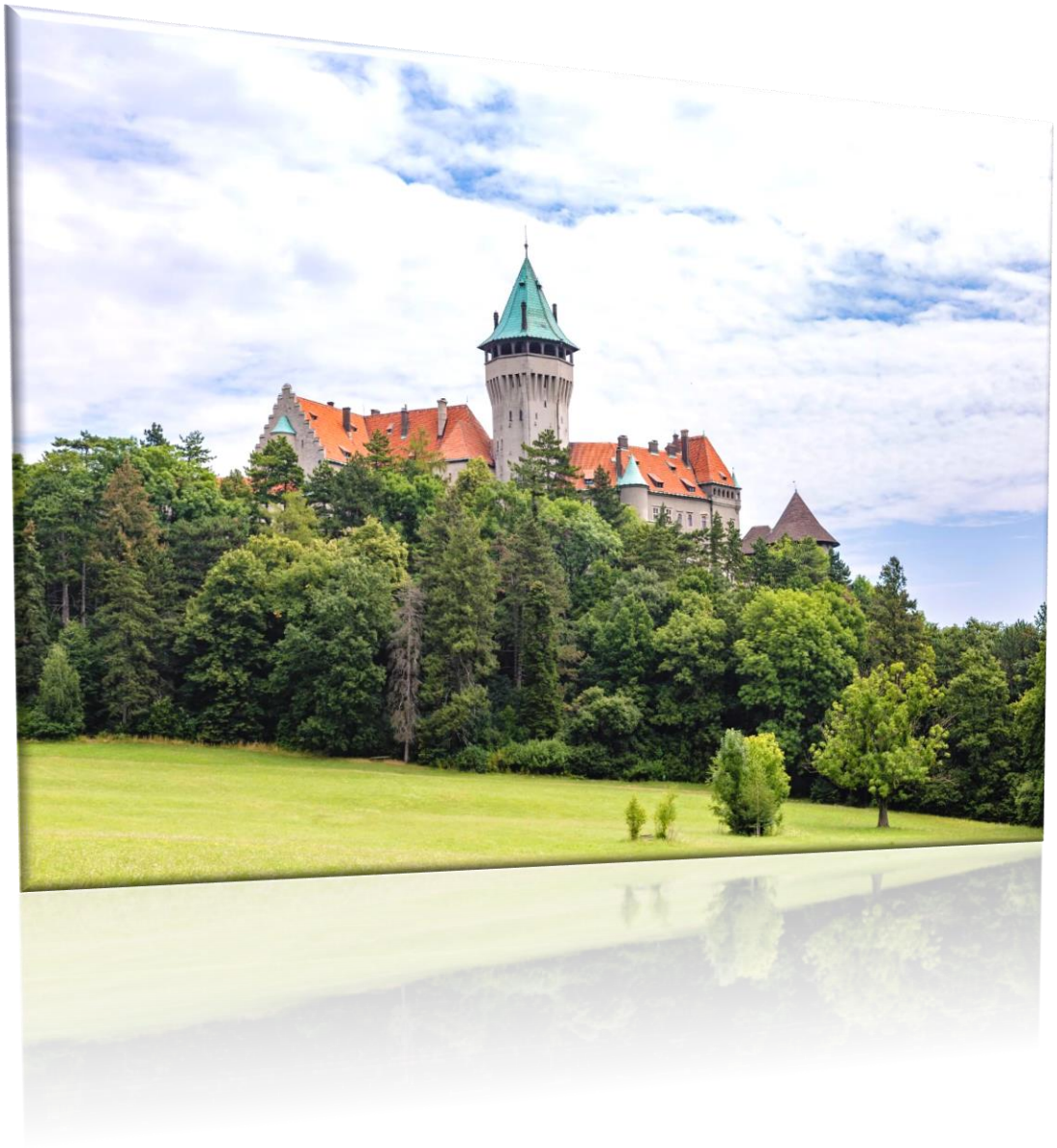

Genetic Toxicology and Cancer Prevention

Book of Abstracts

Smolenice, June 12-15, 2023

Czech and Slovak Environmental Mutagen Society

Book of Abstracts

Genetic Toxicology and Cancer Prevention

Smolenice, June 12-15, 2023

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Published by

ISBN 978-80-972247-9-0

ČESKO-SLOVENSKÁ
BIOLOGICKÁ SPOLEČNOST, z.s.

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This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 857381


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Program

Monday, June 12, 2023

15:00 – 15:10 Opening ceremony

15:10 – 15:30 Laudatio MUDr. Radim Šrám, DrSc.

Chairs: Andrea Bábelová a Jiří Rubeš

15:30 – 16:00 Miroslav Machala
Sphingolipidomics of colon cancer

16:00 – 16:30 Coffee break

16:30 – 16:50 Simona Kajabová
Effects of complex airborne environmental mixtures on sphingolipid and eicosanoid composition in human bronchial epithelial cells

16:50 – 17:10 Katarína Kozics
Effect of thymol and its derivatives on colorectal cell lines in *in vitro* system

17:10 – 17:30 Eva Sedlačková
The chorioallantoic membrane (CAM) assay as a relevant *in vivo* model to study the effect of thymol on colorectal tumor progression

17:30 – 17:50 Monika Šramková
Risk assessment of innovative nanohydrogels developed for skin regeneration applications

17:50 – 18:10 Lucia Bálintová
3D skin *in vitro* model development for human skin wound healing testing

18:10 – 19:30 Dinner

19:30 – Departure from KC Smolenice

20:00 – **Social program: Včelovina**

Tuesday, June 13, 2023

Chairs: **Andrea Rössnerová a Miroslav Machala**

9:00 – 9:30 Andrea Ševčovičová
Mechanisms of action of bisphenol A on eukaryotic cells

9:30 – 9:50 Ivana Ďurovcová
Use of the model organism *Caenorhabditis elegans* to evaluate the toxic effects of poly- and perfluoroalkyl substances

9:50 – 10:10 Jitka Pavlíková
Epigenetic mechanisms of the influence of early maternal care on depressive disorder in adulthood

10:10 – 10:30 Monika Mesárošová
Toxicity assessment of xenobiotics: A comparison of 2D and 3D *in vitro* models

10:30 – 11:00 Coffee break

11:00 – 11:30 Pavel Rössner Jr.
A novel *in vitro* approach to evaluate biological impacts of environmental air pollution

11:30 – 11:50 Kateřina Hoňková
Epigenetic alterations in genome-wide DNA methylation in newborns from air-polluted industrial locality

11:50 – 12:10 Antonín Ambrož
Impact of environmental pollutants to oxidative stress and antioxidant markers in mothers and newborns (Continue of the long-term research in the Czech Republic)

12:10 – 12:20 Anton Bujňák
Chromosome aberrations in selected groups of coke oven workers

12:20 – 13:30 Lunch

Chairs: **Andrea Ševčovičová a Jan Topinka**

13:30 – 13:50 Soňa Marvanová
Neglected polycyclic aromatic sulphur heterocycles: benzo[b]naphtho[d]-thiophenes and naphthylbenzo[b]thiophenes

13:50 – 14:10 Táňa Závodná
Personal exposure monitoring to polycyclic aromatic hydrocarbons bound to dust particles of different size fractions

14:10 – 14:30 Katarína Gerčáková
Novel triorganotin compounds exert a significant cytotoxic effect on breast cancer-derived cells

14:30 – 14:50 Michal Šelc
The antifibrotic effect of silibinin-coated gold nanoparticles against liver fibrosis in mouse

14:50 – 15:00 Klára Červená
Plasma KRAS mutations as early diagnostic biomarkers for pancreatic cancer in high-risk group patients

15:00 – 15:30 Coffee break

15:30 – 16:00 Andrea Rössnerová
Genetic alteration profiling in women acutely exposed during the processing of dental nanocomposites

16:00 – 16:20 Zuzana Šímová
Transcriptome changes in humans acutely exposed to nanoparticles during dental treatment

16:20 – 16:50 Nikoleta Vargová
Advances in transcriptomics for cancer research: unveiling insights through single-cell sequencing

17:00 – 18:30 **Poster presentation**

18:30 – 19:30 Dinner

20:00 – **Social program: Slovak wine tasting – Little Carpathian region**

Wednesday, June 14, 2023

Chairs: **Monika Šramková a Boris Bilčík**

9:00 – 9:30 Alena Gábelová
Residual metal nanoparticles accumulated in the body induce late toxic effects and alterations in transcriptional and miRNA landscape

9:30 – 9:50 Michal Šíma
The effect of metal nanoparticles on gene expression of mouse mesenchymal stem cells

9:50 – 10:10 Barbora Svitková
Plate reader spectroscopy as a possible substituent for atomic absorption spectroscopy in the quantification of the cellular uptake of the nanoparticles

10:10 – 10:20 Majlinda Meta
An avian CAM model of cervical cancer and its diagnosis and treatment using PDT

10:20 – 10:50 Coffee break

10:50 – 11:10 Kristína Jakič
Distribution, accumulation and biological effects of gold nanoparticles *in vivo*

11:10 – 11:30 Petra Mazancová
Effective reduction of SARS-CoV-2 RNA levels using a tailor-made therapeutic oligonucleotide

11:30 – 12:00 Miroslav Piršel
DNA repair: a punishment or reward?

12:00 – 13:30 Lunch

13:45 – Indoor/outdoor activities (Driny cave, hiking)

19:00 – **Conference dinner**

Thursday, June 15, 2023

Chairs: **Hanka Lehocká a Pavel Rössner Jr.**

9:00 – 9:30 Pavel Vodička
Genomic instability in adenomas and colorectal cancer progression

9:30 – 9:50 Soňa Vodenková
Association of mitochondrial DNA copy number and telomere length with colorectal cancer patient outcomes

9:50 – 10:10 Michal Kroupa
Reduced methylation of olfactory receptor genes and amplification of 6p25.1-p22.3 as specific epigenetic and genetic alterations in colorectal cancer liver metastases

10:10 – 10:40 Coffee break

10:40 – 11:10 Jiří Rubeš
Influence of air quality in urban agglomerations on the sperm quality of their residents

11:10 – 11:30 Pavel Svitok
Current possibilities for infertility treatment in oncology patients

11:30 – 12:00 Andrea Bábelová
Periostin – a new candidate for biomarker in CKD progression

12:00 – 12:30 Best poster award
Discussion – elections / new members
Closing ceremony

12:30 – 13:45 Lunch

14:00 – Departure from KC Smolenice

Abstracts

Sphingolipidomics of colon cancer

Miroslav Machala[1], Ondrej Kovac[1], Josef Slavik[1], Simona Strapacova[1], Jana Slovackova[1], Jan Vondracek[2]

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Sphingolipids (SLs) and glycosphingolipids (GSLs) are important components of cell membranes and membrane microdomains; they also act as intracellular signaling molecules. Deregulation of tumor cell lipidome may thus contribute both to the alteration of lipid signaling and modulation of membrane properties in tumor cells. Major genetic changes linked to colorectal cancer (CRC) include mutations in APC, KRAS, TP53, and mismatch repair genes. Additionally, modifications of expression of protooncogenes (c-Fos, c-Jun, c-Myc) and related intracellular signal transduction pathways have been reported in CRC. However, the functional links among regulatory pathways and changes in SL/GSL metabolism remain poorly characterized. We are missing particular information about: 1. differences among SL/GSL levels and expression of genes/proteins related to SL/GSL metabolism in tumor cells vs. adjacent non-tumor tissues; 2. mechanisms regulating expression and activity of genes of SL/GSL metabolism both in normal colon tissue and during colon carcinogenesis; 3. roles(s) of related biosynthetic pathways, including de novo fatty acid synthesis in deregulation of SL/GSL levels; 4. potential functional roles of SLs and GSLs during colon cancer development. So far, only limited characterization of specific changes in lipid metabolites and genes/proteins has been reported. The molecular basis of regulation of SL/GSL metabolism is largely unknown, although several studies suggested possible roles of transcription factors related to epithelial-mesenchymal transition in alterations of gene expression and chaperone proteins in induction of enzymatic activities. Upregulation of fatty acid synthesis in CRC and/or glucose metabolism might be also interconnected with induced SL and GSL biosynthesis. Taken together, specific patterns of SL/GSL levels could be linked with carcinogenesis, and newly identified metabolites and genes could become promising CRC biomarkers and/or potential therapeutic targets. The proposed lecture is intended to summarize recent advances in (sphingo)lipidomics and related SL/GSL metabolic gene expression studies in CRC and colon cancer cells.

Supported by the Ministry of Health of the Czech Republic, grant No. NU21-03-00421.

Effects of complex airborne environmental mixtures on sphingolipid and eicosanoid composition in human bronchial epithelial cells

Simona Kajabova[1,3], Martina Hyzdalova[1], Ondrej Kovac[1], Katerina Pencikova[1], Martina Parenicova[1], Miroslav Ciganek[1], Jan Vondracek[2], Miroslav Machala[1]

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The toxic effects of airborne particles have been evaluated in numerous studies. Profiling of airborne particles and their organic extracts has revealed multiple types of adverse effects, including activation of aryl hydrocarbon receptor (AhR), induction of xenobiotic-metabolizing enzymes, various oxidative, genotoxic and mutagenic effects, inflammatory responses, or cell death. Sphingolipids (SLs) and glycosphingolipids (GSLs) are known as important structural components of cell membranes, including lipid rafts, as well as bioactive lipid signaling molecules playing numerous roles in cell (patho)physiology. SLs and GSLs can regulate cell growth, proliferation, survival, senescence, apoptosis, as well as the processes linked with cancer progression.

