it came to a conclusion that the used concetrations caused practically none and slight effects on the CAM vessel model.

Summary & Conclusion: The lichen species *Cladonia stellaris* (CLA) and *Pseudevernia furfuracea* (PSE) were monitored and the antioxidant, antiangiogenic activity and irritant effect of lichen extracts on CAM vessels were evaluated. From the results obtained in the experimental measurements, it is possible to conclude that the extract from the lichen CLA had a higher content of polyphenols, while the extract from the lichen PSE contained a higher amount of flavonoids. Using the methods of determining antioxidant activity, it was found that both types of analyzed lichens show antioxidant activity, while the PSE extract showed a higher antioxidant activity in both methods compared to the CLA extract. From the point of view of the evaluation of antiangiogenic activity, it was concluded that the CLA extract at a concentration of 200 µg/ml significantly inhibited the vessel density of the CAM model. PSE extract at a concentration of 200 µg/ml significantly inhibited vessel branching. The observed effects of lichens could be beneficial to the treatment of oncological diseases in particular.

References:

1. Luepke, Food Chem Toxicol 23: 287 – 291, 1985.

2. Nowak-Sliwinska et al., Angiogenesis 17: 779 – 804, 2014.
3. Zhao et al., J. Functional Foods, 80, 104283, 2021.

Support & Funding: This study was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic (VEGA 1/0074/24) and Slovak Grant Agency KEGA under contracts No. 003UVLF-4/2024.

P25:

Indole phytoalexins as inducers of oxidative stress-associated cell death: a study on cancer cells *in vitro*

MISKUFOVA VIKTORIA[1*], ZIGOVA MARTINA[1], MARIANA BUDOVSKA[2], RADKA MICHALKOVA [1*]

[1]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia, viktoria.miskufova@student.upjs.sk, martina.chripkova@upjs.sk, radka.michalkova@upjs.sk
[2]Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, Pavol Jozef Šafárik University, Slovakia; mariana.budovska@upjs.sk
* These authors contributed equally to this work

Background: Cervical cancer is one of the most common malignant diseases affecting women worldwide. With an estimated 700 new cases annually in Slovakia and 600,000 new cases globally, it ranks as the fourth most prevalent cancer in women [1]. The majority of cases are caused by the sexually transmitted human papillomavirus (HPV), responsible for the majority of cervical cancer cases [2]. Cervical cancer remains a significant public health issue, especially in developing countries due to limited access to screening and healthcare. Natural compounds originating from plants have proven to be promising scaffolds for the synthesis of new bioactive substances with potential anticancer properties. Among such molecules are indole phytoalexins, secondary metabolites of plants primarily from the Brassicaceae family [3]. These compounds have demonstrated various biological effects, including antimicrobial, antifungal, antiparasitic, antiproliferative, antiangiogenic, and anticancer activities [4-6]. For this reason, studying the mechanisms of these promising molecules can significantly contribute to chemoprevention or the treatment of oncological diseases.

Objective: The main goal of our experimental work is to study the impact of synthetic indole phytoalexin on the proliferation of cervical cancer cells in in vitro conditions. Partial objectives include evaluating the metabolic and antiproliferative activity of the tested compound on tumor cells and analyzing the potential mechanism of its action, which involves monitoring the ability of indole phytoalexin to induce apoptosis using specific markers. Another objective was to study the effect of the selected compound on the progression of the cell cycle of HeLa cancer cells by analyzing changes in cell cycle phases and associated proteins. Significant proteins targeted in our study included apoptosis markers and signaling pathways involved in cell death, proliferation, and survival. Another primary objective was to monitor the involvement of oxidative stress in these mechanisms using the antioxidant agent N-acetylcysteine.

Material & Methods: To assess the antiproliferative activity of indole phytoalexin MB-591 on HeLa, human cervical adenocarcinoma cells, and Bj-5ta cells, DMSO (negative control) and cisplatin (positive control) served as controls and NAC as an antioxidant. Viability changes were monitored using the metabolic MTT test, measuring absorbance changes over cells, where metabolized MTT tetrazolium salt forms insoluble formazan. The absorbance change post-crystal dissolution is proportional to mitochondrial enzyme activity change. Preliminary MTT results guided a BrdU proliferation assay,

confirming changes in proliferation induced by the compound at various concentrations via BrdU incorporation into DNA. For a detailed understanding, flow cytometry evaluated cell viability, cycle, protein levels, and structure/organelle activity using PI, Annexin V, DHR123, TMRE, etc. Western blot assessed protein levels and phosphorylation, utilizing specific primary and HRP-conjugated secondary antibodies, with β-actin as a loading control. These methods provide a comprehensive insight into the impact of indole phytoalexin MB-591 on cervical cancer cell proliferation, shedding light on potential therapeutic applications.