In this study, we used human bronchial epithelial HBEC-12KT cells in order to study the effects of complex organic mixtures derived from standard reference material representing urban airborne particles (SRM1649b). We combined the analyses of the effects of crude extract (CE) with the evaluation of the impact of its chromatographic fractions (F1-3) in cytotoxic and non-cytotoxic concentrations after 24-hour exposure. We examined the effects on SL/GSL and eicosanoid levels, expression of the genes linked to lipid metabolism and cell cycle distribution.

The cytotoxic concentrations of CE and F3 have shown significantly higher AhR activity, resulting in induced degradation of AhR. Metabolites from the AhR metabolomic pathway were observed to have a genotoxic effect by decreasing p21 protein levels, a protein responsible for cell cycle arrest, and an increase in DNA damage marker - histone H2AX, indicating more extensive DNA damage. Next, we investigated the deviations in SL/GSL metabolism. We found a significant increase in dihydroceramides (dhCer) and ceramides, and also a significant decrease in sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P), accompanied by the deregulations of expressions of some corresponding genes (CERS2, ASAH1, CERK). This switch in the ratio of Cer/S1P plus an increase of dhCer could indicate a shift towards apoptosis. We also detected changes in GSL, both hexosylceramides and lactosylceramides were suppressed. These alterations were predominantly observed in the cytotoxic concentrations of CE and F3, but to a certain extent, similar changes were also observed in the non-cytotoxic concentrations.

Altogether, we have observed genotoxic effects, extensive changes in the SL/GSL metabolism, expression of linked genes after the exposure, suggesting that the organic extracts of SRM1694b in cytotoxic concentrations cause strong adverse effects in cells and shift the cell machinery towards cell death.

Funded by the Czech Science Foundation, project no. 17-27669S.

Effect of thymol and its derivatives on colorectal cell lines in *in vitro* system

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The beneficial effects of thymol (TY), a naturally occurring phenol monoterpene of essential oil from thyme, on human health are well known for many years. It is widely used in medical practices, cosmetics, agriculture, and as a natural remedy. However, due to its low solubility in aqueous media, its use e.g. in the food industry is limited. Our study is focused on the synthesis of hydrophilic derivatives of TY (acetic acid thymol ester, thymol β-D-glucoside) while the antioxidative and antiproliferative properties, as well as the effective cellular uptake, will remain intact. The biological activity was studied using colorectal cell models cultured *in vitro* (HT-29 and HCT-116).

The cytotoxic effect of the new derivatives on the colorectal cancer cell lines HT-29 and HCT-116 was assessed via MTT assay. The genotoxic effect was determined by comet assay and micronucleus analysis. ROS production was evaluated using ROS-Glo™ H_2O_2 assay. We confirmed that one of the thymol derivatives (acetic acid thymol ester) has the potential to have a cyto/genotoxic effect on colorectal cancer cells, even at much lower (IC50 ~ 0.08 µg/ml) concentrations than standard thymol (IC50 ~ 60 µg/ml) after 24 h of treatment. On the other side, the genotoxic effect of the second studied derivative - thymol β-D-glucoside was observed at a concentration of about 1000 µg/ml. The antiproliferative effect of studied derivatives of thymol on the colorectal cancer cell lines was found to be both dose and time-dependent at 100 h. Moreover, thymol derivatives treated cells did not show any significantly increased rate of micronuclei formation. New derivatives of thymol significantly increased ROS production too.

The results confirmed that the effect of the derivative on tumor cells depends on its chemical structure, but further detailed research is needed. However, thymol and its derivatives have great potential in the prevention and treatment of colorectal cancer, which remains one of the most common cancers in the world.

This study is based upon work from project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, VEGA grant 2/0055/20 and a Grant program for SAS PhD. students-APP0410.

The chorioallantoic membrane (CAM) assay as a relevant *in vivo* model to study the effect of thymol on colorectal tumor progression.

Eva Sedlackova[1], Maria Makovicka[1], Peter Makovicky[1], Michaela Blazickova[1], Maria Bartosova[2]

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It is obvious that preclinical *in vivo* studies are essential for discovering new therapeutic agents, however, rodent models are time-consuming, expensive, and require approval from the Animal Ethical Committee. Recently, the avian embryo chorioallantoic membrane (CAM) model was well described in human medicine as a cost-effective, easy-to-perform preclinical oncological model for observing pro- and antiangiogenic response, nanotoxicology, tumor biology, and metastasis.

One of the aims of this work was to demonstrate the alternative *in vivo* technique in which the highly vascular and accessible chorioallantoic membrane of Japanese quail was used. The second goal of this work was to monitor the tumor growth from the colon cancer cell line (HCT 116). A new approach to the treatment and prevention of colon cancer may be based on the use of natural substances and because of that, we treated the HCT116 cell line with thymol and applied this on CAM. Subsequently, we observed the changes in tumor growth and in angiogenesis after the application of thymol.

Our study demonstrates that the colon cancer cell line (HCT 116) can form solid, vascularized tumors on the CAM. After the application of thymol the size of the tumor decreased. The thymol also had an effect on the vascular network of the CAM. Tumor invasion could be demonstrated by both histological and optical sectioning. We can conclude that thymol has great potential in the prevention and treatment of colorectal cancer.

This study is based upon work from project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, and VEGA 2/0055/20.

Risk assessment of innovative nanohydrogels developed for skin regeneration applications

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Skin is the largest organ of the human body with diverse functions and any skin impairment leads to a cascade of events, known as wound healing. Infections or pre-existing conditions, such as obesity or diabetes often delay the healing process, resulting in the development of chronic wounds. An ideal wound dressing material should be able to protect the wound from bacterial infections, prevent excessive fluid loss, maintain a moist healing environment and promote the healing process.

Nanotechnology-based materials incorporated into scaffolds allow the creation of nanocomposite smart materials with unique physicochemical and biological properties promoting skin regeneration. The aim of the TENTACLES project is to develop an innovative multifunctional nanogel that integrates the protective (polymer-based nanohydrogel) and healing functions (iron oxide nanoparticles and targeted miRNA) within one nanocomposite smart structure. Our task is a comprehensive assessment of the biological effect of these nanocomposites using different types of skin cells (keratinocytes and fibroblasts) as well as 3D EpiDermFT skin model. The experiments are focused on determining the cytotoxic and genotoxic effect of three hydrogels (Alginate, Pluronic F-127, and Gelatin methacrylate - GelMA) with a different chemical compositions and iron oxide nanoparticles content.

A significant increase in both cytotoxicity and genotoxicity after 24 h nanohydrogel treatment, measured by LDH assay, micronucleus test, and comet assay, was observed only in higher concentrations of GelMA nanohydrogel. Moreover, we noticed a higher amount of apoptotic and necrotic cells after GelMA exposure, and also the percentage of micronuclei was significantly higher. Using H&E staining, we determined that nanohydrogels exposure did not cause any histopathological changes in skin structure.

These results suggest that above mentioned hydrogels loaded with iron oxide nanoparticles could be promising candidates for wound dressings as they do not show any toxic effects, but further investigation is essential for their implementation in practice.

This work was supported by ENM III/2019/861/TENTACLES; DoktoGrant no. APP0316 and project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, CA21108 - European Network for Skin Engineering and Modeling and VEGA No. 2/0121/21.

3D skin *in vitro* model development for human skin wound healing testing

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The use of animal models for various compound testing has contributed to the knowledge of skin diseases, wound healing, and the development of new therapeutic options. However, human *in vitro* skin models, which include essential cellular and structural components would reduce animal experiments during the preclinical evaluation of novel therapeutic approaches.
The development of an HSC (human skin constructs) model is a challenge for many laboratories. HSCs are required for biomaterials, pharmaceuticals, cosmetics *in vitro* testing, and also for the development of complex skin wound therapeutics. The developed model should present a well-differentiated epidermis and dermis similar to the native human skin.

The aim of this work was to develop an HSC composed of all of the epidermis-dermis layers. Different 3D cell culture conditions were tested to optimize HSC maturation, using various combinations of component-free or fully defined media, and air-liquid interface (ALI) culture. Optimized culture conditions allowed the production of HSC by culturing human foreskin fibroblasts (HFFs) embedded in rat tail collagen I for 2–3 days in a fibroblast medium supplemented with FBS and antibiotics. After that culturing keratinocytes (HaCaTs) on top of the collagen for 3 days in a keratinocytes medium. Co-culture was then submerged overnight in a differentiation-formulated medium to stimulate cell-cell contact formation and finally placed at ALI for 15–20 days. Histological analysis revealed uniform distribution of HFFs in the dermal layer and their typical elongated morphology. The epidermal compartment showed a multi-layered differentiated structure.

The developed HSC represents a fundamental *in vitro* tool to evaluate biocompatibility
of biomaterials, safety, pharmacotoxicity, and effectiveness, as well as to investigate skin biology, skin disease pathogenesis, wound healing, and skin infection.

This work was supported by ENM III/2019/861/TENTACLES; DoktoGrant no. APP0316, project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, CA21108 - European Network for Skin Engineering and Modeling and VEGA No. 2/0121/21.

Mechanisms of action of bisphenol A on eukaryotic cells

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Bisphenol A (BPA) is the main component of the most commonly used plastic products, such as disposable plastics, beverage containers, cans, protective sports or medical devices. Its frequent use and ability to be released into the environment have led to BPA becoming an environmental pollutant detectable in soil, water, and air. BPA is known as an endocrine disruptor for its ability to disrupt hormonal balance. However, studies from the last few decades suggest that BPA negatively affects all organisms, including those that lack an endocrine system, such as yeast and plants. In these organisms, BPA mainly acts by increasing oxidative stress, which can subsequently lead to damage to important biomacromolecules in cells.

In our laboratory, research is mainly focused on elucidating the mechanism of BPA action in cells and its genotoxic potential. We mainly use yeast (*Saccharomyces cerevisiae, Schizosaccharomyces pombe*) and plants (*Hordeum vulgare* L.) as model organisms. Our goal is also to search for suitable and effective ways to remove BPA from the environment.

This work was funded by project VEGA 1/0460/21.

Use of the model organism *Caenorhabditis elegans* to evaluate the toxic effects of poly- and perfluoroalkyl substances

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The use of *Caenorhabditis elegans* as a model organism offers many advantages – it has a short life cycle in which it produces a large number of offspring; the constant number of somatic cells; a transparent body; it is a hermaphrodite and, last but not least, it is easy to cultivate. All this predestines it for easy use in toxicological research.
The main objective of the project, which took place at a foreign institution (Environmental Health and Toxicology Laboratory, Vrije Universiteit in Amsterdam), was to study persistent mobile substances known as per- and polyfluoroalkyl substances (PFAS). PFAS are widely used substances that are used in the production of foam fire extinguishers, non-stick kitchen utensils, or waterproof clothing. Consequently, PFAS can be found in the environment where bioaccumulation occurs. The first phase of the research was focused on monitoring the toxic effects of 11 commonly used PFAS. For further analyses, the substances with the strongest effect on the survival of *C. elegans* were selected, which we subjected to microscopic analysis thanks to strains labeled with fluorescent probes. The results showed that most of the studied PFASs have mild toxic effects that reduce the number of offspring and negatively affect nematode movement. In addition, we observed that the selected substances PFOS and PFDA can increase the level of intracellular reactive forms and destroy mitochondrial networks. PFAS can thus negatively affect the internal homeostasis and integrity of mitochondria, which is crucial for providing energy for the events taking place in all cells.

This work was funded by ZeroPM Project, Slovak Academic Information Agency, and project VEGA 1/0460/21.

Epigenetic mechanisms of the influence of early maternal care on depressive disorder in adulthood

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According to WHO data from 2023, approximately 3.8% of the world's population suffers from depression, while even its milder forms can have a significant impact on the quality of life of an individual and his family. In addition, this disorder is also associated with major socio-economic consequences, in the European Union alone the cost of reduced work productivity caused by depression is more than 170 billion euros per year.

Early maternal care is a key factor in later mental health. Children who have been exposed to inadequate care have, among other things, an increased risk of developing depressive symptoms in adulthood. This negative influence may be caused by epigenetic changes that may manifest in adulthood. Insufficient maternal care can affect the expression of many genes associated with stress regulation, serotoninergic and dopaminergic systems, neuroplasticity and cognitive functions. Such changes can increase sensitivity to stressful situations and reduce the ability to regulate emotions, increasing the risk of depressive disorders in adulthood. Understanding the epigenetic mechanisms associated with depression can help in the development of new and more effective therapeutic methods. These treatment methods based on new epigenetic findings include both drugs and less common therapeutic procedures such as transcranial magnetic stimulation or meditation exercises. In any case, treating depression is expensive, requires long-term therapy, and may only be effective temporarily. For these reasons, it is important to try to prevent depression in order to minimize its effects on the individual and society as a whole.

Prevention of inadequate early maternal care can help prevent unwanted epigenetic changes and is one of the important ways to reduce the risk of developing depression.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic project Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798), which is co-financed by the European Union (European Structural and Investment funds; Operation Programme Research, Development and Education).

Toxicity assessment of xenobiotics: A comparison of 2D and 3D in vitro models

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Animal models and conventional two-dimensional (2D) cell culture models have long been used to understand animal and human physiology and pathology. Cell-based assays play an important role in the drug development process and safety assessment of chemicals and drugs as a fast, cost-effective, and straightforward approach to reduce animal testing. 2D *in vitro* test systems based on monolayer cultures are associated with inherent limitations. Therefore, it is essential to develop alternative *in vitro* cell-based systems which offer an alternative platform for predicting the efficacy, toxicity, and pharmacokinetics of new drugs and can represent an innovative approach to screening for xenobiotic effects and reducing animal testing. 3D models (spheroids) have also proven to be very useful and promising tool.
In vivo, the liver and the kidney are the major organs in which xenobiotics are metabolized, transformed and play a key role in detoxification and their elimination from the body. They also represent two primary targets of the toxic effect of xenobiotics.

Two human cell lines, hepatocyte carcinoma cells (HepG2) and renal proximal tubule epithelial cells (TH1) were used to evaluate the biological activity of two chosen xenobiotics, aflatoxin B1 (AFB1) - a potent genotoxic hepatocarcinogen and ifosfamide (IFO) - a synthetic analog of cyclophosphamide that has a nephrotoxic effect, in various *in vitro* test systems (2D – monolayer; 3D model – HepG2 spheroids).
The objective of this study was to evaluate the cytotoxic and genotoxic effect after short-term (2h) as well as long-term (24h) cell exposure to xenobiotics (AFB1 and IFO). The cytotoxic effect was determined by MTT assay in 2D models (TH1 and HepG2) and by LDH assay in 3D spheroids (HepG2). The evaluation has shown that in 2D models 2h and also 24h exposure to IFO decreased the cell viability of both TH1 and HepG2, while the cytotoxic effect of AFB1 was detected only after long-term exposure in both cell lines. In 3D spheroids, the cellular cytotoxicity was measured only after 24h exposure to IFO.

The genotoxic effect was determined by comet assay (2D, 3D) and micronucleus analysis (2D). AFB1 and also IFO were able significantly to increase the level of DNA strand breaks in both *in vitro* systems, while the most damaging was the long-term IFO exposure. Chosen xenobiotics significantly increased the percentage of micronuclei in both cell lines after 2h and 24h treatment in 2D cell systems.
Consequently, we will also determine the changes in the expression of enzymes involved in the metabolism of xenobiotics, especially the P450 cytochrome complex.

As expected, our results showed different cell responses upon AFB1 and IFO treatment, confirming the differences between cell lines along with the culture conditions.

This study is based upon work supported by VEGA grant 2/0121/21, and project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381.

A novel *in vitro* approach to evaluate biological impacts of environmental air pollution

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Ambient air pollution negatively affects human health and contributes to the incidence of pulmonary, cardiovascular, and neurological disorders. Polluted air contains a mixture of gaseous pollutants, particulate matter (PM) and compounds bound to it. The size of the particles, along with the chemical composition of the complex mixture, determines the possible adverse biological effects of air pollutants. The presence of particles themselves contributes to the activation of the immune system, resulting in the production of reactive oxygen species (ROS) by the immune cells. Some of the compounds bound to PM, notably polycyclic aromatic hydrocarbons, are possibly carcinogenic. The presence of transition metals in PM further contributes to ROS generation and the oxidative damage of macromolecules. Gaseous components further potentiate pro-oxidant and pro-inflammatory properties, as well as the overall carcinogenicity of polluted air.

Although the assessments in human organisms provide the most reliable information on the health effects of air pollutants, such experiments are time-consuming, costly, and limited by ethical considerations. Thus, animal tests have been commonly used. However, the application of the 3R concept (replacement, reduction, refinement) in animal experimentation and differences between human and animal organisms necessitate alternatives to *in vivo* tests that use *in vitro* cell models.

For a comprehensive evaluation of the biological effects of air pollutants, the application of exposure experiments that involve a complete mixture of ambient air pollutants is crucial. These experiments are optimally conducted in cell cultures grown at the air-liquid interface (ALI). The ALI exposure method is considered superior to the application of particles in suspension as it exhibits a similar response at a significantly lower dose and allows avoiding artifacts caused by the collection of the particles and their suspension in a culture media.

In our research project, we investigate the toxicity of real-world complete ambient air in lung bronchi and olfactory mucosa (a proxy to brain effects) tissues from healthy and diseased donors (asthma, Alzheimer´s disease). The cells are grown at the ALI in our exposure system in the field conditions of localities differing in air pollution levels. We will analyze cytotoxicity, oxidative stress, immune response, and whole genome mRNA and miRNA expression changes. The results will contribute to understanding the biological effects of polluted air and differences in response in samples from healthy and diseased donors.

This work was supported by Czech Science Foundation (22-10279S).

Epigenetic alterations in genome-wide DNA methylation in newborns from air-polluted industrial locality

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Epigenetics is considered an environmental biosensor in public health. Specifically, DNA methylation is implicated as a mechanism linking early environmental exposures. This study is part of the project “Healthy Aging in Industrial Environment”, where we evaluated gene-specific genome-wide DNA methylation in 500 newborns from air-polluted and control locality.

Air-polluted district Karvina is characterized by coal and metallurgic industry with significant hot spot of air pollution in the Czech Republic and in Central Europe. The control district of Ceske Budejovice is located in the clean air area. During the sampling period (2019-2020), the concentration of benzo[a]pyrene was 3.95 ± 2.02 ng/m^3 in Karvina and 0.85 ± 1:18 ng/m^3 in Ceske Budejovice ($p < 0.01$). The DNA samples were isolated from frozen cord blood by the Miller salted-out method and processed on the microarray platform (Illumina Infinium MethylationEPIC BeadChip). Detailed questionnaires were collected from mothers.

A locality-specific DNA methylation pattern in newborns is slightly different. We found 1094 differentially methylated CpG loci (DML) between newborns from the industrial locality compared to the control locality (698 hypermethylated and 396 hypomethylated). The six CpG loci, all situated in the same region of the CpG island, regulate *PRF1* involved in the regulation of the immune system. The most significant DML was cg00172603 ($log_2FC = 0.27$, adj. p-value = 0.02) in CpG island annotated to *SSBP3*. Groups of DML annotated to the same gene are linked to *RUNX3*, which is a known tumor-suppressor gene and is associated with the alteration of DNA methylation in childhood asthma. Based on genes regulated by significant CpG sites, we found only two KEGG pathways, the Apelin signaling pathway, which plays an important role in the development of hypertension and future heart diseases, and the long-term depression pathway. Biological processes are mostly involved in immune responses.

This study was supported by the European Regional Development Fund under Grant HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798).

Impact of environmental pollutants to oxidative stress and antioxidant markers in mothers and newborns (Continue of the long-term research in the Czech Republic)

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A balance between pro-oxidant and antioxidant mechanisms is responsible for maintaining the physiological levels of reactive oxygen species (ROS) in cells. ROS are necessary for the regulation of signaling pathways involved in cell growth, proliferation, differentiation, and survival [1]. However, if excessive increased ROS occurs, oxidative stress, which contributes to the pathophysiology of many diseases, is induced [2]. The increased levels of ROS can be caused by various endogenous (inflammation) and exogenous factors (exposure to environmental pollutants, lifestyle, diet). Cells are protected against the deleterious effects of ROS by “primary” antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] [3]. However, if the capacity of antioxidant mechanisms is insufficient, ROS induce kinases leading to the activation of transcription factors (NF-κB, AP-1), propagating the pro-inflammatory signaling cascade and release of cytokines (e.g., TNFα, IL-1β, IL-6), chemokines and other inflammatory molecules [4]. Additionally, ROS interact with cellular macromolecules (DNA, lipids) and cause their damage. Oxidative damage to DNA is mostly induced by the attack of ROS on nucleobases. If not repaired, the oxidized nucleobases may induce mutations. 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodG), a predominantly formed oxidized nucleotide that represents the rate of oxidation of guanine in the nucleotide pool, is excreted into urine [5]. Its levels in spot urine samples may be used as a biomarker of short-term exposure to air pollution [6]. A ROS-mediated attack on cell membrane lipids, such as polyunsaturated fatty acids (PUFA), modifies cell membrane properties resulting in the disruption of regular cellular functions [7]. Peroxidation of arachidonic acid (AA), a polyunsaturated fatty acid abundantly contained in cell membranes independent of cyclooxygenases, leads to the formation of a number of products that include isoprostanes (IsoPs) [8]. IsoPs are cleaved from the sites of origin and then either circulated in plasma or excreted in the urine. Quantification of 15-F2t-isoprostane (15-F2t-IsoP) is considered a reliable index of the oxidative stress status *in vivo*. The determination of plasma 15-F2t-IsoP provides a more accurate view of the overall oxidative damage of an organism [9].

This study is a follow-up to our previous work published in Ambroz et al., 2016, where we analyzed the effects of air pollution on “traditional” biomarkers (8-oxodG,15-F2t IsoP) in newborns via maternal exposure. In 2014, while studying the impact of air pollution on oxidative DNA damage [measured via 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodG)] and lipid peroxidation [measured via 15-F2t-isoprostane (15-F2t-IsoP)] in mothers and their newborns from Karvina (a polluted region) and Ceske Budejovice (CB) (a control locality), 8-oxodG and 15-F2t-IsoP levels were expected to increase with increasing concentration of air pollutants. While in winter 2014 in newborns from Karvina the 8-oxodG levels were significantly increased ($P<0.001$) compared to CB, in mothers from Karvina oxidative DNA damage levels were significantly decreased in the same period compared to mothers from the control locality ($P < 0.05$). This may be explained by the adaptation of the adult organism to adverse environmental conditions and the development of protective mechanisms. The 15-F2t-IsoP levels generally followed the same trend as 8-oxodG levels. The exception was observed for lipid peroxidation in samples from newborns collected in the summer of 2013, when 15-F2t-IsoP levels were significantly higher in the control group ($P < 0.001$). This could be a result of the effect of other independent factors (e.g. type of delivery or anesthesia applied during delivery). Multivariate regression analysis of the effect of air pollution on oxidative stress in newborns from Karvina showed PM2.5 concentrations to be a significant predictor for 8-oxodG levels. Exposure to PM2.5 and B[a]P significantly affected lipid peroxidation.

The project “Healthy Aging in Industrial Environment” (HAIE) was carried out in 2018-2022 to verify these results. The aim of this study was to analyze more parameters to provide a comprehensive description of oxidative stress in non-smoking mothers and newborns from the above-mentioned localities. A sampling of biological material (urine, plasma) was performed during the whole year from the summer 2019 until summer 2020. The number of newborns included in the HAIE ($N = 250$/locality) was higher compared to the previous study ($N = 100$/locality). Personal characteristics, concentrations of particulate matter of aerodynamic diameter < 2.5 μm (PM2.5) and benzo[a]pyrene in the ambient air, activities of antioxidant mechanisms (enzymes: SOD, CAT, GPx, antioxidant capacities), levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and the plasma levels of selected persistent organic pollutants (POPs), as well as urinary levels of polycyclic aromatic hydrocarbon (PAH) metabolites were investigated as parameters potentially affecting pro-oxidant and antioxidant processes influencing levels of 8-oxodG and 15-F2t-IsoP. As in the previous study, the morbidity of children will be monitored after 2 years. Comprehensive results, including a comparison of these projects, will be presented at the conference.

Supported by the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798).

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Neglegted polycyclic aromatic sulphur heterocycles: benzo[*b*]naphtho[*d*]thiophenes and naphthylbenzo[*b*]thiophenes

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Polycyclic aromatic sulphur heterocycles (PASHs) belong among ubiquitous environmental pollutants. They originate from similar sources as polycyclic aromatic hydrocarbons (PAHs), and they usually accompany PAHs in the same samples; however, their toxic effects remain poorly understood. So far, IARC evaluated only two PASHs, dibenzothiophene (DBT) and benzo[*b*]naphtho[2,1-*d*]thiophene (BN21T), and categorized them in Group 3, as there is inadequate (for DBT), or limited (for BN21T) evidence for their carcinogenicity in experimental animals.

Here, we studied the aryl hydrocarbon receptor (AhR)-mediated activities of DBT, benzo[*b*]naphtho[*d*]thiophenes (BNTs), and naphthylbenzo[*b*]thiophenes (NBTs), as well as their presence in two types of environmental matrices: river sediments collected from both rural and urban areas, and in airborne particulate matter ($PM_{2.5}$) sampled in cities with different levels and sources of pollution. BN21T, BN23T, 22NBT, and 21NBT were newly identified as efficient AhR agonists in both rat and human AhR-based reporter gene assays, with 22NBT being the most potent compound identified in both species. BNTs were dominant PASHs present in both $PM_{2.5}$ and sediment samples, with BN21T being the most abundant one, followed by BN23T. The levels of NBTs were mostly low or below detection limit. BN21T and BN23T were identified as the most significant contributors to the AhR-mediated activity in the environmental samples evaluated in this study. Both induced nuclear translocation of the AhR and CYP1A1 expression in a time-dependent manner. Independently of their ability to activate the AhR, BN12T, 21NBT, 31NBT, and 32NBT inhibited gap junctional intercellular communication in a model of rat liver epithelial cells.