Results: The study of the antiproliferative effect of the tested indole phytoalexin using the MTT test and BrdU assay revealed a dose-dependent inhibition of HeLa cancer cell proliferation by MB-591 in the concentration range of 1 to 10 μmol/L. The calculated IC50 was 6.87±0.56 μmol/L, and for subsequent experiments, a concentration of 7 μmol/L was used. In combination with NAC at a concentration of 2.5 mmol/L, an antagonistic effect of NAC against the studied substance was observed. Flow cytometry demonstrated dose-dependent MB-591-induced damage to mitochondrial functions, apoptosis induction, and phosphatidylserine externalization after 72 hours of exposure. A significant decrease in cells in the G1 phase occurred after 72 hours, accompanied by cycle arrest in the S phase. Western blot analysis showed a significant increase in apoptosis and DNA damage markers, such as the irreparable increase in cleaved PARP, phospho-histone H2A-X, and others. Cell cycle changes were likely associated with the modulation of cell cycle and proliferation-regulating proteins, including p21, Rb, and PCNA. Furthermore, changes in phosphorylation and protein levels associated with autophagy were monitored, such as the reduction in phospho-PTEN or an increase in LC3A/B levels. Many of these effects of MB-591 were modulated by NAC. The effects of the studied substance NAC and their combination were monitored after 3, 6, 12, 24, 48 and 72 hours, and a significant involvement of ROS in the potential anticancer effect of MB-591 was shown.

Summary & Conclusion: Indole phytoalexins have long been considered promising compounds for their antiproliferative effects. We observed the modulatory impact of indole phytoalexin on the cell cycle, its regulatory proteins, apoptosis, and autophagy in cervical cancer cells [7]. The dose and time-dependent effects of the studied substance on tumor cell survival were demonstrated. The potentially anticancer agent, MB-591, significantly induced the mitochondrial apoptotic pathway, autophagy likely initiated as a protective mechanism, and cell cycle arrest [8-10]. The antioxidant NAC exhibited a protective effect against MB-591-induced cytotoxicity, quenching reactive oxygen radicals generated by the tested substance. In combination with NAC, there was a decrease in levels of proapoptotic proteins and other markers of regulated cell death. NAC antagonized the effects of MB-591 on changes in cell distribution during the cell cycle and modulated MB-591-induced autophagy [11]. Although our study demonstrated excellent antiproliferative effects of the indole phytoalexin with high selectivity against tumor cells, further research is needed to gain a comprehensive understanding of its mechanism and clinical application.

References:

1. Sung H et al., CA Cancer J Clin, 71(3):209-249; 2021.
2. de Martel et al., Lancet Glob Health, 8 (2):e180-e190; 2020.
3. Klein AP and Sattely ES, Proc Natl Acad Sci U S A, 114 (8):1910-1915; 2017.
4. Nguyen et al., Planta 255(6):116; 2022.
5. Chripkova M et al., Molecules, 21(12); 2016.
6. Budovská et al., Tetrahedron, 143:133573; 2023.
7. Budovská et al., Tetrahedron, 120:132898; 2022.
8. Chripkova, M et al, Toxicol In Vitro, 28 (5):909-15; 2014.
9. Tischlerova et al., World J Gastroenterol, 23(24):4341-4353; 2017.
10. Marianna et al., Molecules, 28(1); 2022.
11. Hong T et al., Cells, 10 (2); 2021.

Support & Funding: This research was funded in part by the Grant Agency of the Ministry of the Education, Science, Research and Sport of the Slovak Republic VEGA 1/0653/19, VEGA 1/0498/23 and VEGA 1/0446/22 and the Slovak Research and Development Agency under the contract No. APVV-16-0446. Moreover, this publication is the result of the project implementation: "Open scientific community for modern interdisciplinary research in medicine (OPENMED)", ITMS2014+: 313011V455, supported by the Operational Programme Integrated Infrastructure, funded by the ERDF. Furthermore, this publication is the result of the project implementation: "Medicínsky univerzitný vedecký park v Košiciach (MediPark, Košice—Fáza II.)", kód ITMS2014 + 313011D103 supported by the Operational Programme Research & Innovation, funded by the ERDF. We would like to thank Mgr. Martina Hájiková for her excellent technical assistance.

(picture by Šemeláková Martina, Grindavík, Iceland)

Student scientific international conference "GenICa"
Book od Abstracts

Editor: RNDr. Martina Šemeláková PhD.

Publisher: Pavol Jozef Šafárik University in Košice
Publishing ŠafárikPress

Year: 2024
Pages: 44
Author's sheets: 3,5
Edition: first

DOI: https://doi.org/10.33542/SSI-0333-3
ISBN 978-80-574-0333-3 (e-publication)