In conclusion, not only BN21T, but also other PASHs, such as BN23T should be studied more deeply, and included in risk assessment of relevant exposure scenarios. Importantly, effects of NBTs, although these PASHs do not appear to be ubiquitously distributed, should not be disregarded, as they can be present at significant levels in heavily polluted industrial locations. In particular, 22NBT that elicits very strong AhR-mediated activity should be further studied as potentially relevant environmental AhR-active contaminant. Alternative toxic modes of action, such as inhibition of GJIC could also be relevant for some PASHs, in particular NBTs. More attention should be paid to the potential health impacts of PASHs and their environmental distribution.

This study was supported by the Czech Science Foundation grant (No. 21-00533S) and by the Institute of Biophysics of the Czech Academy of Sciences (RVO: 68081707) and by Ministry of Agriculture of the Czech Republic (RO 0523).

Personal exposure monitoring to polycyclic aromatic hydrocarbons bound to dust particles of different size fractions

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Polycyclic aromatic hydrocarbons (PAHs) represent ubiquitous environmental contaminants. Benzo[a]pyrene (BaP) is listed as a Group 1 carcinogen by International Agency for Cancer Research and a number of other PAHs are categorized as probably or possibly carcinogenic to humans. Long-term inhalation exposure to PAHs has been associated with different types of cancer, cardiovascular, respiratory, and other diseases. PAHs are generated primarily during incomplete combustion of organic matter. In the atmosphere, they are distributed in both gaseous and particulate matter (PM) phases. Personal monitoring of PM-bound PAHs offers an opportunity to obtain more detailed information on exposure histories and PAH sources in studied areas. In the present study, the personal exposure to PAHs bounded to different size fractions of PM (< 0.25, 0.25–2.5, and > 2.5 μm) was investigated in a set of 129 participants from two localities of the Czech Republic: industrial area of the city of Ostrava (n = 65) and a control area of České Budějovice city (n = 64). The personal air sampling was performed for 24h in various seasons from August 2019 to August 2021 and was accompanied by a detailed questionnaire and PM2.5 measurements from the nearest monitoring stations. Extraction of target 20 PAHs was carried out by organic solvent extraction in an ultrasonic bath. The analytical method for PAH determination was developed using gas chromatography coupled to tandem mass spectrometry in electron ionization (GC-EI-MS/MS). The total amount of sum 20 PAHs ranged from 0.02 to 26.13 ng/m^3, with a median of 0.3 ng/m^3. The concentration of BaP, an air quality standard, ranged from 0.01 to 3.27 ng/m^3, with a median of 0.02 ng/m^3. The EU daily average concentration limit of 1.0 ng/m^3 was exceeded in 20 out of 129 volunteers, especially in those exposed in the industrial locality and the winter season. The particle size distribution showed that up to 87% of the total amount of PAHs is bound to the fraction lower than 0.25 μm, and only 1% in the fraction larger than 2.5 μm. Ostrava air had twice as higher mean concentrations of PAHs compared to České Budějovice, which is related to the industrial activities in this area. The highest inhalation exposures to all monitored PAHs were observed in the months of January and February for both sampling seasons, indicating the contribution of local heating and unfavorable metrological conditions. The results of personal monitoring contribute to the understanding of local and seasonal sources of PAHs and the identification of specific activities with high PAH exposure.

This work was financially supported by the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798).

Novel triorganotin compounds exert a significant cytotoxic effect on breast cancer-derived cells.

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Breast cancer is the most prevalent cancer in women. The efficacy of treatment depends on the stage and type of disease. Metallodrugs (cis-platin, oxaliplatin, carboplatin) have been used in breast cancer treatment for many years. However, their use is limited due to toxicity (nephrotoxicity, neurotoxicity) and the development of resistance (McWhinney *et al*., 2009). Therefore, it is necessary to identify new, more effective substances with selective effects. A group of organometallic compounds belonging to non-platinum metal-based chemotherapeutics has shown a promising anticancer effect. It was demonstrated that triorganotin compounds can induce apoptosis in different cell lines at low concentrations (Fickova *et al.*, 2015). Our study aimed to test *in vitro* cytotoxic effect of three triorganotin compounds: N, N-dimethyl dithiocarbamate triphenyltin (PhS2), tributyltin trichloroacetate (Cl3CO), and tributyltin trifluoromethane sulfonate (F3SO3) on a panel of breast cancer cell lines. We used the MTT test, CellTiter Glo® Luminescent Cell Viability assay, and annexin V test to detect the cytotoxic effect. We investigated also the effect of compounds on the cell cycle. The genotoxic effect was evaluated by the comet assay. Cis-platin, a conventional anticancer drug, was used as a reference. All compounds showed a potent cytotoxic effect; the IC_{50} was in the range of 0.65-0.19 μM, while IC_{50} for cisplatin was in the range of 1.5-6 μM. The most significant cytotoxicity exerted PhS2, IC_{50} for all cell lines was 0.05-0.11 μM. Reverse comet assay on MDA-MB-231 cells indicated that PhS2 interacts with nuclear DNA, which could contribute to the cytotoxic effect. Future studies will focus on testing the efficacy and toxicity in xenograft and syngeneic mice models and detecting the mechanism of action.

This work was supported by Slovak Research and Development Agency under the contract APVV-20-0314; by the Operational Programme Integrated Infrastructure for the projects: Integrative strategy in development of personalised medicine of selected malignant tumours and its impact on quality of life, IMTS: 313011V446, co-financed by the European Regional Development Fund; Strengthening of Research, Development and Innovation Capacities of Translational Biomedical Research of Human Diseases, ITMS: 313021BZC9, co-financed by the ERDF and by the TRANSMED project, ITMS codes: 26240120008 and ITMS: 26240220071 supported by the Research & Development Operational Program funded by the ERDF.

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The antifibrotic effect of silibinin-coated gold nanoparticles against fibrosis in mouse

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Chronic liver diseases are responsible for about two million deaths annually worldwide, making them the 11th leading cause of death. Liver cirrhosis is the leading cause of death in Slovakia among individuals aged 35-44. Liver fibrosis is the main cause of chronic liver disease, and reducing hepatic fibrogenesis is crucial for the treatment of chronic liver diseases. Therefore, research focused on developing new antifibrotic drugs is essential.

One potential antifibrotic drug could be silymarin. Studies have shown that silymarin has potential therapeutic effects on liver diseases. Silymarin is an extract from the Milk thistle herb and has been traditionally used for its medicinal properties for centuries, especially for liver-related conditions. It is composed mainly of one flavonoid (taxifolin) and seven flavonolignans (isosilybin A, isosilybin B, isosilychristin, silybin A, silybin B, silychristin and silydianin). About half of the silymarin mixture consists of silibinin (silybin A and silybin B in a ratio of 1:1). Silibinin has the highest antioxidation potential of all silymarin compounds and it seems that silibinin may have a key role in the antifibrotic effects of silymarin.

One of the key aspects of the success of a drug is its targeted transport to the liver. Gold nanoparticles are a promising option for drug delivery due to their unique properties and biocompatibility and may enhance silibinin's efficacy. The aim of the project is to prepare silibinin-coated nanoparticles and use them to suppress liver fibrosis *in vitro* and *in vivo*. Further research is necessary to understand its anti-fibrotic mechanisms, optimal dosage, potential drug interactions, and impact on other organs. Changes in profibrotic markers (e.g. *FN1*, *ACTA2*, *COL1A1*, *COL3A1*, *POSTN*), as well as cell viability, proliferation, changes in cytoskeletol will be monitored using various molecular-biological methods to determine whether our silibinin-coated nanoparticles have a better effect than pure silibinin or silymarin.

This work was supported by a grant APVV-16-0579, APVV-20-0494 and VEGA 2/0116/22. This study was performed during the implementation of the project Building-up Centre for advanced materials application of the Slovak Academy of Sciences, ITMS project code 313021T081 supported by Research & Innovation Operational Programme funded by the ERDF. This work is supported by European Union's Horizon 2020, No 857381, project VISION.

Genetic alteration profiling in women acutely exposed during processing of dental nanocomposites

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Nanoparticles (NPs), a fraction of particulate matter that has one or more external dimensions less than 100 nm, became an important part of everyday life in the last 20 years. Among many applications, nanocomposites are frequently used in dentistry. Apart from their undoubted benefits, the questions related to the risk of NPs inhalation during their processing by milling or polishing for human health, and/or the integrity and function of the genome arise. Despite these facts, human biomonitoring studies relevant to this topic are still rare, and analyses concerning the cytogenetic effects of NPs exposure during the processing of nanocomposites in stomatology are completely missing.

In this project, we analyzed a group of 24 women (age 37±2 years) acutely exposed to dental nanocomposite grinding. All of them were sampled twice: before and after the work. Aerosol exposure monitoring of particulate matter (PM) including nano-sized fractions was performed together with working activities by using stationary and personal monitoring. The whole-chromosome painting (WCP) for autosomes #1 and #4 and gonosome X was applied for estimating the range of cytogenetic damage. The frequencies of both stable chromosomal aberrations, as well as numerical alterations, were evaluated. Preliminary results show differences in genetic alterations related to the type of chromosomal aberrations. While the genomic frequency of translocations remained stable after the short-time acute exposure, the frequency of numerical aberrations differed. Moreover, differences between the alterations of autosomes and gonosomes were observed. In conclusion, exposure to NPs is followed by the increased frequency of numerical aberrations which can be dangerous, especially for pregnant women.

This work was supported by the Grant Agency of the Czech Republic #22-08358S.

Transcriptome changes in humans acutely exposed to nanoparticles during dental treatment

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The rapid expansion of nanotechnology brings many benefits, but also may negatively affect human health. Nanoparticles (NPs) are particles with a range of size from 1 to 100 nanometers in two or three dimensions. They are commonly used in commercial products including dental nanocomposites. These materials developed as highly aesthetic materials for teeth reconstruction may contain up to 75 wt. % of nanoparticles to match requirements on their long-term durability in the oral cavity. While the NPs in the composites are chemically bonded to the polymer matrix and do not present any adverse effects, their grinding and polishing during dental treatment produce an aerosol containing a high concentration of NPs. Their inhalation raises questions regarding the risk to human health and genome function. Due to the lack of information on the toxicological impact of NPs on humans, more research is needed to fill these gaps also on the transcriptomic level.

In this project, we searched for the possible impact of inhalation exposure to NPs released during grinding dental nanocomposite on transcriptome changes in 24 human subjects. Subjects were sampled twice per day, before (pre-shift) and after exposure (post-shift). Total RNA was isolated from collected blood and used for miRNA and mRNA libraries preparation. After sequencing, analyses of the differential mRNA and miRNA expression was performed.

In conclusion, we detected a high number of deregulated miRNA and mRNA between studied groups when compared post- and pre-shift exposure to NPs. Our results indicate that the impact of exposure to NPs is dependent on the style of working with nanocomposites as well as on the biological variability among studied subjects.

This work was supported by the Grant Agency of the Czech Republic as #22-08358S.

Advances in transcriptomics for cancer research: unveiling insights through single-cell sequencing

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For several decades, transcriptomic approaches have proven to be a reliable tool for analyzing and better understanding the highly complex nature of cancer. Common transcriptomic approaches, such as RNA-seq, have allowed us to glimpse into the transcriptomic profile of a sample, enabling the identification of differential gene expression and molecular pathways associated with cancer onset and development.

However, the advent of single-cell technologies has completely transformed our understanding of cancer biology. These approaches provide a comprehensive view of tumor tissue, allowing us to explore the transcriptomic and epigenetic profile of every single cell. It is through these approaches that the characterization of cellular diversity has become significantly more accessible, serving as a powerful tool for identifying rare and previously undiscovered cell populations. Most importantly, these approaches unveil what conventional methods struggle to reveal—the underlying complexity and heterogeneity of tumor tissue.

Residual metal nanoparticles accumulated in the body induce late toxic effects and alterations in transcriptional and miRNA landscape

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Our study investigated the potential adverse biological effects of residual gold and titanium oxide nanoparticles (PEG-AuNPs and TiO_2NPs) determined in rats 28 days after a single vein tail administration. The liver was the primary organ of residual metal nanoparticle (MNP) deposits. Although the relative liver weight of PEG-AuNPs-exposed rats was significantly lower compared to control animals, no histopathological lesions in hepatic tissue were observed. However, changes in serum biomarkers associated with alteration in hepatic functions were determined. Hematological and immunological profiling revealed unintended biological outcomes of residual MNPs. In addition, integrated transcriptomic analysis was performed to get comprehensive information about potential exposure-induced effects on rat lungs, liver, and kidneys. Most deregulated genes with functional classification in lipid metabolism, cell cycle, and cell proliferation pathways were identified in hepatic tissue, mainly in PEG-AuNPs-exposed rats. The number of deregulated miRNAs was relatively low compared to mRNA expression changes. However, both MNPs deregulated miR-203a associated with liver injury, and miR-18a-5p and miR-32-5p linked to kidney damage.

Our study emphasizes the need for a more thorough biosafety assessment of poorly soluble MNPs accumulating in the body.

This study was supported by the European Union's Horizon 2020 research and innovation program under GA No. 857381 (VISION project) and GA No. 685817 (HISENTS project), DAAD project (Epigenotoxicity of nanomaterials), and NANOGOLD project (APVV 16-0579).

The effect of metal nanoparticles on gene expression of mouse mesenchymal stem cells

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Several metal nanoparticles (NPs) are known for their antimicrobial properties. However, they may negatively impact human organism, including mesenchymal stem cells (MSC), a cell population contributing to tissue growth and regeneration. For the first time, we performed a comprehensive evaluation of the toxic effects of selected NPs (Ag, ZnO, and CuO) in mouse MSC.

In the initial phase of this project, multiple endpoints such as reactive oxygen species production, lipid peroxidation, DNA alterations, and cell cycle were analyzed. Our results indicated the negative biological impacts of these NPs on MSC. The effects depend on the specific NP and exposure conditions: in our experimental setup, ZnO NPs had the least adverse impacts on MSC, while CuO NPs tended to be the most toxic compound. The tested NP induced intracellular ROS generation, which was most likely partly eliminated by the antioxidant mechanisms, resulting in limited effects on oxidative DNA damage. Lipid peroxidation seemed to be induced in an alternative way, presumably by extracellular ROS. All the tested compounds increased the sensitivity of MSC to apoptotic signals, apoptosis was induced only after the Ag NPs exposure. The tested NP affected the G1 and S phases of the cell cycle after 24 h exposure.

In this contribution, I will focus on the next part of the project, where we searched for the changes in mRNA and miRNA expression caused by the exposure of mouse stem cells to these metal nanoparticles in three different doses. After RNA isolation from cell lysates, mRNA and miRNA libraries were prepared, sequenced, and differential expression was analyzed.

Exposure of MSC to various NPs lead to expression changes most pronounced in the case of silver NPs with a stronger effect on mRNA than on miRNA. The weakest reaction was observed after the treatment with zinc oxide NPs. In conclusion, the metal antimicrobial NPs tested in our study exert a negative response in MSC and might not be optimal for combined wound treatment with MSC.

This work was supported by the Czech Science Foundation (21-17720S).

Plate reader spectroscopy as a possible substituent for atomic absorption spectroscopy in the quantification of the cellular uptake of the nanoparticles

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Interest in utilizing nanoparticles (NPs) for biomedical applications requires a correct assessment of their intracellular concentration. The concentration of internalized NPs by the cells is becoming crucial for cell targeting and drug delivery. The knowledge of the intracellular concentration of NPs is especially important as most of the biological effects manifest in a dose-dependent manner.

Ultrasensitive atomic absorption spectroscopy (AAS) is seen as one of the gold standard methods for quantifying internalized NPs. Besides its limitation to metal-based NPs, AAS also requires a specific expensive instrument. Despite being a sensitive method, the sample preparation and handling is tedious, which makes it time-consuming and cost-intensive in many cases.

In this study, we report a solid, fast, and accessible alternative to AAS – plate reader spectroscopy (PRS), which offers a susceptible option for daily laboratory use without the need for sophisticated equipment. We investigated the cellular uptake of magnetic iron oxide nanoparticles coated with sodium oleate and bovine serum albumin (BSA-SO-MNPs) in human alveolar epithelial cancer cells A549 assessed by PRS and AAS in parallel with a remarkable correlation coefficient of R = 0.9914.

This work was supported by the European Union's Horizon 2020 research and innovation program under grant agreement No. 857381 (project VISION), and by the Slovak Research and Development Agency under Contract No. APVV-16-0579, APVV-15-0215 and APVV-19-0070. This work was further supported by VEGA grants 1/0069/20 and 2/0160/21.

Distribution, accumulation and biological effects of gold nanoparticles *in vivo*

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Gold nanoparticles (AuNPs) are widely studied for their unique physicochemical properties as a very promising tool for biomedical applications. They can be utilized as drug delivery systems, anticancer treatment agents, imaging boosters, etc. AuNPs are in general considered biocompatible and safe but in most of the studies they were tested in *in vitro* conditions, which do not reflect the response of the whole organism. Another issue is the lack of long-term studies.

Four types of gold nanoparticles were used in our *in vivo* study with animal model. Gold nanospheres with 10 or 20 nm in diameter, coated with bovine serum albumin (BSA) or polyethylene glycol (PEG) were applied to C57BL/6 mice by systemic administration. We investigated the biodistribution, accumulation, and changes in investigated organs (liver, kidney, and spleen) during 120 days.

Our results show that nanoparticle treatment did not affect the appearance, behavior, or growth of mice during the experiment even though all types of nanoparticles were still detectable in all investigated organs four months post-application. We also detected various patterns of accumulation and distribution of these types of AuNPs as well as various responses of the organism to their presence.

This work was supported by the Slovak Research and Development Agency under Contract No. APVV-16-0579, APVV-20-0494 and by VEGA grant no. 2/0116/22. The authors thank the Slovak Cancer Research Foundation, for the OLYMPUS BX46 microscope. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 857381, project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers).

Effective reduction of SARS-CoV-2 RNA levels using a tailor-made therapeutic oligonucleotide

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More than three years after the outbreak of the COVID-19 pandemic, the SARS-CoV-2 virus is still causing lasting damage around the world, impacting the health and lives of millions. Preventive measures, such as the widespread administration of vaccines, are playing a role in reducing the severity of cases. For those who require treatment, clinicians can try to treat the disease and its symptoms with existing drugs. However, an effective therapy specifically targeted against SARS-CoV-2 is still lacking. Therapeutic oligonucleotides have attracted great interest due to their potency and potential for changing the therapeutic landscape of many pathological conditions, including those of viral origin. Targeting conserved SARS-CoV-2 RNA sequences essential for viral replication offers a rational approach to inhibit viral infection and thereby halt disease progression.

Here, we report the antiviral potential of a tailor-made oligonucleotide-based inhibitor targeting SARS-CoV-2, called ASC1R, which showed spontaneous cellular uptake, remarkable >94% efficacy in reducing RdRp RNA levels in transfected lung cell lines, and >98% efficacy in reducing SARS-CoV-2 RNA levels in samples from patients hospitalized with COVID-19 following a single application.

The therapeutic potential of ASC1R could translate into substantial clinical benefits for patients with COVID-19. Furthermore, in the context of infectious diseases, our results provide implications for the research and development of analogous antivirals for other diseases of viral origin. The findings could help to meet the global challenge of developing new and safe treatment modalities.

This work was supported by the Slovak Research and Development Agency under Contracts No. PP-COVID-20-0007 and APVV-21-0220.

DNA repair: a punishment or reward?

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A personal retrospective view of the studies in the field of DNA repair and mutagenesis will be presented. The phenomenon of tolerance of the unrepaired lesions was studied in bacteria, rodent and human primary cells, and cell lines.

Unlike other biologically active macromolecules, DNA is neither disposable nor recyclable. The origin of the human being is accompanied by millions of cell divisions, i.e. millions of DNA replications and despite that the very last DNA copy is remarkably similar to the original one created during egg fertilization. From a chemical point of view, it seems to be impossible. All chemical processes tend to create accidental mistakes. Add to this the limited chemical stability of DNA and its spontaneous degradation, DNA damage caused by reactive species of cell metabolism, the effects of various compounds from the environment like radiation and genotoxic chemicals, and you get the picture of total chemical chaos which should predominate in our cells.

DNA, however, stays remarkably unchanged due to the existence of many DNA repair mechanisms. Various organisms as well as various tissue cells within the same organism seem to choose different strategy for survival. To our experimental experience the shorter the life span of the organisms or the tissue cells the less effective is the DNA repair.

It is well known that once the evolution develops a successful design or process it will reuse them wherever it is suitable. Various mechanisms of DNA damage tolerance are such an example.

Genomic instability in adenomas and in colorectal cancer progression

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In solid cancers both impaired DNA repair and disrupted telomere length (TL) homeostasis are key culprits in cancer initiation, progression, and prognosis. Altered DNA repair leads through the accumulation of mutations into genomic instability. Telomere attrition resulting in replicative senescence, simultaneously by-passing cell cycle checkpoints, is a hallmark of cellular malignant transformation. Telomerase, ubiquitous in advanced solid cancers, is fundamental to cell immortalization. Human solid neoplasms often exhibit chromosomal instability (CIN), both structural and numerical. CIN generates either abnormal aneuploid karyotypes, or continually expands phenotypic heterogeneity as tumor cell populations undergo consecutive cell divisions. We searched for the CIN markers in the adenoma-adenocarcinoma transition and in CRC progression. Understanding the mechanisms and dynamics of tumor genomic diversification, where DNA damage response and telomere homeostasis are important players, is critical in understanding carcinogenesis and overcoming drug resistance. Mitochondrial dysfunction, another cancer hallmark is linked with DNA repair capacity and compensates for damage by increasing the mitochondrial DNA copy number (mtDNA-CN). Current studies on the mtDNA-CN reported ambiguous and inconsistent results for various cancer types. Telomere shortening has a dual role in tumorigenesis. It promotes cancer initiation by inducing CIN, while TL maintenance characterized by telomerase expression is required for cancer cell proliferation and tumor growth. The reports on TL as a biomarker for cancer risk, patient therapy response, and/or survival are contradictory as well. Our investigations were also focused on mtDNA_CN in CRC tissues and adjacent mucosa.

Acknowledgment: AZV NU21-03-00145, NU21-03-00506; GAČR: 21-27902S, The project National Institute for Cancer Research (Programme EXCELES, No. LX22NPO5102).

Association of mitochondrial DNA copy number and telomere length with colorectal cancer patient outcomes

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The dysfunction of mitochondria is one of the cancer hallmarks. Mitochondria evince a limited DNA repair capacity and compensate for damage by increasing the mitochondrial DNA copy number (mtDNA-CN). Current studies on the mtDNA-CN in cancer have reported ambiguous results; most were based on a case-control design and were inconsistent for various cancer types. Telomere shortening has a dual role in tumorigenesis. It promotes cancer initiation by inducing chromosomal instability, while telomere length (TL) maintenance characterized by telomerase expression is required for cancer cell proliferation and tumor growth. Similar to mtDNA-CN, the reports on TL as a biomarker for cancer risk, patient therapy response and/or survival are contradictory.

mtDNA-CN and TL are highly variable across cell types but maintained within a constant range according to the specific tissue type. It has been demonstrated that mitochondrial biogenesis and energy production were decreased in telomerase-deficient mice with severe telomere dysfunction. It thus has been hypothesized that telomere alteration affects not only oxidative defense mechanisms but also mitochondrial functions. The deregulation of the telomere-mitochondria axis, as caused by aging or other physiological factors, triggers carcinogenesis. We, therefore, investigated mitochondria and telomere changes in colorectal cancer (CRC), one of the leading causes of cancer-related deaths. Our study particularly aimed to look closely at mtDNA-CN, TL, and the expression of mitochondrial transcription factor A and telomerase reverse transcriptase in association with CRC patient outcomes.

Our cohort included deep-frozen tumor tissue, adjacent non-tumor tissue, and blood from 163 untreated sporadic CRC patients. We isolated DNA and RNA from these samples and measured particular molecular biomarkers using a quantitative-polymerase chain reaction assay. Currently, the experiments are running and after collecting the experimental data, comprehensive statistical analysis using patient clinical and follow-up data will be performed. The results will be presented during the conference and we believe that they may aid improvements in the current understanding of CRC, by identifying the role of mtDNA-CN and TL in CRC pathogenesis.

This study was financially supported by the Ministry of Health of the Czech Republic (NU22J-03-00033), by the Grant Agency of the Czech Republic (21-04607X and 21-27902S), and by the Programme EXCELES (ID Project No. LX22NPO5102).

Reduced methylation of olfactory receptor genes and amplification of 6p25.1-p22.3 as specific epigenetic and genetic alterations in colorectal cancer liver metastases

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Colon and rectal cancers, referred to as colorectal cancer (CRC), collectively present the third most common cancer worldwide and the second leading cause of cancer death. Aberrant DNA methylation and chromosomal instability (CIN) both play a pivotal role in the pathogenesis of CRC. Mapping both phenotypes during the disease progression can help to identify potential targets for pharmacological intervention and improve the management of CRC metastasis.
In the discovery set of nine patients diagnosed with advanced CRC, high-throughput DNA methylation and DNA somatic copy number alterations (SCNAs) analyses within primary tumors and corresponding liver metastases were performed. Methylation profiles and the most frequent SCNAs identified in the DNA were verified on a validation set of eight CRC patients by whole-exome sequencing, genome-wide DNA methylation measurement, and gene expression profiling. Bioinformatical analysis was done using R language.

In the discovery set, promoter regions of 2395 genes were methylated differently between tumors and metastases. All the metastases had an increase in hypomethylated and a decrease in hypermethylated CpGs. Enrichment analysis of the differently methylated gene promoters showed the olfactory receptors pathway as the most frequently represented (P = 1.06E-8), with all the identified promoters (n = 120) less methylated in metastases. Irrespective of the tissue, the degree of CIN correlated with the hypomethylation level (P = 0.026). DNA regions amplified in primary tumors were highly concordant with those in liver metastases and represented loci on chr7 (p22.3-p13), chr8 (q21.13-q24.3), chr13 (q12.11-q13.3), chr20 (q11.1- q13.33), and chrX (q11.1-q28), while the most frequently lost region was on chr8 (p21.3-p12). Gain unique for metastases (observed in 89% of samples) was located on Chr6 (p25.1-p22.3).

Taken together, herein, we identified a decrease in methylation within promoters of odorant receptors as the most prominent epigenetic difference occurring in metastatic tissues. Furthermore, several genomic hot-spot regions with SCNAs possibly important for the metastatic process were identified. Validation of the results is currently underway.

The study was funded by the Ministry of Health of the Czech Republic (NU22J-03-00028).

Influence of air quality in urban agglomerations on the sperm quality of their residents

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Exposure to air pollution is associated with a range of adverse health effects, including reproductive toxicity. Several studies have investigated the link between outdoor air pollution and semen quality. There are many different factors that can affect sperm development in the cohorts studied, so results on the effect of air pollution on sperm quality are inconsistent. We examined the sperm quality of a group of city policemen in Ostrava and Prague at the end of a period of high air pollutant concentration (winter period) and the same group at the end of a relatively low exposure summer period. Ceske Budejovice was a control area with low pollution levels. The average daily concentrations of air pollutants recorded by stationary monitoring 90 days before semen sampling were evaluated for each site. Examination of the same group of city policemen at the end of a period of high air pollutant concentration and at the end of a period of relatively low exposure limited the influence of age, different lifestyles, different occupational exposures, location, and genetic polymorphism on the results of the effect of air pollution on sperm quality.

Standard semen parameters were assessed according to the World Health Organization guidelines (2010). These parameters included semen volume, sperm concentration, sperm morphology, sperm motility, acrosomal reaction, and sperm plasma membrane integrity. Sperm DNA damage was analyzed after acridine orange staining using the sperm chromatin integrity assay (SCSA). Sperm movement characteristics were determined by computer-assisted sperm analysis (CASA).

Ejaculate volume and sperm concentration did not differ between study periods at either site. The percentage of live spermatozoa, normal spermatozoa, and spermatozoa with intact acrosome in Ostrava and Ceske Budejovice did not differ significantly between the study periods. All these sperm quality indicators are significantly worse in Prague in autumn than in spring. The total number of motile and the number of progressively motile sperm is significantly higher in Prague and Ostrava in March after the winter period. This is due to seasonal variation in sperm activity. In Ostrava, chromatin integrity disturbance (%DFI) is significantly higher after the winter period ($p = 0.003$). In Prague, there is no significant difference in % total DFI between the periods studied, but within the DFI, the proportion of highly disturbed versus moderately disturbed chromatin is significantly higher in autumn ($p = 0.004$). In Ceske Budejovice, we found no significant differences.

Air pollution in both Ostrava and Prague reduces sperm quality in the observed officers, but the nature of the disruption varies according to the representation of individual pollutants in the air. We believe that the basic problem in Ostrava is mainly the high concentration of B[a]P in the air and in Prague NO_2.

This work was supported by the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798).

Current possibilities for the infertility treatment in oncology patients

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Assisted reproduction is a medical field that deals with eggs, sperm, and embryos outside the body (*in vitro*) to achieve pregnancy and successful childbirth (treatment of infertility). In reproductive medicine centers, a clinical embryologist is responsible for various activities, including the examination and processing of semen, the process of in vitro fertilization, and the evaluation of early embryonic development. Additionally, assisted reproduction now includes the aspects of freezing (cryopreservation) of gametes and embryos, as well as genetic testing of embryo cells before their transfer into the uterine cavity.

A current trend is that women often postpone their plans for pregnancy to later ages due to various reasons such as career and social factors. As a result of this trend, there is an increasing population of women with cancer before completing their reproductive years. Early diagnosis and treatment options contribute to a higher survival rate for female cancer patients. However, anti-cancer treatments, although aimed at improving quality of life and reducing long-term consequences, negatively affect women's reproductive functions.

Fertility preservation is crucial for women with oncological diseases to prevent infertility that may occur after cancer treatment. Chemotherapy and radiotherapy have a detrimental impact on ovarian reserve and significantly contribute to premature ovarian failure. The management of treatment is multidisciplinary and tailored individually to each patient. It is essential for women who plan to have a family in the future to receive accurate and up-to-date information about fertility preservation options that can impact their entire lives.

Unfortunately, only 10% of women currently utilize fertility preservation options. On the other hand, men have been using sperm cryopreservation for various medical indications for a long time, such as working in toxic environments or undergoing planned chemotherapy. Freezing oocytes provides women with an equal chance to build their own families and ensures gender equality. The reimbursement of costs for oocyte cryopreservation requires professional and societal discussions for women with medical indications.

Periostin – a new candidate for a biomarker in CKD progression

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Chronic kidney disease (CKD) belongs to a major public health problem, which increased by approximately about 30% in the last decades [1]. Due to the tight connection between renal and cardiovascular systems, renal insufficiency importantly contributes to cardiovascular disease [2]. In combination with cardiovascular complications CKD results in premature mortality before the life-saving transplantation [3]. Therefore, early identification of the processes that likely progress to complete loss of kidney function has become increasingly important. A pathological feature and manifestation of CKD is renal fibrosis. Efforts have been made to identify novel targets associated with renal fibrosis development. One such marker is periostin. It has been observed that periostin expression is absent in a healthy adult kidney and is triggered de novo in case of kidney injury. In our study, we chose a murine model of unilateral ureteral obstruction (UUO) to investigate the role of periostin in the renal fibrotic process. UUO is a well-characterized model of renal fibrogenesis and after 3, 7, 14, and 21 days of UUO, mRNA and protein expression of periostin will be estimated together with known markers of fibrotic progress like fibronectin, collagen, α-SMA, etc. We will use histological and immunohistological analyses to detect periostin localization in the renal tissue and link it to the estimated fibrosis grade.

This work was supported by the Slovak Research and Development Agency under Contract No. APVV-20-0494 and by VEGA 2/0116/22 grant. This study was performed during the implementation of the project Building-up Centre for advanced materials application of the Slovak Academy of Sciences, ITMS project code 313021T081 supported by Research & Innovation Operational Programme funded by the ERDF. This work is supported by European Union's Horizon 2020, No 857381, project VISION.

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Posters

Genotoxic effect of selected hydrogels loaded with superparamagnetic iron oxide nanoparticles potentially applied in regenerative medicine

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Advanced methods in regenerative medicine are trying to develop suitable materials that can reproduce and restore the favorable, natural environment needed for skin regeneration. The development of advanced multifunctional materials for injured skin treatment with the ability to provide multiple functions at once is crucial for clinical application. The utilization of hydrogels loaded with nanoparticles in regenerative medicine provides an innovative way to treat skin injuries.

It is important to evaluate the biosafety of nanohydrogels as a degradable biomaterial for use in the biomedical field. The aim of this study was to determine the genotoxic effects of newly prepared nanocomposites. The model system represents different types of skin cell lines, keratinocytes (HaCaT), and fibroblasts (HFF-1). The experiments were focused on determining the genotoxic effect of nanocomposites in *in vitro* conditions. For nanohydrogel build-up, three different hydrogels (Alginate, Pluronic F127, and Gelatin metacryloyl) with different chemical compositions and iron oxide nanoparticles were used. For genotoxicity determination, we used three different methods: comet assay, fpg-modified comet assay, and micronucleus test to determine aneugenic, clastogenic, and DNA damage. We also determine nanoparticle release from hydrogel structure using Prussian blue staining.

Initial results after 24 h nanohydrogel exposure, measured by comet assay showed a significant increase in DNA damage in the case of higher concentration of gelatin metacryloyl nanohydrogel. We didn't observe any DNA damage in the two other nanohydrogels. Subsequently, we used an fpg-modified comet assay to determine if this damage is caused by base oxidation. However, we did not observe any differences between samples with and without fpg-enzyme treatment. We assume that DNA damage is a result of single or double-strand breaks incurred as the attempted repair of UV irradiation-induced base damage in DNA structure. From the results of the micronucleus test, we noticed a higher amount of apoptotic and necrotic cells after gelatin metacryloyl exposure, also the presence of micronuclei was significantly higher. Results of Prussian blue staining showed that nanoparticle release from hydrogel structure depends on hydrogel concentration.

This work was supported by ENM III/2019/861/TENTACLES; DoktoGrant no. APP0316, project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, CA21108 - European Network for Skin Engineering and Modeling and VEGA No. 2/0121/21

Cytotoxic and genotoxic effect of thymol derivatives on colorectal carcinoma spheroids

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Thymol is a monoterpene phenol with a characteristic odor. Thymol has potential uses in the pharmaceutical, cosmetic, food, agronomic industries, and so on. It has a proven bioactive effect on tumor cells, including colorectal cancer tumor cells.
However, its properties such as low solubility, absorption, and cell penetration prevent its wider application. Therefore, new hydrophilic derivatives – acetic acid thymol ester and thymol α-D-glucoside - were synthesized. In our study, we treated spheroids of colorectal cancer tumor cell lines (HT-29 and HCT-116) with thymol, acetic acid thymol ester, or thymol α-D-glucoside on a concentration scale for 24 hours. Cytotoxicity was determined by the MTT method. The genotoxic effect of substances was analyzed by the single-cell gel electrophoresis (comet assay).

For a comprehensive assessment of the effect of thymol and the newly synthesized derivatives - acetic acid thymol ester and thymol α-D-glucoside, the cytotoxic and genotoxic effect was also determined in 3D culture on colorectal cancer tumor cells. 3D cell culture ensures greater stability, while better representing real cell aggregation, morphology, and mutual cell interaction. As a result, the creation of a more complex microenvironment was ensured, which to a greater extent corresponds to the real conditions *in vivo*. Spheroids were formed after 5 days using ULA (ultra-low attachment) microplates. Subsequently, the cytotoxic and genotoxic effects of thymol, acetic acid thymol ester, and thymol α-D-glucoside were analyzed and compared using the methods mentioned above.

Our results demonstrated that a newly synthesized derivative - acetic acid thymol ester - with targeted chemical structure modification acts more effectively on both colorectal cancer cell cultures in 3D at much lower concentrations than thymol alone. Comet assays have shown a significant increase in DNA damage for the newly synthesized derivative even at non-cytotoxic concentrations. The HCT-116 cell line showed higher DNA damage values than HT-29. Incucyte Zoom noted the effect of thymol and acetylthymol on the proliferation of both tumor cell lines. The results confirmed our assumption that the newly synthesized hydrophilic derivative can act more effectively than thymol. In the future, we would like to focus on determining the expression of selected proteins using the Western blot method.

This work was supported by a European Union's Horizon 2020, No 857381 project VISION, grant VEGA 2/0055/20, and a Grant program for SAS PhD. students- APP0410.

Plasma KRAS mutations as early diagnostic biomarkers for pancreatic cancer in high-risk group patients

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Pancreatic cancer (PanCa) has one of the lowest 5-year survival rates – less than 10%. To improve the prognosis of PanCa, it is necessary to develop tools that will enable earlier diagnosis. More than 90% of PanCa cases are characterized by the presence of *KRAS* mutations in tumor tissue that play a critical role in the initiation of PanCa. Recently, attention has been focused on liquid biopsy which can better reflect the whole genetic profile of the tumor and may help with early diagnosis.

Our study aims to discover whether *KRAS* gene mutations can be detected in the plasma isolated from the PanCa risk group patients - diabetes mellitus II (DMII) and chronic pancreatitis (CP). The *KRAS* mutation was analyzed in 34 PanCa, 68 DMII, and 26 CP patients by droplet digital PCR. Plasma cell-free DNA was investigated for three mutations hotspots: *KRAS* p.G12D c.35G>A, p.G12V c.35G>T, p.G12R.
Detailed results of the study will be presented during the meeting.

We believe that these results discover whether plasma analysis of *KRAS* mutation can serve as a biomarker for early diagnosis in risk group patients.

Supported by grant AZV NU-21-07-00247, by Czech Science Foundation 22-05942S and by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102).

Innovations in the objective evaluation of chromosomal aberrations in cytogenetic analyses (CAHPL)

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"Cytogenetic analysis of human peripheral lymphocytes" (CALPL) is a sophisticated medical analysis with a demanding procedure at every step. Objective microscopic analysis of numerical and structural changes is its main pillar. Objective evaluation of changes and damage to lymphocyte chromosomes requires long-term practice and compliance with professional criteria. The reason for digital innovations is the high variability of morphological shapes, both healthy and damaged chromosomes, their extremely small dimensions (in nanometers), and the occurrence of pseudo-formations that must be distinguished and excluded from the evaluation. In complicated and low-quality mitoses with deformed or overlapping morphological structures, the assessment of the finding is ambiguous and dubious, with several possible interpretations. In such cases, subjective error occurs more often during examination and evaluation. Therefore, because of doubts, mitosis is better excluded or removed from the evaluation. Digital recording of optical images of mitoses and chromosomes, which are electronically fixed, enlarged, and modified, makes multiple details of even complicated morphological structures visible and refines their objective assessment. It allows to further enlarge the image recording, adjust brightness, contrast, and saturation, save it in databases, send it to colleagues immediately for assessment in the form of MMS messages and e-mails, etc. Saving images in RAW format enables the enlargement of the image and sections without loss of sharpness and details, thus improving the evaluation of the enlarged image. All the chromosomal structures in the image can be counted very precisely. Digital recordings of images of mitoses and chromosomes improve and make the whole work on the morphological assessment of chromosomal aberrations more pleasant.

Activity of the national reference center for the evaluation of late effects of chemical substances by methods of genetic toxicology

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The National Reference Center for the Evaluation of Late Effects of Chemical Substances by Methods of Genetic Toxicology (NRC for GT) was established on October 15, 2021, at the Regional Public Health Authority (RPHA) with a seat in Kosice, and it is currently the only department of genetic toxicology in Slovakia. The NRC for GT continues the activity that was previously provided by the department of the Public Health Authority of the Slovak Republic in Bratislava. Since the risk factors of the occupational and living environment must be under control, as they increase the risk of developing new malignant tumors, it is important to continue the NRC activity for GT. In addition to conventional cytogenetic and microbiological methods, the specialized department of genetic toxicology at RPHA, with a seat in Kosice, has developed counseling for increasing awareness of the given issue since 1986. It provides advice to people with oncological diseases or to people who are interested in the prevention of oncological diseases. The department provides analysis of anamnestic data as well as counseling for early cancer prevention for individuals or groups. The NRC for GT conducts laboratory diagnostics in the field of assessment of the late effects of chemical substances and other genotoxic factors by methods of genetic toxicology. It examines biological material (blood, urine) and also the genotoxic effects of various chemical substances and complex mixtures. The NRC for GT also analyzes the effects of other mutagenic factors from individual components of the living, occupational environment, and lifestyle factors.

Introduction to occupational diseases in the Czech Republic

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Occupational diseases have a significant impact on medical fitness to work, both from the perspective of the affected individual and from a societal perspective. For some diseases, the occupational origin is clear (silicosis, pneumoconiosis), but there are also diseases that occur simultaneously in the population as general diseases. They arise under non-occupational conditions also (peripheral neuropathy, bronchial asthma, skin eczema etc.). For the majority of occupational diseases, it is not possible to reassign the worker to the work that led to the disease, even if the clinical and laboratory signs of the disease have disappeared. This does not apply in cases where it is possible to ensure the discontinuation of contact with the nox. Rational preventive measures have the best health effect and economic impact while minimizing costs.

Occupational diseases are regulated by a number of labor regulations, in particular the Labor Code (Act No. 262/2006 Coll.), the Government Regulation No. 276/2015 Coll., on compensation for pain and difficulty of social integration caused by an occupational accident or occupational disease, and Government Decree No. 321/2018 Coll., on the regulation of compensation for loss of earnings following incapacity for work caused by an occupational accident or occupational disease. The enabling provision for the establishment of the list of occupational diseases is set out in Section 107(1)(b) of Act No 155/1995 Coll., on Pension Insurance. Government Decree No 290/1995 Coll. establishing the list of occupational diseases (hereinafter referred to as the **'List of Occupational Diseases'**) was issued on the basis of this Decree. The List of Occupational Diseases regulates in more detail both the definition of the concept of occupational diseases and their listing. The actual process of assessment and recognition of occupational diseases is regulated in Section 61 et seq. of Act No 373/2011 Coll., on Specific Health Services.

In order for the concept of occupational disease to be met, the conditions in the List of Occupational Diseases must be met. These conditions are of a clinical nature, i.e. the diagnostic criteria, including the severity of the disease. Furthermore, it must be verified that the work in question is work in which, according to the List of Occupational Diseases, such a disease can arise (hygiene condition).

What the individual conditions contain, who is responsible for their assessment and a brief analysis of the number of applications submitted for the assessment of possible occupational diseases compared to the final number of recognized occupational diseases will be discussed in more detail in the paper itself.

This work was supported by the grant IGA_LF_2023_007.

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Occupational diseases with a focus on cancer in the Czech Republic

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The link between certain occupations and the incidence of cancer has been known historically. The first references describe the symptoms of these diseases, later data were obtained by retrospective epidemiological studies. A list of carcinogens and their target organs can be found on the International Agency for Research on Cancer (IARC) website. Here, chemicals, physical agents and work processes are classified into 4 groups, ranging from carcinogenic to humans to not classifiable as to its carcinogenity to humans.

Situation in the Czech Republic

In the current **List of Occupational Diseases**, cancer can be classified under various chapters and headings, specifically under Chapter I (caused by chemicals, specifically arsenic, beryllium, cadmium, chromium, nickel and their compounds, halogenated hydrocarbons, formaldehyde, benzene, aromatic nitro and amino compounds, polychlorinated dibenzodioxins, PAHs, inorganic acids, ethylene oxide and other oxiranes, halogenated alkyl ethers, aryl ethers, other substances or mixtures). In addition, these diseases may also be classified under Chapter II (caused by physical factors, specifically under the heading of diseases caused by ionizing radiation), Chapter III (relating to the respiratory tract, lungs, pleura and peritoneum, specifically asbestos-related cancers, beryllium, radioactive substances, coke-oven gases, wood dust, lung cancer in association with pneumoconiosis caused by dust containing free crystalline silica). Chapter IV then includes skin cancers caused by physical factors, especially UV radiation, and those caused by soot, tar, pitch, mineral oils and arsenic). Chapter V, which represents communicable and parasitic diseases, includes, for example, hepatocellular carcinoma following hepatitis B or C.

The number of reported cases of occupational cancers according to the National Registry of Occupational Diseases is significantly lower than the number of reported cases of cancers in the general population according to the National Cancer Registry. This includes cases of cancers that are primarily associated with working conditions (e.g. mesothelioma arising in association with asbestos exposure).

The statistics show that the issue of occupational cancers is not a matter of history and that the emphasis on prevention in the area of working conditions is still relevant. If a suspected occupational cancer has already occurred, an objective assessment of the working conditions in accordance with current scientific knowledge is required for official recognition of the occupational disease. It is necessary to draw attention to the possibility of biological monitoring

of exposure and the effect of genotoxic factors in the working environment. At the same time, an assessment of the impact of a combination of different noxes can be required, which is challenging.

This work was supported by the grant IGA_LF_2023_007.

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Genetic changes in mitochondria: a potential marker of testicular tumor chemoresistance?

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Genetic changes in tumor cells play an important role in response to treatment of multiple human malignancies. Recently, considerable efforts have been made to better define the consequences of mtDNA genetic alterations in tumorigenesis.
In this work, we determined the mutational profile of the mitochondrial genome of the resistant TGCT cell lines 1777NRpmet and 1411HP in comparison with the sensitive TGCT cell line 2102EP. Sequencing comparison of resistant and sensitive TGCT cell lines showed significant differences in identified mutations and most mutations were identified in respiratory genes.

Metabolic dysfunction is one of the main features of malignant cells, and it is clear that mutations of the mitochondrial genome lead to a metabolic deficit. The accumulation of mitochondrial mutations is related not only to disease progression but also to treatment resistance. Analysis of respiratory chain function revealed differences between the sensitive 2102EP cell line and the resistant 1777NRpmet and 1411HP cell lines. We also observed a significant difference in respiration between both resistant cell lines.
By focusing on genetic variability and gene changes in nuclear and mitochondrial genomes, we hope to contribute to the identification of new genetic biomarkers associated with chemoresistance in TGCTs.

This work was supported by grants: APVV-19-0286, VEGA2/0056/21, MVTS COST CA21154. TGCT cell lines were kindly provided by Dr. Thomas Mueller (Martin Luther University Halle-Wittenberg, Halle, Germany).

Association of the C*677T* polymorphism in the *MTHFR* gene and male infertility

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The study aimed to investigate the existence of a relationship and to determine the degree of association between the *MTHFR* gene and the occurrence of male infertility. The analysis set consisted of 273 individuals (173 males with fertility disorder and 100 fertile individuals were the control set). We determined the hormonal profile in all probands (TEST, LH, FSH, ProL, TSH, EST, SHBG) using a Cobas e411 biochemical analyzer. We detected the presence of an SNP (*rs1801133*) in the *MHTFR* gene by real-time PCR genotyping analysis and evaluated the genotypic and allelic frequencies. We found a statistically significant difference in the mean levels of LH, ProL, TEST, SHBG (***p < 0.001), and EST, TSH (**p < 0.001) between the set of probands diagnosed with fertility disorder and the set of healthy fertile individuals. A statistically significant difference in the genotypic and allelic frequency of the *rs1801133* polymorphism between fertile and infertile individuals was not confirmed (p = 0.859). Significantly significant differences were found in the mean levels of individual biochemical parameters depending on the genotype of CC; LH, ProL, EST, SHBG (***p < 0.001), TEST (**p < 0.001), genotype of CT; TEST, SHBG (***p < 0.001), and genotype of TT; SHBG (***p < 0.001). The results of our study did not confirm the association of *C677T* polymorphism with the occurrence of male infertility.

This work was supported by KEGA project no. 002PU-4/2021 "University Teaching of Genetics by Innovative Forms and Methods."

Biological safety assessment of 10 nm gold nanoparticles with BSA coating in a mouse model *in vitro* and *in vivo*

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Targeted drug delivery systems offering countless benefits to human health represent a hot topic in the field of nanomedicine. Such interest in this type of therapy is caused also by the close cooperation between medicine and nanotechnology, which, thanks to dynamic technological progress, is now able to generate a wide range of nanoparticles that differ in size, shape, and coating material. However, the ever-increasing available spectrum of nanoparticles potentially usable in medical practice lacks information about their biological safety. To expand the accessible data on the biosafety of nanomaterials, a closer look at the impact of 10 nm BSA-coated gold nanoparticles (since their basic biological, chemical, and physical properties meet the criteria for carriers in targeted drug delivery systems) was chosen and performed on a mouse model - both *in vitro* and *in vivo*.

Despite the fact that *in vitro* experiments did not reveal any serious adverse effects of the tested nanoparticles (after 24 hrs of exposure), results from *in vivo* experiments showed changes at the mRNA level in the expression patterns of genes related to the inflammatory process, oxidative damage, and tissue fibrosis both 1 and 30 days after treatment (a single intravenous injection of nanoparticles in 5% glucose solution at a dose of 1 mg (Au)/kg (mouse)). Moreover, a higher representation of fibronectin-positive cells in the spleen and kidney of mice from 30-day variant of the *in vivo* experiment was captured by immunohistochemical staining. The fact that overexpression of fibronectin at the protein level can be detected by immunohistochemistry *in situ*, but not quantitatively by western blotting, leads to the conclusion that pathological processes may appear in the organism later in time, and therefore it is important to supplement the obtained data with experiments focused on the impact of the tested nanoparticles in longer periods of time.

This work was supported by the Slovak Research and Development Agency under Contract No. APVV-16-0579, Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and Slovak Academy of Science under Contract No. VEGA 2/0116/22 and the H2020 Twinning project VISION under Contract No. 857381.

An avian CAM model of cervical cancer and its diagnosis and treatment using PDT

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Photodynamic therapy (PDT) is a promising method for the treatment of cancer. Its principle is to deliver a photoactive compound, a photosensitizer (PS), to target tissue and activate it with light, leading to the formation of cytotoxic reactive oxygen species. Tumors can be destroyed by direct damage to the tumor cells, damage to the vasculature, and the subsequent immune response of the host. The aim of this study was to develop and verify a uterine cervix cancer model using C-33A cell line, for the research and optimization of highly selective PDT using quail and turkey chorioallantoic membrane (CAM).

C-33A cells were cultured in an EMEM medium or in an extracellular protein mixture, Cultrex®. As an experimental model, *ex ovo* quail CAM and *in ovo* turkey CAM were used. On the embryonic day (ED) 7 in quail, and ED14 in turkey embryos, the cell suspension was added to the silicone rings (Ø7 mm) placed on the CAM surface. On ED10 in quail, and ED18 in turkey embryos, PS hypericin (HYP) was applied and photodynamic diagnosis (PDD) using 405 nm LED light at various time intervals was performed. PDT was performed using laser light of 405 nm for 2 min (285 mW/cm^2), 5 h after HYP application. The effect of C-33A cells and subsequent PDD and PDT on the CAM vasculature and tissue was evaluated by measuring vessel growth parameters, qPCR, and histological analysis.

All methods of cell suspension application (in medium or Cultrex®) resulted in visible coating or amorphous formations on the CAM surface. In addition, Cultrex® led to formations that attracted blood vessels. In PDD, the formed amorphous structures were visible and distinguishable on the CAM thanks to fluorescence immediately after HYP application. Parameters of the vascular network were in general decreased after the PDT application and we observed a visible damaging effect on the CAM vessels. 24 h after local PDT we didn't observe changes in gene expression (angiogenic, apoptotic, and oxidative stress genes). Histological analysis showed the invasiveness of the C-33A cell line in the area of the mesodermal layer of the CAM. PDT led to the thickening of the chorion into the mesodermal layer, which we consider a characteristic effect of PDT in our laboratory conditions. However, we did not observe a negative effect of PDD or PDT on the ability of C-33A cells in combination with Cultrex® to penetrate CAM tissue.

We were able to establish a cervical cancer model using C-33A cell line in both quail and turkey CAMs. Cultrex® proved to be a suitable support medium for C-33A cells. PDD allowed differentiation of tumor foci on CAM, whereas PDT likely requires modification of conditions to completely inhibit the growth of tumors formed by C-33A cells.

This research was supported by grants VEGA 2/0042/21, APVV 20-0129, Doktogrant APP0242, and NCN Grant MINIATURA 2017/01/X/NZ8/00094.

Chromosome damage in regions with different levels of air pollution

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The environment is one of the important factors influencing human health. High levels of air pollution have been linked to the risk of cardiovascular, respiratory, and neurodegenerative diseases and cancer, and can interfere with fertility and affect the outcome of pregnancy. Various biomarkers are used to study the impact of the environment on health. Among the most appropriate cytogenetic markers is the analysis of structural chromosome aberrations, which have been shown to predict cancer risk. In this study, we compared chromosome damage in city policemen from three cities in the Czech Republic: industrial Ostrava characterized by high levels of benzo[a]pyrene, Prague with heavy traffic emitting nitrogen oxides, and relatively clean Ceske Budejovice located in an area with predominantly agricultural activity. Chromosomal aberrations in lymphocytes were evaluated by fluorescence in situ hybridization with painting probes for chromosomes 1, 2, 3, and 4. To assess differences in chromosome damage between the regions, cytogenetic parameters were adjusted for age. An increase in the frequency of unstable chromosome aberration, i.e. dicentric chromosomes and acentric fragments, was observed in Ostrava ($p = 0.014$ and $p = 0.044$, respectively) and in Prague ($p = 0.002$ and $p = 0.006$, respectively) in comparison with Ceske Budejovice. The difference was significant only for spring samples taken after the winter period when the concentration of pollutants in the air increases due to poor dispersion conditions. Samples collected after the summer period did not differ in any cytogenetic parameter. The different levels of pollution during the year were also reflected in the difference between spring and autumn samples. In both Ostrava and Prague, an increase in the frequency of dicentric chromosomes ($p = 0.017$ and $p = 0.023$, respectively) was observed after the winter period. More breakpoints were observed on chromosome 1 than on the other chromosomes examined ($p < 0.001$). The number of breakpoints in the heterochromatin region 1p11-q12 was lower than in other parts of chromosome 1 ($p < 0.001$). The chromosome aberration test used differentiated between populations exposed to low and high pollution and also between seasons with different levels of pollution.

This work was supported by the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798).

Human glioblastoma spheroids as a preclinical model for the study of photodynamic therapy

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Aspects related to the response of cells to photodynamic therapy (PDT) have been well studied in cell cultures, which often grow in monolayers. In this work, we propose a spheroidal model of human glioblastoma U87MG cells, designed to mimic superficial tumor tissue. Spheroids can be xenografted onto Japanese quail chorioallantoic membrane (CAM) to study the effects of photodynamic diagnosis and therapy in real time. CAM is a suitable model for research in the field of PDT due to its morphological and functional properties.

Embryos were incubated at 37°C and 50-60% relative humidity. On day 7 of embryonic development (ED7), prepared spheroids were transferred on the CAM surface and incubated until ED11. At this time the spheroids were bounded with silicone rings into which were injected two concentrations of hypericin (30 µl of 500 nM or 10 µM). They were irradiated by laser (405 nm, 285 mW/cm^2) for 4 min. The tissue was photographed and histopathologically processed on ED12. Gene expression of proangiogenic and inflammatory factors was evaluated by qPCR.

After application on the CAM surface, the U87MG spheroids were visible to the naked eye. Pharmacokinetics of fluorescence in ultraviolet light (405 nm) during 0, 1, 3, 5, and 24 h were observed after the application of a higher dose of hypericin, and photodiagnosis was possible from 1h after topical application of hypericin. At the lower hypericin concentration, detachment and relaxation of peripheral spheroid cells and their photodamage were observed in the area of hypericin application after PDT, but no significant difference in spheroid size compared to the control was detected. qPCR analysis revealed some significant differences in the expression of genes related to the immune response. Higher expression of IL-8 and TLR-7 after hypericin application was observed, especially when hypericin groups were stored in dark conditions. No significant changes in the expression of VEGF-A and Quek1 genes were observed 24 hours after treatment, but an increase in their expression was observed after the application of spheroids compared to tissue without spheroids.

A quail CAM model of glioblastoma spheroids was developed in this work that can be used in future PDT studies. The mechanism of PDT in individual spheroid layers should be better investigated in the future. The preclinical model of glioblastoma spheroids on CAM is a suitable model for drug screening and study of delivery systems.

The work was supported by grants VEGA 2/0042/21, APVV-20-0129, and VVGS-2023-2554.

Chromosome aberrations in selected groups of coke oven workers

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Polycyclic aromatic hydrocarbons (PAHs) belong to a group of environmental contaminants. PAHs represent a special class of organic compounds that are widely studied for their genotoxic, carcinogenic, and teratogenic properties. Inhaled PAH particles cause respiratory complications and increase the risk of lung cancer. Coke oven plant workers are at increased risk of kidney and prostate cancer. In addition to the direct mutagenic effect, reduced efficiency of DNA repair mechanisms has been reported in individuals exposed to PAHs. The aim of our study was to determine the frequency of aberrant cells and the degree of association between the selected variables and the average percentage of aberrant cells in the two observed workgroups of coke oven plant. A comparison of aberrated cells in the entire exposed set of workers with the control group showed a significant difference ($p < 0.05$). Individual categories of chromosome breaks were not shown to be statistically significant in the entire exposed group compared to the unexposed group. Gaps were significantly more frequent ($p < 0.001$) in the overall group of coke oven workers compared to the control group. The WG1 workgroup had a higher frequency of aberrant cells ($p < 0.01$) compared to the control group. Likewise, chromatid breaks occurred significantly more often in WG1 ($p < 0.01$) compared to the control. Gaps occurred in both monitored subgroups (WG1 and WG2) with a statistically higher frequency ($p < 0.001$) compared to the control group. Spearman's correlation analysis did not show a significant association between the selected variables (age, duration of occupational exposure, and duration of smoking) and the average percentage of aberrant cells.

This work was supported by KEGA project no. 002PU-4/2021 „University Teaching of Genetics by Innovative Forms and Methods“.

Concordance between telomere length and mitochondrial DNA copy number in solid colorectal adenomas

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Colorectal adenomas (CA) are abnormal growths that arise from the intestinal epithelium. These CA can be precursors to colorectal cancer (CRC), which is the second cause of cancer-related deaths worldwide. To reduce mortality from CRC, it is important to identify new biomarkers that can help discern the disease at an early stage when it is more treatable.

The study hypothesizes the relationship between telomere length (TL) and mitochondrial copy number (mtDNA CN) could serve as a potential biomarker for CA formation. Both TL and mtDNA CN were studied in 145 patients, always in CA and its paired normal tissue (NT). We also followed the gene expression of the enzyme responsible for renewing telomere ends (*TERT*) and a regulator of mtDNA CN (*TFAM*). We performed the isolation of nucleic acids from fresh frozen tissues and multiplex RT-qPCR using highly specific Taqman probes.

The results showed that TL in NT was significantly longer than in CA ($p < 0.0001$). This is also related to the fact that *TERT* expression was higher in NT than in CA ($p < 0.0001$), although the expression levels in both tissues were found only at low levels. Increased mtDNA CN was observed in CA compared to NT ($p < 0.0001$). Dysregulation in *TFAM* expression was also seen between both tissues ($p < 0.001$). Future research plans will follow these parameters in a cohort of patients in CRC stage I in order to monitor adenoma-to-tumor progression.

By identifying these biomarkers, new screening tests for CA and CRC can be established. This could lead to earlier detection of CA formation and progression to CRC and brings new insights into a less-studied field of processes in precancerous stages of CRC.

Acknowledgments: 21-04607X, 22-05942S, and NU22J-03-00033

Novel oligonucleotide based therapy for effective reduction of TKI-resistant CML cells

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Chronic myelogenous leukemia (CML) is a slow-progressing type of blood cancer, characterized by the presence of the BCR-ABL1 oncogenic driver protein. Over the past two decades, the development of TKIs has revolutionized the treatment of CML. Despite their sophistication, TKIs still have limitations. Up to 30% of patients either can't tolerate or develop resistance to the medication, which eventually results in the development of cancer that can evade treatment. Here, we report the results of a therapeutic oligonucleotide of the original design, namely ASP210, that showed the ability to reduce *BCR-ABL1* mRNA levels with remarkable efficiency by >99% after a single application and to induce cell death in TKI-resistant cells, including those with the clinically relevant T315I mutation, by day 5 after re-dosing. The effect was selective for cancer cells, indicating a favorable safety profile for this therapeutic modality. Our findings suggest that ASP210 is a promising therapeutic avenue for CML patients who fail to respond to TKI therapy.

This work was supported by the Slovak Research and Development Agency under Contracts No. APVV-15-0215, APVV-19-0070, and by VEGA Grants No. 1/0069/20, 2/0160/21, and 2/0116/22. The research was further supported by the Ministry of Health, Czech Republic via a project for conceptual development of research organization No. 00023736. The study was performed during the implementation of the project Building up Centre for Advanced Materials Application of the Slovak Academy of Sciences, ITMS project code 313021T081

supported by Research & Innovation Operational Programme funded by the ERDF.

An original therapeutic oligonucleotide effectively reducing the leukemic burden in a humanized mouse model of CML

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Chronic myelogenous leukemia (CML) is conventionally treated with tyrosine kinase inhibitors (TKIs). Despite their sophistication and therapeutic benefit, TKIs still have limitations such as being neither selective for CML cells nor specific for BCR-ABL1, leading to clinically relevant side effects. In addition, up to 30% of patients either can't tolerate or develop resistance to the medication, which eventually results in the development of cancer that can evade treatment.

The need for precision therapeutics led to the development of an original, highly potent, and safe oligonucleotide-based modality against *BCR-ABL1* mRNA, called ASP210. This drug candidate was shown to be effective in inducing cell death in *BCR-ABL1* positive cells while completely sparing *BCR-ABL1* negative cells. The therapeutic potential of ASP210 was demonstrated *in vivo* when a 10-day systemic *i.v.* administration of ASP210 (1 mg/kg/day) resulted in a reduction of leukemic burden in JAX mice by up to 99% and a significant reduction of the leukemic mass in the infiltrated organs by up to 50%. The favorable safety profile of ASP210 in terms of acute and sub-acute toxicity will also be presented.

This work was supported by the Slovak Research and Development Agency under Contracts No. APVV-15-0215, APVV-19-0070, and by VEGA Grants No. 1/0069/20, 2/0160/21, and 2/0116/22. The study was performed during the implementation of the project Building up Centre for Advanced Materials Application of the Slovak Academy of Sciences, ITMS project code 313021T081 supported by Research & Innovation Operational Programme funded by the ERDF.

Cytotoxicity and genotoxicity hazard of hospital wastewaters evaluated by alternative *in vitro* assay

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Hospitals and medical facilities use a wide range of different chemicals and preparations for therapeutic purposes, diagnosis, research, disinfection and daily operations, which are discharged into the sewer (sewage) system, either after pretreatment or without treatment. Some chemicals are not effectively removed in wastewater treatment plants and can therefore be a source of pollution for surface water and groundwater, thus posing an increased risk to human health and the environment. Many scientific studies have confirmed that wastewaters (WWs) from medical facilities are often cytotoxic, genotoxic, or both. However, the evaluation of the cyto/genotoxic effects of given compounds in WWs is not a simple matter, mainly due to the variable characteristics of the WWs which depend on the type of hospital activity. We monitored the cyto/genotoxic potential of WWs from 5 hospitals in the Czech Republic and the evaluation was performed by means of various in vitro methods, i.e. single cell gel electrophoresis assay (Comet assay), bacterial reverse mutation test (Ames test), chicken egg genotoxicity assay, *Allium cepa* test, cell transformation assay, and hen's egg test for micronucleus induction. Our study confirmed that all tested WWs samples can be assessed as potentially genotoxic or mutagenic regarding the results of the specific in vitro tests, with the exception of the Ames test. The evaluation of samples in the Ames test might be affected by the adjustment of the samples before testing, particularly sample filtration or sterilization which may lead to the removal of genotoxicants. Contamination by microbiological agents of chlorinated WWs appears to be a key phenomenon causing significant cellular and nuclear damage recorded in this study.

Supported by ERDF/ESF project "International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860).